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AUGUST 30 - SEPT. 1, 1981
AUSTRALIAN SOCIETY FOR REPRODUCTIVE BIOLOGY

THIRTEENTH ANNUAL CONFERENCE

University of Canterbury
Christchurch, New Zealand

AUGUST 30 - SEPTEMBER 1, 1981

PROGRAM AND ABSTRACTS OF PAPERS

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**AUSTRALIAN SOCIETY FOR REPRODUCTIVE BIOLOGY**

**PROGRAMME GUIDE**

**Location A1 - Lecture Room A1; A2 - Lecture Room A2**

**AUGUST 30TH, 1981 - SUNDAY**

1. SYMPOSIUM
   - Fetal Growth & Development
   - Robinson/McDonell/Leggins/Thorburn
   - (1-4)

2. ESA Mini Symposium "CLONING"
   - A1 Lecture Room
   - (1100-1130)

3. LODING LECTURE
   - Prof. B.K. Follett - 'Photoperiodism'
   - A1 Lecture Room
   - (1200-1230)

4. III. FERTILITY ORAL
   - A2 Lecture Room
   - (1600-1700)

**AUGUST 31ST, 1981 - MONDAY**

5. V. SPECIAL LECTURE ASRB
   - Prof. W. Snooks - Epitopes
   - MALE oral
   - (50-56)

6. VI. MALE POSTERS
   - 11:00-11:30

7. ESA SYMPOSIUM
   - A1 Lecture Room
   - (11:30-12:00)

8. LUNCH/TRADE/POSTERS
   - (12:00-13:30)

9. ESA SPECIAL LECTURE
   - Prof. Edis - Parathyroid
   - (57-75)

10. VII. LUTEAL FUNCTION ORAL
    - A2 Lecture Room
    - (76-81)

**SEPTEMBER 1ST, 1981 - TUESDAY**

11. VIII. PREGNANCY ORAL A2
    - (100-103)

12. IX. LACTATION ORAL A2
    - (110-113)

13. X. PREGNANCY LACTATION POSTERS
    - (94-106)

14. XI. ADS/ESA KELLON LECTURE
    - The control of carbohydrate metabolism in pregnancy
    - A1 Lecture Room
    - (1545-1645)

15. XII. FERTILITY ORAL
    - A2 Lecture Room
    - (1730-1800)

16. XII. FERTILITY POSTERS
    - (with refreshments) (17:49-18:20)

**SESSION I - SYMPOSIUM: FETAL GROWTH AND DEVELOPMENT**

Chairman: Prof. T. Robinson

**SESSION II - FERTILITY: ORAL PRESENTATION**

Chairman: Prof. T. Robinson

**SESSION III - FERTILITY: ORAL PRESENTATION**

Chairman: Prof. T. Robinson

**SESSION IV - FERTILITY: ORAL PRESENTATION**

Chairman: Prof. T. Robinson

**PROGRAMME**

1. 0900 - 1000 Registration

2. 1000 - 1100 Welcome and Opening

3. 1100 - 1200 Invited Paper: "The Placental Lactogen"

4. 1200 - 1300 Symposium: "Fetal Development and Growth"

5. 1300 - 1400 Symposium: "Fetal Development and Growth"

6. 1400 - 1500 Symposium: "Fetal Development and Growth"

7. 1500 - 1600 Symposium: "Fetal Development and Growth"

8. 1600 - 1700 Symposium: "Fetal Development and Growth"

9. 1700 - 1800 Symposium: "Fetal Development and Growth"
SESSION III - FERTILITY: ORAL PRESENTATION
Chairman: Dr. K. McMillan

1600 11 An isolated pituitary model in the sheep: effects of oestradiol-17β (E2) on Gn-RH stimulated LH and TSH release
I.P. Clarke, J.T. Cummins, J.K. Findlay, D.M. de Kretser

1615 12 Assessment of blood flow to individual ovarian follicles: relative levels in ewes of high and low fecundity
B.W. Brown, P.E. Hattner, B.M. Bindon

1630 13 Ovine follicle stimulating hormone stimulates secretion of inhibin in immature female rats
V.W.K. Lee, J. McMaster, H. Quigg, L. Leversha

1700 14 Regulation of ovarian inhibin production by steroids
K.M. Henderson, P. Franchimont

1715 15 Learning self-recognition of the fertile period: results in a prospective multicentre trial
H.G. Burger

1730 16 Biochemical and ultrastructural studies of normal and hostile cervical mucus
P.J. Lutjen, B. Daunter

SESSION IV - FERTILITY: POSTER PRESENTATIONS

17 Is a particular body composition related to first oestrus in Romney hoggets?
R.W. Moore, J.J. Bass, G.W. Winn

18 Ram-induced oestrus in pre-puberal ewes
A.H. Williams

19 Effect of diet and live weight on FSH and oestradiol concentrations in Romney ewes
T.W. Knight, E. Payne, A.J. Peterson

20 Sensitivity to GnRH in Booroola and control ovariectomized ewes
T. O'Shea, L.J. Cummins, B.M. Bindon, L.R. Piper

21 Ram-induced ovulation in seasonally anoestrous Merino ewes actively immunized against an oestrone-gelatin conjugate
R.J. Scaramuzzi, R.M. Hoskinson, M.T. Hinks, H.M. Radford, K.E. Turnbull

22 Ovulation in the goat after intra-vaginal sponge and PMSG treatment
A.J. Biter, S. Salamon, W.M.C. Maxwell

23 Ovarian and pituitary activity of three breeds of ewe subjected to artificial photoperiod
A.L. Poulton, T.J. Robinson

24 The effect of melatonin feeding on plasma prolactin levels and the onset of oestrus activity in sheep
D.J. Kennaway, T.A. Gilmore, R.F. Seamark

25 The control of active immunization against oestrone or androgens to increase fecundity of sheep
R.I. Cox, Patricia A. Wilson, R.J. Scaramuzzi, R.M. Hoskinson, J.M. George, B.M. Bindon

26 Seasonal changes in LH secretion in normal and clover infertile ewes
W.A. Chamley, I.J. Clarke, A.R. Moran

27 Stimulation of ovulation rate in Merino ewes by immunization against steroids
R.M. Hoskinson, R.I. Cox, R.J. Scaramuzzi, H.T. Hinks, M.A. Hillard, K.E. Turnbull, M.S.P. Mong

28 Duration of action of PMSG for super-ovulation in sheep and cattle
B.M. Bindon, L.R. Piper, H.A. Cheers, Y.M. Curtis, E.J. Holland, R.D. Nethery

29 Progesterone and prostaglandin E and F secretion by human corpora lutea in long-term organ culture
T.P. Dehany, A.O. Trownson, A.L.A. Boura, W.A.M. Walters

30 Effects of cloprostenol on the corpus luteum of the guinea pig
T.I. Azmi, J.D. O'Shea, C.S. Lee, R.J. Rodgers

31 Factors influencing interpretation of the zona-free hamster ovum test for human fertility
J.P.P. Tyler

32 High pressure liquid chromatography: a new approach for the purification of inhibin from ovine follicular fluid
M. Dobos, H. Burger

33 Inhibin levels in individual sheep follicles
C.G. Tsonis, H.R. Quigg, V.W.K. Lee, L. Leversha, A.O. Trownson, J.K. Findlay

34 Suppression of plasma FSH in ovariectomized ewes given follicular fluid
J.K. Findlay, L. Cummins, T. O'Shea, B. Bindon

35 Suppression of induced ovulation by inhibin
L.J. Cummins, T. O'Shea

36 Development of a new system capable of sustained release of non-steroidal anti-inflammatory compounds
P.V. Pеплов, P.R. Hurst

37 The delivery of indomethacin from silastic rods into the uterus of the rat
P.R. Hurst, P.V. Pеплов

38 Histochemical changes in the reproductive tracts of ewes injected with progesterone early in the oestrous cycle
Suree Chartwaingam, T. O'Shea, J.F. Hecker

39 Effect of oestradiol-17β and progesterone on the incorporation of D-[U-14C]glucose into secretions from tissue explants in organ-culture
B.E. Murdoch
40 Prostaglandins in the ovine endometrium and uterine lumen
N. Colvin, J. Swaney, B. Doughton, J.K. Findlay

41 A technique for the study of prosta­
glandin production by human decidual tissues
Janet Keast, G.C. Liggins, R.P. Sefton

42 Cervical histology as a measure of the response of ewes to large amounts of oestrogen
N.R. Adams

43 Anti-uterotrophic action of progesterone in the oestrus stimulated uterus: comparison of the sheep and mouse
B.G. Miller, R. Tassell, G.M. Stone

44 Action of progesterone on uterine steroid receptor level: comparison of the sheep and mouse
G.M. Stone, C. McCaffery, B.G. Miller

45 The source of uterine 'luminal fluid' proteins in pregnant mice
L.A. Hinds, C.H. Tyndale-Biscoe

46 The response in plasma progesterone to hypophysectomy of the female tammar
J.D. Curlewis, G.M. Stone, A.L. Poulton, M. Axelson

47 The major steroids in ovarian and adrenal venous plasma of Trichosurus vulpecula
C.A. Horn

48 Luteinising hormone in the brush tail possum
G. Shaw

49 Myometrial activity in the tammar wallaby

MONDAY, AUGUST 31:

SESSION V - MALE : ORAL PRESENTATION
Chairman: Professor I. White

0900 50 SPECIAL LECTURE
The role of cyclic nucleotides and of epididymal proteins in the initiation of sperm motility
Dr. D. Hoskins, Oregon Primate Centre, U.S.A.

0930 51 The period of DNA synthesis by primary spermatocytes, and the duration of the cycle of the seminiferous epithelium of the dog
P.B. Davies, T.C.A. Martin

0945 52 Regulation of the synthesis and secretion of epididymal proteins
D.E. Brooks

1000 53 Effect of cauda epididymal fluid and proteins on the metabolism and motility of ram sperm
J.G. White, P.P. Goh, J.K. Voglmaier

1015 54 Alterations in the flagellar plasma membrane of ram spermatozoa during post-testicular maturation
M.P. Bradley, I.T. Forrestor

1030 55 Human seminal plasma and spermatozoal CMP-sialic acid synthetase and sialyltransferase activities
B. Daunter

1045 56 Plasma membrane changes induced in ejaculated ram sperm following selective removal of seminal plasma
M.T.W. van Eerten, G.J. Jansen, I.T. Forrestor, K.I. Memillan

SESSION VI - MALE : POSTER PRESENTATIONS

1100 57 Chromosomal and reproductive studies of bulls evaluated for artificial breeding
W.L. Potter, A.W. Blackshaw

1230 58 Effects of a phosphodiesterase inhibitor on the survival of chilled and frozen bull semen
P. Situmorang, T.C.A. Martin

59 Fertility of frozen-thawed goat semen
A.J. Pitar, S. Salmon

60 Acrosome reactions in motile human spermatozoa at low and high pH
J.M. Cummings, T.M. Green

61 Some chemical parameters of goat semen
G. Henda-Ruguel, P.Y.W. Chow, I.D. White, S. Salmon

62 Removal of seminal plasma inhibition of PHA stimulated lymphocytes following group A streptococcal absorption
S. Phillipson, B. Boettcher

63 The development of an animal model for epididymovasostomy
P.D. Temple-Smith, G.J. Souttivick

64 Testosterone treated wethers and ewes used as teaser
K.B. Croker, L.G. Butler, M.A. Johns, S. McColl

65 Oestrogen-androgen interaction facilitating male sexual activity
P.E. Mattner

66 Responses of rams to electro-ejaculation during epidural anaesthesia and after treatment with compounds acting on the autonomic nervous system
T.C.A. Martin

67 LH and testosterone responses to graded doses of gonadotropin releasing hormone (GnRH) in post pubertal bulls
T.B. Post, M.H. Reich, B.N. Binds

68 Compensatory hypertrophy of the boar testis following hemigonadectomy
D.K.R. Putra, A.W. Blackshaw
Testicular function in the marsupial mouse Antechinus minutus parthenitus

Observations on gonadal and endocrine activity of three breeds of ram subjected to artificial photoperiod

Glucose metabolism in cultured sertoli cells

Relationship between levels of testicular inhibin and serum FSH in rats after bilateral efferent duct ligation

Leydig cell hyperplasia and gonadotrophin responsiveness following cryptorchidism in the mouse

Comparison of the in vitro biological activity and anterior pituitary and interstitial cell receptor affinity of LH/H and its analogues

Identification of HCG antibodies after chronic daily HCG treatment in adult male rats

SESSION VIII - PREGNANCY: ORAL PRESENTATION

0900  82  Deep-freezing of human embryos
       A.O. Trounson, L.R. Mohr, P.A. Pugh, J.P. Leeton, E.C. Wood

0915  83  Incorporation of $^{14}$C-glucose into inner cell masses isolated from mouse blastocysts by immunosurgery
       W.R. Edirisinghe, R.G. Wales

0930  84  Amino acid transport during pre-implantation development

0945  85  Pregnancy associated proteins in the mare
       A.A. Gidley-Baird, B. Tei, N.W. Davey, J.G. Gradzinskas

1000  86  Progesterone production by sheep, goat and sheep x goat foetuses
       N.W. Moore, G.R. McDowell

1015  87  Residual effects of low nutrition during early pregnancy in the ewe
       R.A. Farr, A.R. Williams, I.P. Campbell

SESSION IX - LACTATION: ORAL PRESENTATION

1100  88  Sucking behaviour of lambs from high fecundity Booroola longwool cross ewes
       G.N. Hinch, R.W. Kelly, G.H. Davis, B.A. Veenvliet

1115  89  Calving date and nutrition effects on interval from calving to first oestrus in beef cattle
       G.M. Hinch, R.W. Kelly, G.H. Davis, B.A. Veenvliet

1130  90  Breeding of sows during lactational anoestrus
       G.W. Montgomery

1145  91  Calving performance to fixed-time insemination of dry and suckled Hereford cows
       N.E. Johnston, T. Stelmasiak

1200  92  LH release and luteal function in post-partum ewes after the pulsatile administration of LH-RH
       R.K. Munro, N.W. Moore

1215  93  Artificial induction of lactation: the importance of glucose
       D. Lesanaru, G.H. McDowell

SESSION X - PREGNANCY/LACTATION: POSTER PRESENTATIONS

1400-1645  94  Mixed species (Bos indicus-Bos taurus) cattle twins
       J.H. Shelton, P.M. Summers

1400-1645  95  Comparative ultrastructure of the hatched human, mouse and bovine blastocysts
       L.R. Mohr, A.O. Trounson, P.A. Pugh

1400-1645  96  A serum factor in pregnant pigs detected by a rosette inhibition test
       A.S. Greaves, A.L.C. Wallace, T.Y. Pan, N.W. Rigby

ANNUAL GENERAL MEETING (ASRB)

SESSION VII - LUTEAL FUNCTION: ORAL PRESENTATION

Chairman: Dr. R. Fairclough

1400  76  The corpus luteum of the brush possum does not require luteotropic support
       C.M. Tyndale-Biscoe, L. Hinds

1415  77  Luteal size and function in the Booroola Merino
       R.J. Searsmuzzi, K.M. Turnbull, J.A. Downing, B.M. Bindon

1430  78  Protein binding of progesterone in rat plasma
       D.J. Willox, N.W. Bruce

1445  79  Relation between ovulation rate and plasma progesterone in Merinos with natural and induced high fecundity
       B.M. Bindon, L.J. Cummings, L.R. Piper, T. O'Shea

1500  80  Plasma concentrations of progesterone and oestadiol about oestrus in red deer hinds
       R.W. Kelly, K.P. McNatty, G.H. Moore

1515  81  Clinical studies of progesterone production using non-radioactive isotopes

1600  82  Glucose metabolism in cultured sertoli cells
       C.E. Cross, A.W. Blackshaw

1615  83  Relationship between levels of testicular inhibin and serum FSH in rats after bilateral efferent duct ligation
       C.L. Au, D.M. Robertson, D.M. de Kretser, B. Jagou

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1700  86  Identification of HCG antibodies after chronic daily HCG treatment in adult male rats
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Rate of blood flow to the corpora lutea of pregnancy and to those of previous cycles throughout pregnancy in the rat

Differentiation of pregnancy and non-pregnancy in pigs from plasma oestrone sulphate levels

HPLC determination of specific radioactivity of lactate in plasma

The movement of iron across the rat placenta

Observations on the activity of 20a-hydroxysteroid oxidoreductase in ovine foetal erythrocytes

The concentration of progesterone and 13,14-dihydro-15-keto prostaglandin F-2α (PGFM) in the possum Trichurus vulpecula during pregnancy

The ultrastructure and cortisol concentration of adrenal glands in the marsupial neonate

Electron microscopy and lymphocyte subpopulations of ovine milk cells

Mammary lactose in tammar wallabies (Macropus eugenii)


SESSION VIII - PREGNANCY : ORAL PRESENTATION Chairman: Professor G. Liggins

0900 82 Deep-freezing of human embryos A.O. Trounson, L.R. Mohr, R.A. Parr, A.H. Williams, I.P. Campbell

0915 83 Incorporation of 14C-glucose into inner cell masses isolated from mouse blastocysts by immunosurgery M.W. Edirisinghe, A.G. Walls

0930 84 Amino acid transport during pre-implantation development P.L. Kaye, H.B. Pratt, M.H. Johnson, R.B. Chruch, G.A. Schultz

0945 85 Pregnancy associated proteins in the mare A.A. Gidley-Baird, B. Teisner, N.W. Moore, G.H. Davis

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1100 88 Suckling behaviour of lambs from high fecundity Booroola longwool cross ewes G.N. Hitch, R.W. Kelly, G.H. Davis, E.A. Veenvliet

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102 The concentration of progesterone and 13-14-dihydro-15-keto prostaglandin F-2α (PGFM) in the possum Trichurus vulpecula during pregnancy

103 The ultrastructure and cortisol concentration of the serratolne in the mammal

104 Electron microscopy and lymphocyte subpopulations of ovine milk cells

105 Effect of temporary calf removal on beef cow reproduction

106 Mammary lactose in tammar wallabies (Macropus eugenii)

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INTRAUTERINE GROWTH RETARDATION


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Perinatal mortality and morbidity are increased in fetuses which are small-for-gestational age in both man and in all other species studied. Even when major congenital malformations are excluded the growth retarded newborn is more likely to have persistent physical and neurological handicap. Many factors have been shown to influence fetal growth adversely, e.g. maternal or fetal disease, nutrition, drugs and environmental influences.

The natural incidence of growth retarded fetuses is low making investigation of the small fetus difficult. Thus a variety of different techniques have been devised to increase the proportion of growth retarded infants. Maternal undernutrition has been used extensively to induce growth retardation and the rapidity with which fetal growth rate is reduced, has only been demonstrated recently(I).

The utero-placental circulation has been disturbed in a variety of ways. Ligation of uterine or umbilical vessels results in an abrupt change in fetal growth rate(2,3). Creasy, Barrett, deSwiet, Kahanpää & Rudolph (1972)(4) produced a more gradual change in placental function by embolising the maternal side of the placenta throughout the latter stages of pregnancy. Alexander (1964)(5) devised a method for restricting the growth of the placenta from the time of attachment in sheep. Excision of endometrial caruncles before pregnancy results in the formation of a placenta with fewer cotyledons than normal. Alexander demonstrated that the resulting small placenta was associated with an increase in the incidence of fetal death, premature labour and intrauterine fetal growth retardation.

We have utilized Alexander's technique to induce intrauterine growth retardation. At about 100 days of pregnancy catheters were implanted into the ewe and fetus to enable collections of blood samples and recording of various physiological variables from the fetus. Three groups of fetuses were followed; growth retarded fetuses and fetuses of normal birthweight from ewes which had endometrial caruncles excised, and control fetuses. At the end of the experiment the body proportions of each fetus were determined. In the growth retarded fetuses the brain, kidneys and adrenals were significantly greater in proportion to bodyweight than in controls. These small fetuses were hypoxaemic (\(P_{O_2}\)=20.6 (mean ± S.E.M.)mmHg vs 23.2±0.7mmHg in controls) and polycythaemic (packed cell volume 37.3±1.67 vs 29.8±0.7%). The normal sized fetuses in ewes from which endometrial caruncles were removed were hypoxaemic only in late gestation. The growth retarded fetuses were hypoglycaemic; plasma lactate concentrations were lower than normal and the lactate-pyruvate ratio increased(6). Endocrine changes were also observed in the growth retarded fetuses. Plasma insulin concentrations correlated with the concentration of glucose(7). Similarly somatomedin-like activity was reduced in plasma of fetuses in ewes which had endometrial caruncles excised(8). In contrast the concentration of cortisol, adrenaline and noradrenaline were increased. Induced hypoxaemia had a more profound effect on the growth retarded fetuses with more substantial rises in ACTH, cortisol and catecholamines than in controls.
These results indicate that intrauterine fetal growth retardation due to restriction of placental growth is associated with altered oxygenation and metabolism. It is suggested that the endocrine changes observed are secondary to these but probably in turn, account for the altered pattern of fetal growth.

(2) Wigglesworth, J.S. Pathology and Bacteriology, 88: 1-13 (1964)


LUNG DEVELOPMENT

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University of Auckland, Auckland, New Zealand.

(Abstract not received)
FECUNDITY IN BOOROOLA MERINO SHEEP - FURTHER EVIDENCE OF A MAJOR GENE
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Invermay Agricultural Research Centre, Mosgiel

It has been postulated, using data from the original CSIRO sample of 14 Booroola ewes and their 19 daughters that the exceptional fecundity of this breed is due to a major gene or closely linked group of genes (1). The segregation criterion was a set of triplets at least once in the lifetime of a ewe. The present study analyses ovulation rate and litter size data from daughters of eight Booroola type rams. The rams were mated to Merino ewes in single sire groups. Each sire progeny group comprised 25-46 ewes which were laparoscoped 17 days after joining each year. The triplet segregation criterion seemed appropriate as only 1 percent of the ewes in the Merino flock have triplet ovulations. The results are summarized in Table 1.

Table 1: Proportions of ewe progeny with and without one record of triplet litters/ovulations in 3 - 6 years' observations.

<table>
<thead>
<tr>
<th>Sire</th>
<th>Litter Size</th>
<th>Ovulation Rate</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>&lt;3</td>
<td>&gt;3</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>A</td>
<td>41</td>
<td>37</td>
</tr>
<tr>
<td>B</td>
<td>44</td>
<td>39</td>
</tr>
<tr>
<td>C</td>
<td>46</td>
<td>32</td>
</tr>
<tr>
<td>D</td>
<td>41</td>
<td>39</td>
</tr>
<tr>
<td>E</td>
<td>31</td>
<td>36</td>
</tr>
<tr>
<td>F</td>
<td>40</td>
<td>27</td>
</tr>
<tr>
<td>G</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>H</td>
<td>41</td>
<td>38</td>
</tr>
<tr>
<td>Mean</td>
<td>0.59 0.41</td>
<td>0.47 0.53</td>
</tr>
</tbody>
</table>

Classification according to litter size was not totally consistent with the major gene hypothesis (1). Three progeny groups had fewer triplet litters (rams B and D (P<0.01), ram H (P<0.05)) than the expected ratio of 0.5:0.5 for a heterozygous ram, but the incidence of triplets was greater (P<0.01) than for the Merino flock.

Using triplet ovulations as the segregation criterion, two progeny groups differed significantly from the ratio of 0.5:0.5 expected for a heterozygous ram, but the incidence of triplets was greater (P<0.01) than for the heterozygous ram. Progeny of ram G had more (P<0.01) triplets than expected from a heterozygous ram, and did not differ significantly from a ratio of 0:1.0 that would be expected for progeny of a high fecundity homozygous ram. Progeny of ram D has less (P<0.05) with triplet ovulations than would be expected for a heterozygous ram. The mean lifetime ovulation rate of the ewes with at least one triplet ovulation was 2.80 and for the other ewes it was 1.54 (contemporary Merino ewes = 1.39).

These data largely support the hypothesis that fecundity in Booroola Merinos is affected by a major gene, although the performance of the progeny of ram D is an exception. Undoubtedly the segregation criterion to identify the genotype of ewes and rams needs further clarification since factors including embryonic mortality, environmental effects and high levels of fecundity in the base breed could markedly influence the interpretation of results.

It is well known that ovulation rate in ewes is stimulated by improved nutrition. The effects of quality rather than quantity of feed have not been extensively investigated although, where this has been done (1-3), energy rather than protein intake has been the important component of feed affecting ovulation rate. However, the recent findings that supplements with high protein content such as lupins can cause increases in ovulation rate (4,5) has necessitated further evaluation of the roles of dietary protein and energy levels.

Eight hundred mixed age Coopworth ewes weighing about 46 kg were allocated to 16 groups and used in an experiment with a 4 x 4 factorial design. Four pelleted diets that had different levels of crude protein (12, 15, 18 and 22%) but similar levels of energy (11 MJ/kg DM) were formulated using various combinations of barley, peas, grass meal and maize gluten and these were fed at four levels (0.5, 1.0, 1.5 and 2.0 kg/ewe/day) for a period of 19 days. All ewes were treated with intraovaginal sponges to synchronize oestrus and following a 10 day introductory period ewes were fed solely on their assigned ration until laparoscopy 5 days after the second oestrus. The ovulation rate is shown in Table 1.

### TABLE 1. Ovulation rate and protein intake (g/day).

<table>
<thead>
<tr>
<th>Intake kg DM/e/d</th>
<th>Diet (%) CP</th>
<th>A (12)</th>
<th>B (15)</th>
<th>C (18)</th>
<th>D (22)</th>
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<tr>
<td>0.5</td>
<td></td>
<td>1.26 (60)</td>
<td>1.13 (75)</td>
<td>1.04 (90)</td>
<td>1.22 (110)</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td>1.23 (120)</td>
<td>1.23 (150)</td>
<td>1.12 (180)</td>
<td>1.46 (220)</td>
</tr>
<tr>
<td>1.5</td>
<td></td>
<td>1.23 (180)</td>
<td>1.54 (225)</td>
<td>1.41 (270)</td>
<td>1.51 (330)</td>
</tr>
<tr>
<td>2.0</td>
<td></td>
<td>1.62 (240)</td>
<td>1.47 (300)</td>
<td>1.51 (360)</td>
<td>1.66 (440)</td>
</tr>
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</table>

Analysis of percent ewes having multiple ovulations (% EMO) on a group basis indicates that there are significant effects of both protein (P) and energy (E) intakes.

The model giving the best fit is one which includes a dichotomy effect of protein intake and an additional significant effect of energy.

\[
\text{logit } \% \text{ EMO} = (1) P \geq 200 \text{ g/d} - 1.85 + 0.0495 \\
(11) P \geq 200 \text{ g/d} - 0.88 (20.021) \epsilon
\]

This indicates that there is a trigger level of protein intake above which the slope of the response to energy intake is similar. The level of protein intake at which the dichotomy occurred in this trial (about 200 g/day) is much higher than the levels at which a similar type of response has been seen with lupin supplemented diets (6). This indicates that the rate of ruminal degradation of various sources of protein could influence the absolute level at which the trigger effect of protein intake occurs.

EFFECTS OF GnRH ANALOGUE AND PROSTAGLANDIN ON THE BOVINE OESTROUS CYCLE

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When either prostaglandin F2α (PGF) or its analogues is injected into lactating dairy cows during dioestrus (Days 7 to 16; oestrus = Day 0), the variation in the post-injection interval to oestrus is influenced by the stage of dioestrus (1). In PGF-treated cows detected in oestrus within 120 h of injection, and inseminated after detection, pregnancy rates are 10% higher than in untreated herd mates (1). A field trial was completed to test whether an injection of GnRH analogue could improve the precision of synchrony of oestrus to a subsequent PGF injection while still maintaining the fertility advantage.

Cows in eight dairy herds were injected i.m. with PGF (a) when at Days 7 to 16 of the cycle preceding insemination. In any one herd half of these PGF-treated cows had been injected with GnRH (b) 72 h, 24 h or within 15 min before the PGF injection. The post-treatment interval to oestrus for each cow was carefully monitored by each herd owner with the aid of tail-paint (2). Animals which failed to show oestrus within 10 days were examined per rectum.

TABLE 1. Percentage distribution of interval to oestrus.

<table>
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<tr>
<th>TREATMENT</th>
<th>INTERVAL INTERVAL TO OESTRUS AFTER PGF (days) NO. OF COWS</th>
</tr>
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<tr>
<td></td>
<td>(h) 3 4 5 6 7 8 9-12 NS</td>
</tr>
<tr>
<td>+ 0.25</td>
<td>+ 24 - 14 8 12 10 20 16 19 80</td>
</tr>
<tr>
<td>+ 24</td>
<td>+ 72 - 5 15 3 6 21 22 12 17 78</td>
</tr>
<tr>
<td>+ 12</td>
<td>- - - - - - - - - - - -</td>
</tr>
</tbody>
</table>

NS = not seen in oestrus

Injecting GnRH before PGF did not improve the precision of synchrony to the latter injection (Table 1). Amongst PGF and GnRH + PGF cows, the interval to oestrus was influenced by the stage of the cycle at which a cow was injected. For example, the percentage of cows in oestrus within 3 days (46-70 h) after a PGF injection declined from 64% among cows injected on Day 7 of the cycle to 21% on Day 12 and then increased again to 69% on Day 16. These trends were reversed with the GnRH-treated cows. The pregnancy rate for PGF cows was 71.4% compared to 61.4% in GnRH+PGF cows.

The results demonstrate that a low dose of GnRH analogue injected during dioestrus has a potent short-term effect on ovarian function.

(a) Lutalyse, Upjohn NZ Ltd (25 mg PGF-tham salt per i.m. injection).
(b) Receptal, Hoechst NZ Ltd (5 mcg Hoe 766 per i.m. injection).

EFFECT OF SHORT DAYLENGTH AND MELATONIN TREATMENT ON PLASMA PROLACTIN AND ON THE BREEDING SEASON AND PREGNANCY IN PINEALECTOMISED EWES

Department of Obstetrics and Gynaecology, University of Adelaide and Department of Agriculture and Fisheries, Kybollite, South Aust.

The role of photoperiod in controlling the onset of the breeding season in sheep is well established. Another more recently observed phenomenon involves an increase in ovulation rate in ewes subjected to short daylength just prior to the normal breeding season (1). This increase in ovulation rate does not occur in pinealectomised sheep (E. A. Dunstan, unpublished). The aim of the current experiment was to determine whether treatment with melatonin, a putative pineal hormone, could mimic the effects of shortened daylength.

On December 4th, 1979, 22 pinealectomised (Px) and 22 sham-operated (Sh) Saxon Merino X Border leicester ewes, run as a single flock, were assigned to 4 treatment groups viz; 1) 5 Px and 5 Sh ewes which were run into a darkened shed at 1600 h daily (i.e. 4 hours before sunset) for 9 weeks 2) 5 Px and 5 Sh ewes which were injected with 100 ug melatonin daily at 1600 h 3) 5 Px and 5 Sh ewes were implanted with melatonin capsules (release rate 150 ug/day) and 7 Px and 7 Sh ewes which were left untreated in the field as a control group. On December 24th a fertile ram was introduced into the flock and estrous observations and blood samples obtained daily for oPrL and progesterone analysis.

Forty of the 44 ewes exhibited a mating pattern consistent with a "Ram effect" and 43 ewes became pregnant. The presence of a pineal gland and treatments had no discernable effects on the timing of ovulation, gestation length or number of lambs. There were however marked differences between groups in plasma prolactin levels during the last six weeks of treatment (Table 1).

TABLE 1. Plasma prolactin levels (median and range ng/ml)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sham (Sh)</th>
<th>Pinealectomised (Px)</th>
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<tbody>
<tr>
<td>Control</td>
<td>82 (50-180)</td>
<td>41 (22-130)</td>
</tr>
<tr>
<td>Short Day</td>
<td>21 (8-26)</td>
<td>51 (37-86)</td>
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<tr>
<td>Melatonin Injection</td>
<td>8 (3-15)</td>
<td>58 (40-90)</td>
</tr>
<tr>
<td>Melatonin Implant</td>
<td>27 (16-47)</td>
<td>9 (3-22)</td>
</tr>
</tbody>
</table>

* P <0.01 compared to relevant control group

As is shown, short day treatment and both modes of melatonin administration suppressed PrL in intact (Sh) ewes whilst only the melatonin implants had effects in Px ewes. These data may be interpreted to further implicate the pineal in influencing temporal changes in end organ sensitivity and highlight the complexity of the interactions between seasonal changes in the pineal, melatonin, prolactin and ovulation rate.

PULSATILE SECRETION OF LH IN OVARIECTOMIZED EWES: EFFECTS OF OESTRADIOL AND THE INTRODUCTION OF RAMS

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Department of Animal Science and Production, University of W.A.
CSIRO, Division of Animal Production, Prospect, N.S.W.

In entire ewes, the frequency of luteinizing hormone (LH) pulses is lowest during anoestrus, probably due to increased sensitivity of the hypothalamo-hypophyseal system to negative feedback by oestradiol. The frequency of pulses can be increased in anoestrus ewes by (1). The frequency of LH pulses in ovariectomized ewes is (2). Ovariectomized ewes also are more sensitive to the inhibitory effects of oestradiol during the anoestrus season (3) and may respond to the introduction of rams in a similar fashion (4). It was therefore proposed to test if the introduction of rams to ovariectomized ewes would increase the secretion of LH and if the effect was dependent on oestradiol.

Fifteen ewes were ovariectomized and given empty 10 mm implants (n=5) or 5 or 10 mm implants (n=5) containing oestradiol-17β. The implants were constructed of Silastic Medical Grade tubing (1.98 mm outside diameter for the 10 mm implants, 1.57 mm for the 5 mm implants), and were inserted subcutaneously six weeks prior to the experiment.

Blood was collected every 15 minutes for 12 hours on the day of the experiment. After six hours of sampling rams were placed among the ewes. Plasma was assayed for LH and effects of treatments on LH levels were tested by analysis of variance.

**TABLE 1: EFFECTS OF OESTRADIOL (E2) AND THE INTRODUCTION OF RAMS ON THE LEVELS OF LH IN OVARIECTOMIZED EWES**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before Rams</th>
<th>Rams Present</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pulses/h</td>
<td>Basal level</td>
</tr>
<tr>
<td></td>
<td>(ng/ml)</td>
<td>(ng/ml)</td>
</tr>
<tr>
<td>Controls (n=5)</td>
<td>1.18 ± 0.07</td>
<td>5.80 ± 0.57</td>
</tr>
<tr>
<td>Sem E2 (5)</td>
<td>0.40 ± 0.22</td>
<td>2.47 ± 0.10</td>
</tr>
<tr>
<td>10 mm E2 (5)</td>
<td>0.40 ± 0.21</td>
<td>1.52 ± 0.39</td>
</tr>
<tr>
<td>*mean of 10 lowest points in profile (2)</td>
<td>0.87 ± 0.18</td>
<td>3.87 ± 1.05</td>
</tr>
</tbody>
</table>

Prior to the introduction of rams the basal levels and frequency of LH pulses were lowest in the ewes treated with oestradiol (P=0.003). After the introduction of rams, secretion of LH was increased within 15 minutes in eight oestradiol-treated ewes. These responses were similar to those seen in entire ewes (2). There was a significant increase in the frequency of LH pulses only in oestradiol-treated ewes (effect of interaction P=0.039). The basal levels also increased in all groups of ewes after introduction of rams (P=0.009).

It was concluded that the increase in LH secretion seen on the introduction of rams is principally due to an inhibition of the negative feedback action of oestradiol. A relatively small component of the increase may have been due to time, or due to an increase in LH secretion independent of oestradiol.


AN ISOLATED PITUITARY MODEL IN THE SHEEP: EFFECTS OF OESTRADIOL-17β (E2) ON Gn-RH STIMULATED LH AND FSH RELEASE.

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Medical Research Centre, Prince Henry's Hospital, Melbourne, 3004
*St. Vincent's Hospital, Melbourne, 3065 and *Department of Anatomy, Monash University, Clayton, 3168.

It has been suggested that oestrogen has a direct effect on the pituitary to regulate Gn-RH stimulated LH and FSH secretion(1). The aims of the present study were to develop an isolated pituitary model in the sheep, and to use this model to examine the effects of E2 on Gn-RH stimulated LH and FSH secretion.

Using ovariectomized (OVX) Merino ewes, a surgical procedure was developed that involved transection entry into the spinal cord bundle along the mid-line. Initially, we disconnected the pituitary from the hypothalamus by sectioning the stalk median eminence (SME) at the and proximal to the pituitary stalk median eminence (SME) at the (Model 1). Subsequently the introduction was performed by section between the infundibulum and the SME, above the superior hypophyseal arteries (Model 2). Blood samples were collected via jugular venous cannulae and plasma LH and FSH levels were measured by RIA. Hourly pulses of 250 ng Gn-RH were administered via jugular venous cannulae.

In Model 1 (n=4) plasma LH and FSH levels on the day after stalk section were 29±11 and 25±4 ng/ml (mean±S.E.) of levels prior to stalk-section; plasma FSH levels were 0.8-2.5 ng/ml and plasma FSH levels ranged from non-detectable to 87 ng/ml. In these ewes an i.v. injection of E2 caused a biphasic production, maximal 0.57±1.32 ng/ml (5.33±7.38 SEM) to develop an isolated pituitary model

**TABLE 2: LEVELS OF LH IN OVARIECTOMIZED EWES**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Basal level (ng/ml)</th>
<th>LH pulses/h</th>
<th>Basal level (ng/ml)</th>
<th>LH pulses/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=5)</td>
<td>1.02 ± 0.90</td>
<td>1.0</td>
<td>7.33 ± 0.78</td>
<td>1.02</td>
</tr>
<tr>
<td>Sem E2 (5)</td>
<td>0.90 ± 0.22</td>
<td>2.47</td>
<td>5.55 ± 1.91</td>
<td>0.90</td>
</tr>
<tr>
<td>10 mm E2 (5)</td>
<td>0.87 ± 0.18</td>
<td>1.52</td>
<td>3.87 ± 1.05</td>
<td>0.87</td>
</tr>
</tbody>
</table>

In summary, this study demonstrates that E2 has direct effects on the pituitary regulating LH and FSH release in the ewe. These effects are qualitatively similar to those seen in ovariectomized ewes. Model 2 provides a viable isolated pituitary model but higher doses of Gn-RH appear to be necessary to restore plasma LH and FSH to the concentrations measured before surgery.

of hormone stimulates glycolytic activity in the Booroola dextran-coated charcoal (lmg/ml) using procedures described previously(1). In this study, we report the time-course of changes in the concentration of peripheral blood inhibin of rats injected with various doses of FSH.

Immature female rats were injected ip with 0.2µg or 1.0µg of purified ovine FSH (S1555BR, 100xNIH-FSH-S10) and sacrificed at 3 and 6h after injection. The blood samples collected were allowed to clot and centrifuged at 1300g x 15min at 4°C. For the measurement of inhibin in serum, 400µl of each serum sample was extracted with 5% polyethylene glycol and dextran-coated charcoal (lg/ml) using procedures described previously(1). The steroid-free serum extracts were assayed using an in vitro pituitary cell culture system(3); the reference standard used was an ovine testicular lymph protein preparation (OTLP4) with a designated potency of 10µg.

Table 1. Serum inhibin levels after injection with various doses of FSH.

<table>
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<tr>
<th>Time after injection (h)</th>
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<th>3</th>
<th>6</th>
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<tbody>
<tr>
<td>Dose of FSH (µg)</td>
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<td></td>
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<tr>
<td>0.2</td>
<td>3.7(0.7)</td>
<td>3.0(0.4)</td>
<td>3.6(0.5)</td>
</tr>
<tr>
<td>1.0</td>
<td>3.8(0.7)</td>
<td>9.1(1.6)</td>
<td>8.3(0.9)</td>
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<tr>
<td>2.0</td>
<td>4.4(0.9)</td>
<td>16.7(3.1)</td>
<td>13.5(2.6)</td>
</tr>
</tbody>
</table>

Values represent mean(SE); n=4 per group; *Data from previous study(2), potency (59kC.L.)

It is clear that a dose-related increase in circulating inhibin levels occurred at 6h after injection with varying doses of FSH. No increase was observed at any time in animals injected with the lowest dose (0.2µg) of FSH.

In conclusion, these studies demonstrate that the secretion of inhibin is increased when FSH is administered to intact females. Observation, together with previous reports of lack of response of inhibin secretion with oLH(2), suggests that the ovarian follicles in these ewes may be an important regulator of ovarian inhibin secretion in immature female rats.

References:
REGULATION OF OVARIAN INHIBIN PRODUCTION BY STEROIDS
E.M. Henderson and P. Franchimont*

Department of Biochemistry, University of Western Australia, Nedlands, W.A. 6009 and Laboratoire de Radiométrie Nucléaire, Université de Liège, Liège, Belgium.

The regulation of ovarian inhibin production is poorly understood. In this study, the regulation of inhibin production by bovine ovarian cells in vitro, and the relationship of inhibin production in individual bovine follicles to their hormonal environment was investigated. The specific suppression of FSH production by rat pituitary cells in culture was used as a biosays for inhibin. Inhibin activity of dextran-charcoal treated ovarian cell culture medium and follicular fluid (FF) was expressed relative to the potency of a preparation derived from ovine testicular lymph (OTLP) assigned a potency of 1 U/mg. Preliminary studies showed that the dose response curves for OTLP, ovarian cell culture medium and FF were parallel in basal and LH-RH stimulated conditions.

Inhibin production by GC during 24 h culture periods was inhibited up to 50% by exogenous progesterone added at concentrations of 1 and 5 ng/ml. In contrast, androstenedione, testosterone and 5α-dihydrotestosterone at 1 and 5 ng/ml stimulated GC inhibin production 2-5 fold in a dose dependent manner. Oestradiol-17β and oestrone at 1 and 5 ng/ml had no effect. Inhibin production by control cultures in these experiments ranged from 375 ± 16 to 470 ± 21 U/mg protein/24 h (mean ± s.e.m. of 6 replicates cultures). Bovine luteal cells while producing substantial amounts of progesterone (37-95 µg/mg protein/24 h) failed to produce detectable amounts of inhibin.

Luteinization of follicular granulosa cells (GC) in culture, as indicated by increased progesterone production from 4 ± 0.5 to 18 ± 1.7 µg/mg protein/24 h was accompanied by a reduction in their production of inhibin from 1500 ± 29 to 100 ± 14 U/mg protein/24 h (means ± s.e.m. of 6 replicate cultures). Bovine luteal cells while producing substantial amounts of progesterone (37-95 µg/mg protein/24 h) failed to produce detectable amounts of inhibin.

Inhibin production by GC during 24 h culture periods was inhibited up to 50% by exogenous progesterone added at concentrations of 1 and 5 ng/ml. In contrast, androstenedione, testosterone and 5α-dihydrotestosterone at 1 and 5 ng/ml stimulated GC inhibin production 2-5 fold in a dose dependent manner. Oestradiol-17β and oestrone at 1 and 5 ng/ml had no effect. Inhibin production by control cultures in these experiments ranged from 375 ± 16 to 470 ± 21 U/mg protein/24 h (mean ± s.e.m. of 5 replicates cultures).

Measurement of steroids and inhibin concentrations in bovine FF (n = 63) showed that as follicle size increased (from 0.3 to > 0.8 ml FF/follicle), oestradiol-17β concentrations increased significantly (P < 0.01) from 9 ± 2 to 181 ± 45 ng/ml (mean ± s.e.m.) and testosterone and inhibin concentrations decreased significantly (P < 0.01) from 43 ± 5 to 16 ± 5 ng/ml and from 308 ± 22 to 155 ± 15 U/ml respectively (means ± s.e.m.). Progesterone concentrations remained unchanged (< 50 ng/ml).

These findings indicate that androgens may regulate ovarian inhibin production through modifying GC inhibin production. The inhibitory effect of progesterone on GC inhibin production may be important at the time of GC luteinization and corpus luteum formation.

*Author is in receipt of a Queen Elizabeth II Research Fellowship.

LEARNING SELF-RECOGNITION OF THE FERTILE PERIOD: RESULTS IN A PROSPECTIVE MULTICENTRE STUDY

Henry G. Burger

(Medical Research Centre, Prince Henry's Hospital, Melbourne) on behalf of the Task Force on Methods for the Determination of the Fertile Period, World Health Organization, Special Programme of Research, Development and Research Training in Human Reproduction. It has been reported previously that women can observe a sequence of changes in cervical mucus at the vulva, which correlate with periovulatory hormonal events. The present study was undertaken to determine the percentage of normal women able to make such observations and the factors influencing this ability.

Teachers were selected in five centres, in Auckland (New Zealand), Bangalore (India), Dublin (Ireland), Manila (The Philippines) and San Miguel (El Salvador). They assisted in subject recruitment and had prime responsibility for teaching and follow-up of all cycles. Teachers were allocated to each centre on the basis of their skill in teaching and their ability to conduct the study, and the programme was standardized. Teachers were selected in five centres, in Auckland (New Zealand), Bangalore (India), Dublin (Ireland), Manila (The Philippines) and San Miguel (El Salvador). They assisted in subject recruitment and had prime responsibility for teaching and follow-up of all cycles. The fertile period was defined as occurring at the onset of mucus secretion or of a sensation of wetness detectable at the vulva. The "peak day" was the last day on which mucus resembling raw egg white was noted or the last day on which a wet lubricant sensation was felt. This day has been shown previously to correlate closely with the hormonal estimate of the day of ovulation. The fertile period ended on the third night after the peak.

Eight hundred and sixty-nine subjects, mean age 30.1 ± 5.2 years, were admitted (Auckland 112, Bangalore 205, Dublin 234, Manila 146, San Miguel 162). Eighty-three per cent of all subjects were housewives while their partners had occupations varying widely between centres. Their educational level varied from illiterate to tertiary, and they had an average of 1.9 pregnancies. The first cycle following instruction, a mean of 93.1 ± 5.2% showed an interpretable ovulatory mucus pattern, with a range of 93.7% and 94.1% for the two subsequent cycles. Understanding of the method was assessed by a test, which showed that 90.8%, 94.2% and 97.1% in these three cycles respectively. Ability to learn mucus self-recognition was independent of educational, cultural and socio-economic characteristics and was uninfluenced by a previous history of vaginal discharge or evidence of vaginal or cervical infection. The overall mean lengths of the 3 teaching cycles were 29.4 (±3.5 SD), 29.3 and 29.2 days respectively. The fertile period averaged 9.6 days in length and was 10.8 in Auckland, 9.7 in Bangalore, 10.5 in Dublin, 9.6 in Manila and 7.3 in San Miguel.

It is concluded that almost all women in the present study, from five centres and of various educational and socio-economic levels, were able to recognize the fertile period after one cycle of teaching. These observations have important implications for fertility regulation and the management of infertility.


BIOCHEMICAL AND ULTRASTRUCTURAL STUDIES OF NORMAL AND HOSTILE CERVICAL MUCUS

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At ovulation, the structural integrity of cervical mucous facilitates the easy access of spermatozoa to higher reaches of the female genital tract. In certain cases of infertility, spermatozoa become immobilized on tract. Recently, a biochemical interaction has been postulated for such a reaction, suggesting that hostile cervical mucous is deficient in sialic acid. This result was in desialylation and immobilization of the spermatozoa (1).

In the present investigation, both normal and hostile midcycle mucus samples (as determined by sperm penetration tests) were analysed for mucus (as determined by sperm penetration tests) and for ultrastructural uptake of NeuNAc (NeuAc) and examined by scanning electron microscopy. Variations in three parameters were compared, namely the structural porosity of the cervical mucous, the percentage of NeuNAc uptake, and the absolute sialic acid content.

Freeze-dried samples of midcycle cervical mucus were reconstituted in 25 ml 0.05 M Tris-HCl buffer (pH 6.5) and incubated at 37°C for 1 hr in the presence of 25 μl of radioactively labelled (C-14) 277 Ci/ml. The precipitate was washed with 10% TCA glass fibre discs (Whaitem; GF/A). The precipitate was collected and used for liquid scintillation to determine the absolute sialic acid content. Mucus samples for ultrastructural analysis were fixed in 0.6% gluteraldehyde and 0.2 M sodium phosphate buffer prior to preparation.

The action for scanning electron microscopy was then performed, using the results in Table I following statistical analysis using the students' t-test.

### TABLE I. Comparison of the histochemical and ultrastructural properties of normal and hostile midcycle cervical mucus.

<table>
<thead>
<tr>
<th>MUCUS</th>
<th>DEPTH OF SPERM PENETRATION (μm)</th>
<th>UPTAKE OF NeuNAc (μg/100 mg mucus)</th>
<th>PORE SIZE (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt; 2.5</td>
<td>1.47 ± 0.4</td>
<td>10 - 30</td>
</tr>
<tr>
<td>Hostile</td>
<td>&gt; 2.5</td>
<td>4.24 ± 1.47</td>
<td>0 - 0.5</td>
</tr>
</tbody>
</table>

It was found that infertility mucus had significantly more receptor sites for NeuNAc (P < 0.001). In addition, scanning electron microscopy revealed that the structural porosity common to normal midcycle analysis was diminished in hostile mucous samples. These results suggest that mucus structure could play a role in sialic acid in maintaining the structural integrity of sialic acid mucous. Modification of mucous structure by modulating sialic acid has important ramifications to the understanding of human infertility and the development of a non-hormonal intracervical contraceptive.


**IS A PARTICULAR BODY COMPOSITION RELATED TO FIRST OESTRUS IN ROMNEY HOGGOTS?**

R.W. Moore, J.J. Bass* and G.W. Winn*

Whatawhata Hill Country Research Station, Hamilton, New Zealand
* Ruakura Agricultural Research Centre, Hamilton, New Zealand

The Romney hogget oestrous season extends from late April to mid-July in New Zealand. Although live weight is a useful indicator of the incidence of hogget oestrus in a group (1) there is a wide range in the individual weight at first oestrus.

It is possible that body composition may improve the prediction of these hoggets that will show oestrus. Frisch has claimed that menarche in girls is associated with a minimum level of body fat (17%) (2) and rats fed on high and low fat diets had the same weight of fat in their bodies at first ovulation although those on the high fat diet showed oestrus earlier and at a lighter weight (3). The aim of this experiment was to test the Frisch hypothesis which relates fat levels with puberty for young ewes. More specifically we aimed to find out by means of logistic discriminant functions if oestrus in hoggets occurs when the amount of fat and protein reaches a particular level or an organ reaches a particular size.

Meaned were fed at pasture at two levels of nutrition from December 20 until April 12. During this time the low nutrition group (L) grew from 20 to 25 kg and the high nutrition group from 20 to 31 kg. Both groups were well fed thereafter. Harnessed vasectomised Rams were introduced on April 1, the mean date of first oestrus being June 3 for the L group and May 28 for the H group. Ewes were killed 0 - 5 days after first ovulation. The non-oestrous ewes were killed on July 17. All carcasses were dissected and chemically analysed.

Seventy-four % (62/84) of the L group showed oestrus and all (24/24) of the H group. There was a relationship between oestrous and non-oestrous animals. Percentage protein content of the uteruses of oestrous animals was 18.0 ± 4.24 g, non-oestrous animals 9.4 ± 1.47 gm. The weight of the spleen was also related, the mean weight of the spleens of the oestrous animals was 44.6 ± 10.7 gm and the non-oestrous animals 9.1 ± 4.19 gm. A good discrimination between oestrous and non-oestrous animals was achieved by a combination of the variation in weights of spleen, rumen, liver and lungs, only 2/22 of the non-oestrous animals were wrongly classified and 5/86 of the oestrous animals.

This study has disproved the Frisch hypothesis for young ewes. The biological reason for the discriminatory power of the spleen, rumen, liver and lungs complex are not clear.

EFFECT OF DIET AND LIVE WEIGHT ON FSH AND OESTRADIOL CONCENTRATIONS IN ROMNEY EWES

T.W. Knight, E. Payne* and A.J. Peterson*
WhaWha Hill Country Research Station, P.B., Hamilton, N.Z.
* Ruakura Agricultural Research Centre, P.B., Hamilton, N.Z.

Increased FSH concentrations 3 to 5 days before oestrus have been associated with increased ovulation rates in lupin supplemented ewes (1). This experiment aims to determine the effect of lupin feeding and live weight on plasma FSH and oestradiol (E2).

Two groups of ewes weighing 39 kg (LLW) and 48 kg (HLW) were fed, at maintenance for 34 days, diets containing 50% of either lupins (L) or barley (B) (2). Mean ovulations at the second oestrus after synchronisation were 1.15 and 1.39 for LLW and HLW ewes and 1.13 and 1.38 for B and L ewes (2). A sample of 8 ewes per treatment were bled 3 times daily from Day -5 to +1 (Day 0 = oestrus). The 3 daily blood samples were pooled and assayed for FSH and E2.

FSH concentrations increased from Days -5 to -4, then declined to a minimum by Day -1 (Table 1). The L ewes had higher (P<0.01) mean FSH concentration than the B ewes, especially on Day -4 (Table 1). FSH concentrations were significantly higher in the LLW than HLW ewes for Days 0 and 1 but not for Days -5 to -1.

** p < 0.01; * p < 0.05; TP < 0.1

E2 concentrations were higher (P<0.01) in L ewes than B ewes and tended to be higher in HLW than LLW ewes (Table 1).

While ovulation rates are increased by both lupin feeding and increased live weight the present results suggest the mechanisms are not the same. The increased FSH over the 4 days before oestrus in lupin fed ewes causes more follicles to be ovulated which is reflected in higher E2 levels. The presence of higher FSH levels coincident with elevated oestrogen levels suggests that the lupin feeding has elevated the threshold to the negative feedback between E2 and FSH. The lower ovulation rate in the barley fed ewes is reflected by lowered E2 and FSH levels. With high live weights there is an increase in the number of follicles (2) producing more E2 which depresses FSH, suggesting there is no effect of live weight status on the negative feedback between E2 and FSH.


### TABLE 1. Mean plasma FSH and E2 concentrations

<table>
<thead>
<tr>
<th>DAY</th>
<th>FSH (ng/ml)</th>
<th>L</th>
<th>B</th>
<th>LLW</th>
<th>HLW</th>
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<tr>
<td></td>
<td>27</td>
<td>55</td>
<td>22</td>
<td>36</td>
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<td>42</td>
<td>78</td>
<td>44</td>
<td>19</td>
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<td>44</td>
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SENSITIVITY TO GnRH IN BOOROOLA AND CONTROL OVARIECTOMIZED EWES

T. O'Shea, L.J. Cummins, B.M. Bindon and L.R. Piper
Physiology Department, University of New England and
CSIRO, Division of Animal Production, Armidale.

In ovariectomized ewes pituitary function is not modulated by gonadal feedback. In one study of ovariectomized ewes of 3 breeds differing widely in genetic capacity for fecundity, GnRH-induced LH release did not seem to be related to fecundity (1).

Merino ewes of the high fecundity Booroola strain and a random bred control strain were ovariectomized in August. Two months later (Expt 1) and again, after reallocation, six months later (Expt 2) the LH response to varying doses of GnRH (Lutal Hoechst) was examined. GnRH was given i.v. and plasma LH levels measured by RIA. Blood samples were taken prior to treatment and at 10 to 30 minute intervals after treatment for 200 minutes in Expt 1 and 140 minutes in Expt 2. Peak concentrations were measured 10-20 minutes after GnRH.

TABLE 1. LH response in ovariectomized ewes (retransformed least squares means ± S.E.)

<table>
<thead>
<tr>
<th>Dose (μg)</th>
<th>Booroola</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>92 ± 15</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>2.0</td>
<td>301 ± 48</td>
<td>60 ± 7</td>
</tr>
<tr>
<td>8.0</td>
<td>396 ± 70</td>
<td>83 ± 9</td>
</tr>
<tr>
<td>32.0</td>
<td>175 ± 34</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>0.75</td>
<td>490 ± 97</td>
<td>106 ± 13</td>
</tr>
<tr>
<td>10.00</td>
<td>869 ± 172</td>
<td>169 ± 21</td>
</tr>
<tr>
<td>30.00</td>
<td>1108 ± 243</td>
<td>111 ± 14</td>
</tr>
</tbody>
</table>

The data (shown in Table 1) were examined by least squares analysis after log transformation. In both experiments the amount of LH released increased as the dose of GnRH increased (P < 0.005). In Expt 1 there was a strain by dose interaction (P < 0.005) for both peak concentration and area under the curve. In Expt 2 a similar interaction (P < 0.05) was observed for area under the curve. These interactions were principally due to a tendency for the Booroolas to release more LH in response to the lowest dose of GnRH. The increased pituitary sensitivity of Booroolas to GnRH may be related to their ability to continue to ovulate during the normal anoestrus period (2).

These results show that the dose of GnRH used to assess pituitary function is of critical importance. The difference between the present results and those in (1) may be due to the choice of dose or to the possibility that Booroola and Finn ewes have evolved different endocrine mechanisms associated with high fecundity.


RAM-INDUCED OVULATION IN SEASONALLY ANOVULAR MERINO EWES ACTIVELY IMMUNIZED AGAINST AN OESTRONE-GELATIN CONJUGATE

R.J. Scaramuzzi, R.M. Hoskinson, N.T. Hinks,
R.M. Radford and K.E. Turnbull
C.S.I.R.O., Division of Animal Production, Prospect, N.S.W.

To optimize ovulation rate (OR) in steroid-immunized ewes it is essential to control antibody responses. Thymus-dependent antigens establish a T-cell memory that may be provoked into an unacceptably great IgG antibody response by booster immunizations. Thymus-independent antigens do not elicit an IgG memory response. This study evaluates the immune memory established in the ewe by an oestrone-gelatin conjugate, thought to be a thymus-independent antigen; additionally, it was concerned with the ovarian response to the ram of seasonally anoestrous ewes, so immunized.

3-Carboxymethyltestosterone linked to gelatin by the diimide method gave a conjugate with 65 moles steroid per 10^5 g gelatin. Ewes were immunized with the conjugate in Freund's complete adjuvant (Group (a), 4 ewes) or DEAE-dextran (Group (b), 5 ewes) and given booster immunizations at 1 and 3 months. During this period, the immunized and 18 control ewes were isolated from rams. Seven days after the third immunization, 9 control ewes were separated from the flock and 2 vaccinated rams introduced to the remainder. The OR was recorded at laparoscopy 7 days later. Blood samples were obtained 7 days after booster immunization and 1 hr before and after the introduction of rams. The plasma was assayed for steroid antibody titre and LH levels.

TABLE. Antibody titre, LH level, OR and ovulatory responses of anoestrous oestrone-immunized ewes following the introduction of rams.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Antigen*</th>
<th>Antibody*</th>
<th>LH (ng NIH S15/m)</th>
<th>Before</th>
<th>After</th>
<th>Ewes Ovulating OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunized</td>
<td>(a)</td>
<td>287 (71-550)</td>
<td>11.98</td>
<td>16.42</td>
<td>7/9</td>
<td>1.71</td>
</tr>
<tr>
<td>Immunized</td>
<td>(b)</td>
<td>23 (2-27)</td>
<td>0.18</td>
<td>3.93</td>
<td>5/9</td>
<td>1.00</td>
</tr>
<tr>
<td>Control with rams</td>
<td></td>
<td>0.24</td>
<td>0.13</td>
<td>1/9</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

* Mean reciprocal values (tertiary responses)

There were no detectable primary antibody responses. Generally weak secondary and large tertiary antibody responses indicated that the gelatin conjugate was capable of establishing a strong immune memory in sheep. The results show that the ewe flock was anoestrous and most ewes were induced to ovulate by the introduction of rams. The LH levels of immunized ewes were elevated (P < 0.05) and a further non-significant rise occurred within 1 hr of the introduction of rams. The OR in the immunized group was increased (P = 0.07) suggesting that the factors controlling OR are independent of those controlling the breeding season.
OVULATION IN THE GOAT AFTER INTRAVAGINAL SPONGE AND PMSG TREATMENT

A.J. Ritar, S. Salamon and W.N.C. Manwell
Department of Animal Husbandry, University of Sydney, N.S.W. 2006.

Progestagen imregnated intravaginal sponges used in conjunction with PMSG have been successful in controlling oestrus in the goat (1). This communication presents data on time and rate of ovulation after sponge-PMSG treatment. Two experiments were conducted, the first in the non-breeding season and the second in the breeding season. Non-lactating cross-bred Angora (Expt.1) and feral does (Expt.2) were treated with sponges (Chrono-gest, Intervet) for 16-18 days and PMSG (0=control, 200, 400, 600 I.u. Batch 509, Gravamen, Breaford Lab.) was injected either 48 hr before or at sponge removal (n=10). After sponge removal, the ovaries of each animal were examined four times by laparoscopy for recent ovulation(s) and corpora lutea (CL). At each time of examination the dose of PMSG had no affect on the number of animals which had ovulated, therefore the data for doses of PMSG within sides of two experiments were pooled, and are presented in Table 1.

TABLE 1. Number of doses (2) with ovulation(s) and CL at different times of laparoscopy (n=30, control n=10)

<table>
<thead>
<tr>
<th>Season</th>
<th>PMSG Injection</th>
<th>Time of laparoscopy after sponge removal (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-breeding</td>
<td>0 hr</td>
<td>46, 51, 56, 61, 66, 85</td>
</tr>
<tr>
<td>(Expt.1)</td>
<td>48 hr</td>
<td>11(37), 19(63), 22(73), 28(92), ***</td>
</tr>
<tr>
<td>Control</td>
<td>0 hr</td>
<td>0, 1, 2, 3, 3, 3</td>
</tr>
<tr>
<td>(Expt.2)</td>
<td>48 hr</td>
<td>21(70), 27(90), 28(93), 30(100)</td>
</tr>
<tr>
<td>Breeding</td>
<td>0 hr</td>
<td>5(17), 15(50), 23(77), 27(90)</td>
</tr>
<tr>
<td>(Expt.1)</td>
<td>48 hr</td>
<td>16(53), 22(73)</td>
</tr>
<tr>
<td>Control</td>
<td>0 hr</td>
<td>0, 0, 0, 1, 1, 1</td>
</tr>
<tr>
<td>(Expt.2)</td>
<td>48 hr</td>
<td>10(37), 16(53), 22(73)</td>
</tr>
</tbody>
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Injection of PMSG 48 hr before sponge removal increased the number of animals which had ovulated at the first three examinations in the non-breeding season and at 36 hr in the breeding season. There was a linear relationship between dose of PMSG and final (85 hr) ovulation rate; the mean values (± SE) for doses of 200, 400, 600 I.u. were 1.8±0.16, 2.0±0.21, 3.0±0.36 (P<0.01) in the non-breeding season, and 1.5±0.29, 2.3±0.33, 3.2±0.48 (P<0.05) in the breeding season. Injection of PMSG 48 hr before sponge removal increased the mean ovulation rate in the non-breeding season (2.5±0.27, 48 hr v. 1.1±0.16, 0 hr, P<0.05), but not in the breeding season (2.1±0.33, 48 hr v. 2.5±0.31, 0 hr). There was no interaction between dose of PMSG and time of injection in either season.

Due to the infrequency of the blood sampling, no attempt was made to estimate the number of episodic pulses of LH. However, the mean basal concentrations and amplitudes of LH discharge observed during anoestrus were greater than during the period of cyclic activity (P<0.05). Prolactin levels were also higher during anoestrus (P<0.01).

The ovarian activity of the ewes of all breeds used in this experiment was influenced by the artificial light regime. The Romney and Dorset ewes exhibited a less intense seasonality with cyclic activity commencing earlier, during the long photoperiod, and terminating later. This pattern is similar to that seen in the field under conditions of natural photoperiod.

REFERENCES


OVARIAN AND PITUITARY ACTIVITY OF THREE BREEDS OF EWE SUBJECTED TO ARTIFICIAL PHOTOPERIOD

A. L. Poulton and T. J. Robinson
Department of Animal Husbandry, University of Sydney, N.S.W. 2006.

Four Merino, four Poll Dorset and four Romney Marsh ewes were kept in two environmentally controlled rooms from May 1979 to February 1981. Two ewes of each breed were assigned to each room.

The ewes were subjected to an artificial light regime of an initial sixteen week period of "constant light" days (12 hr light:12 hr darkness) followed by four alternating sixteen week blocks of "short" days (6 hr light:18 hr darkness) and "long" days (18 hr light:6 hr darkness). Each room had an identical light cycle operating sixteen weeks out of phase. Estimates were made of ovarian and pituitary activity in response to the artificial light regime. Ewes were bled twice weekly: peripheral plasma progesterone >1 ng/ml was taken as indicative of ovarian activity. Luteinizing hormone (LH) measurements were performed on peripheral plasma samples collected at two-hourly intervals over twelve hour periods, eleven conducted between February 1980 and February 1981. Pooled plasma samples from six of these sampling periods, held between February 1980 and September 1980, were assayed for prolactin. These data were pooled for comparisons between periods of cyclic activity (breeding season) and anoestrus (Table 1).

The cyclic activity of all ewes was influenced by the artificial light regime. Generally the Romney and Dorset ewes began cycling 4-8 weeks after the photoperiod shift to "short" days while the Merino ewes began to cycle 4 weeks prior to the shift.

During one complete cycle, encompassing two sixteen week blocks of "short" and "long" days, the Merino, Dorset and Romney ewes exhibited an average season of cyclic activity of 22, 18 and 16 weeks respectively.

TABLE 1. Mean plasma concentrations of basal and peak LH and of prolactin during the breeding season and anoestrus (ng/ml)

<table>
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<tr>
<th>BREEDING SEASON</th>
<th>ANOESTRUS</th>
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<tr>
<td>Basal LH</td>
<td>Peak LH</td>
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<tr>
<td>Merino</td>
<td>1.57</td>
</tr>
<tr>
<td>Dorset</td>
<td>1.78</td>
</tr>
<tr>
<td>Romney</td>
<td>1.49</td>
</tr>
</tbody>
</table>

Due to the infrequency of the blood sampling, no attempt was made to estimate the number of episodic pulses of LH. However, the mean basal concentrations and amplitudes of LH discharge observed during anoestrus were greater than during the period of cyclic activity (P<0.05). Prolactin levels were also higher during anoestrus (P<0.01).

The ovarian activity of the ewes of all breeds used in this experiment was influenced by the artificial light regime. The Romney and Dorset ewes displayed an intense seasonality (P<0.05), but not in the breed of the ewes; the Merino ewes exhibited a less intense seasonality with cyclic activity commencing earlier, during the long photoperiod, and terminating later. This pattern is similar to that seen in the field under conditions of natural photoperiod.

REFERENCES

THE EFFECT OF MELATONIN FEEDING ON PLASMA PROLACTIN LEVELS AND THE ONSET OF OESTROUS ACTIVITY IN SHEEP

D.J. Kennaway, T.A. Gilmore and R.P. Seamark

Department of Obstetrics and Gynaecology, The University of Adelaide.

There is now compelling evidence implicating the pineal in the transfer of photoperiod information to the gonadal axis in a variety of small laboratory mammals (1). The data relating the pineal to seasonal changes in breeding activity in ruminants is less clear. In the present experiment we have examined whether we could mimic the effects of shortened day-length on seasonally anestrous animals by raising blood concentrations of melatonin 8 hours before the onset of darkness. This was done by feeding the animals pelleted food onto which melatonin had been adsorbed (2). We have previously reported changes in serum prolactin levels in goats following daily melatonin treatment 4 hours prior to darkness (3).

Ten maiden Border Leicester x Merino ewes were acclimatized to 16 h light daily (0300-1900 h) for four weeks. Five of the animals were then fed pellets treated with melatonin (2 mg per day) and the remainder untreated pellets daily at 1100 h for 103 days. Seventeen days after the commencement of treatment, serum prolactin levels were found to be significantly lower (p<0.05; between 1500 and 1900 h and 2400-0800 h) in the melatonin fed compared to untreated animals. By day 31 prolactin levels were lower in the melatonin fed animals throughout the entire 8 h sampling period (0900-1700 h, 52 ± 5 ng/ml vs 9.6 ± 4 ng/ml). The prolactin levels remained suppressed up to Day 99.

The feeding of melatonin had no apparent effect on the baseline serum levels of either FSH or LH nor on the frequency and amplitude of the LH peaks or on the changes evoked in serum prolactin following prolactin releasing hormone treatment (4). The LH peaks in both groups 1 and 2 were affected the hypothalamo-pituitary response to estradiol as, when tested after 52 days, the LH response of the treated animals to an injection of 15.5 up estradiol-17β was significantly diminished when compared with control animals.

Following the administration of the estradiol (Day 53) and the introduction of a ram (Day 71) the 5 melatonin fed ewes commenced cyclic ovulatory activity on Days 61,68,69,79 and 82 compared to Days 75,97,>103 in the 5 untreated control ewes. These results indicate that melatonin administered orally to seasonally anestrous sheep induced changes in circulating prolactin and influenced the time of onset of the breeding season in a manner, which mimicked at least partially, the effects of altered photoperiod.

SEASONAL CHANGES IN LH SECRETION IN NORMAL AND CLOVER INFERTILE EWES

W.A. Chamley,* I.J. Clarke# and A.R. Moran*

Victorian Department of Agriculture,* Animal Research Institute, Werribee and Regional Offices, Bendigo. # Medical Research Centre, Prince Henry's Hospital, Melbourne.

Ewes having a high intake of isoflavones, originating from some cultivars of subterranean clover, can develop "Clover Disease" which is associated with reduced reproductive performance. Several physiological changes and clinical manifestations have been described and it was suggested (1) that the "clover diseased" ewe had altered neuro-endocrine function. Detailed studies of LH secretion during the breeding season (May) and the anoestrous (September) have been carried out with normal Merino ewes (from Werribee) and Merino ewes which had grazed Yarloop clover for several years in central Victoria. The average lambing percentage for the flock grazing Yarloop was <40%.

One jugular vein of each ewe was cannulated one day before each experiment. During each experiment, 10ml blood samples were taken every 20 min. during an interval of 5-7 hrs. Plasma was harvested and later assayed for LH using double antibody RIA. Results are shown in the table.

### TABLE 1. Antibody titre, ovulation rate and oestrus behaviour in steroid-immunized Merino ewes.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Carbon Atom</th>
<th>Mean Reciprocal Antibody Titre (Range)</th>
<th>Group OR</th>
<th>% Oestrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>3*</td>
<td>440 (240-1650)</td>
<td>1.63</td>
<td>100</td>
</tr>
<tr>
<td>Testosterone</td>
<td>3*</td>
<td>1630 (190-5600)</td>
<td>1.63</td>
<td>100</td>
</tr>
<tr>
<td>Testosterone</td>
<td>17t</td>
<td>160 (100-330)</td>
<td>1.38</td>
<td>88</td>
</tr>
<tr>
<td>Testosterone</td>
<td>17t</td>
<td>360 (100-900)</td>
<td>1.50</td>
<td>100</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>16</td>
<td>170 (100-330)</td>
<td>1.57</td>
<td>71</td>
</tr>
<tr>
<td>Oestrone</td>
<td>3§§</td>
<td>1050 (280-2500)</td>
<td>1.75</td>
<td>88</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>1.00</td>
<td>1.00</td>
<td>100</td>
</tr>
</tbody>
</table>

*Carboxymethyloxime; §hemisuccinate; §carboxyethylthioether; §§carboxymethyl ether

Overall the ovulation rate was stimulated \((p < 0.01)\) in the immunized ewes and a high level of oestrus behaviour was maintained. Accordingly, the corresponding antibody responses must have approached optimum values. The slightly lower OR due to the testosterone hemisuccinate compared to the carboxymethyloxime derivative is attributed to the lower degree of antibody stimulation rather than the steroid used. The increase in OR due to oestrone antibody confirms the utility of its 3 carboxymethyl derivative as an alternative to oestrone-6-CMO (1) for controlled physiological studies, thus greatly simplifying the related chemical synthesis.

DURATION OF ACTION OF PMSG FOR SUPEROVULATION IN SHEEP AND CATTLE

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Ovine hyper-immune serum to PMSG may be used to effectively terminate the action of PMSG in vivo in sheep and cattle (1). This technique is being used to oblate the undesirable side-effects of PMSG which arise from its prolonged half-life in vivo. The present study examines the time intervals between administration of PMSG and anti-PMSG that are compatible with maximum ovulatory responses to PMSG in sheep and cattle.

Control Merinos of mixed ages were injected with 1200 i.u. PMSG (Folligon) on day 14 of a normal oestrous cycle and sub-groups injected 0, 24, 48 or 72 hr later. The ovarian response was assessed at recovery following barbiturate anaesthesia 3 days after oestrus. Mixed ages Hereford cross cattle were subjected to a similar experimental design: 2400 i.u. PMSG injected on +120 hr later. Ovulation rate was measured by mid-ventral laparoscopy and luteal anasthesia 3-8 days after oestrus. The results in Table 1 indicate that when PMSG action was terminated as late as 72 hr in the Tabelle 1. Ovulation rates (0/8) after PMSG and anti-PMSG in sheep and cattle.

<table>
<thead>
<tr>
<th>INTERVAL PMSG</th>
<th>ANTI-PMSG(SR)</th>
<th>n O/R + SE*†</th>
</tr>
</thead>
<tbody>
<tr>
<td>T nil(control)</td>
<td>25 5.8 ± 5.6(1-27) (7)</td>
<td>24 ± 24 12.60 ± 3.3(1-20) (4)</td>
</tr>
<tr>
<td>+24</td>
<td>20 3.2 ± 2.6(13-11) (1)</td>
<td>96 9 ± 6.0 ± 6.1+1-11(1)</td>
</tr>
<tr>
<td>+48</td>
<td>23 5.8 ± 5.9(12-20) (2)</td>
<td>120 12 ± 4.50 ± 4.61-19(1)</td>
</tr>
</tbody>
</table>

† Bracketed values refer to the number of cases of excessive follicle development

INTERVAL PMSG ANTI-PMSG(SR) n HEREFORD CROSS CATTLE O/R + SE*†

b) Duration of action of PMSG for superovulation in sheep and cattle.

Sheep and 120 hr after PMSG injection in the cow ovulation rates less than the maximum superovulatory responses were observed. This is surprising since it has been shown (2) that the induction of follicle growth and oestrogen secretion occurs within a few hours of PMSG administration. It appears that the additional follicle development stimulated by PMSG in the late luteal phase of the cycle requires continued PMSG support to at least onset of oestrus to achieve maximum ovulation rates. It may be necessary to give anti-PMSG after the preovulatory LH discharge to ensure ovulation of all follicles.


PROGESTERONE AND PROSTAGLANDIN E AND F SECRETION BY HUMAN CORPORA LUTEA IN LONG-TERM ORGAN CULTURE.


As part of a study investigating the role of prostaglandins E and F (PGE and PGF) in the function of the human corpus luteum (CL), the present experiments were carried out to relate the secretion of progesterone (P) to that of PGE and PGF by luteal tissue in vitro. Luteal tissue was cut into thin slices of approximate dimensions 2x2x1mm, placed on stainless steel culture grids (4 slices/grid) and incubated in small petri dishes in 2.5 ml medium 199 containing foetal calf serum (10%), Hepes (24mM) and Kanamycin Sulphate (25µg/ml). Luteal slices were cultured for up to 36 hours at 37°C in a humidified atmosphere of 5% CO₂ in air. The medium was changed every three hours for the first 12 hours and every 6 hours thereafter. Preliminary experiments in which the medium was changed at regular intervals of from 1 to 12 hours showed the P secretion rate to be constant for at least 8 hours following the change of medium. The levels of P, PGE and PGF in the medium were measured by radioimmunoassay.

Secretion of P by early CL's (n=3) was initially 3 to 30 times greater than that by late CL's and declined progressively with time in culture. Secretion of P by late CL's (n=5) remained constant during culture and, in two experiments, increased during culture. Late CL's secreted higher levels of PGE and PGF than early CL's. All CL's secreted PGE from the start of culture, but PGF could only be detected in the medium 3 to 6 hours after the start of culture. Both PGE and PGF secretion then tended to increase with time in culture. The increase in PGE secretion was greater than that of PGF. All late CL's secreted PGF, but only one early CL (producing the highest progesterone secretion), released detectable amounts of PGF.

In two additional experiments, luteal slices were incubated with indomethacin (1µg/ml). This abolished the rise in PGE and PGF and significantly reduced progesterone secretion within 12 hours of the start of culture in one experiment (P<0.05) and within 24 hours in the other (P<0.01).

The data show that late human CL's have a greater capacity for PG secretion in vitro than early CL's and apparently produce PG in preference to PGE. The data also suggest that increased PG production may be related to the maintenance of progesterone secretion in vitro.

EFFECTS OF CLOPROSTENOL ON THE CORPUS LUTEUM OF THE GUINEA PIG

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Although the guinea pig is believed to have a uterine luteolytic mechanism involving PGF2α, systemic administration of PGF2α is relatively ineffective in inducing luteolysis. In seeking a means for artificial induction of luteal regression, we have studied the effects of the synthetic prostaglandin analogue cloprostenol ('Estrumate' - IC1).

Stage of oestrous cycle was determined by daily examination for vaginal opening, and vaginal smears. Cloprostenol was administered on day 9 of the oestrous cycle by a single I-P injection, at various dose rates, and effects observed at 3 and 48 hours after administration. Control (C) animals received saline I-P. Blood flow was measured by the radioautographic microsphere method (85Sr-labelled, 15μm spheres), and progesterone by RIA. Tissues for light microscopy were fixed in glutaraldehyde/osmium and embedded in araldite. Data were analysed by analysis of variance and the Student-Newman-Keuls method, or by t-test.

Cloprostenol at 250μg reduced the length of the cycle when compared to preceding cycle length in the same animals (14.6 ± 1.3d vs 17.6 ± 1.4d, P<0.01, n = 5). However, doses of 10 or 50μg were without effect (17.4 ± 1.16 vs 16.4 ± 1.4d and 17.0 ± 1.2d vs 17.2 ± 0.8d respectively).

Histological changes at 48 hours included an increase in number and size of lipid droplets in luteal cells, which was more marked at the higher dose levels; however, even at 250μg the majority of luteal cells appeared viable.

Effects on luteal weight and blood flow, and plasma progesterone, are shown in Table 1. Weight and blood flow are expressed per single CL: individual animals possessed 2-5 (mean 3.5) CL.

Table 1. Effects of Cloprostenol on Guinea Pig Corpus Luteum

<table>
<thead>
<tr>
<th>Duration (h)</th>
<th>Treatment (μg)</th>
<th>Mean ± SD Luteal Weight (mg)</th>
<th>Mean ± SD Luteal Blood Flow (μl/min)</th>
<th>Mean ± SD Plasma Progesterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0 (C) 7</td>
<td>3.61 ± 0.72</td>
<td>20.4 ± 10.46</td>
<td>4.00 ± 1.70</td>
</tr>
<tr>
<td>3</td>
<td>250 7</td>
<td>3.12 ± 0.43</td>
<td>9.40 ± 4.2*</td>
<td>2.58 ± 1.17*</td>
</tr>
<tr>
<td>48</td>
<td>0 (C) 7</td>
<td>5.52 ± 0.62</td>
<td>18.47 ± 8.27</td>
<td>4.67 ± 0.59</td>
</tr>
<tr>
<td>48</td>
<td>10 7</td>
<td>5.06 ± 0.33</td>
<td>25.0 ± 10.5</td>
<td>3.21 ± 0.38*</td>
</tr>
<tr>
<td>48</td>
<td>50 7</td>
<td>1.82 ± 0.41**</td>
<td>5.23 ± 1.90**</td>
<td>2.69 ± 0.69**</td>
</tr>
</tbody>
</table>

Significant differences from control (C) *P<0.05 **P<0.01

Cloprostenol at 50 or 250μg caused a drop in luteal weight by 48 hours. Both luteal blood flow and peripheral plasma progesterone levels were reduced as early as 3h after I-P injection of 250μg cloprostenol, and remained low at 48h. No adverse side-effects were observed at any dosage of cloprostenol.

It is concluded that cloprostenol by I-P injection is luteolytic in the guinea pig, but only at high dose levels. Effects of 250μg cloprostenol on plasma progesterone and luteal blood flow are present within 3 hours of injection, and luteal weight is reduced by ≤ 50% within 48 hours.

FACTORS INFLUENCING INTERPRETATION OF THE ZONA-FREE HAMSTER OVUM TEST FOR HUMAN FERTILITY

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The zona-free hamster ovum at present provides the only model for testing the fertilizing ability of human spermatozoa. In order to assess factors that could affect interpretation of the results from this bioassay the heterologous gamete penetration test has been evaluated. Results have shown that:

(i) the induction of superovulation in immature hamsters provides the most convenient method of obtaining mature ova for study (42.6 ova per animal (n=40) of which 93.7% showed no visible signs of degeneration);

(ii) motile spermatozoa were best recovered by the technique of T-I (150μm) (from 32 semen samples (range of counts 10-158 M/ml): range of motilities 10-75% the geometric mean percentage recovery was 13.8% but the motility was 90%);

(iii) semen with counts < 10.0 M/ml and/or motilities < 20% were best prepared by centrifugation & resuspension of the whole ejaculate;

(iv) an 18 hour pre-incubation of spermatozoa at 37°C (which is associated with capacitation) in an atmosphere of air (final pH of medium 8.2) is preferable to 5% CO2 in air (pH of medium 7.2) since in the latter atmosphere the motility of spermatozoa and their attachment to, and penetration of, zona-free ova were significantly decreased (P<0.05);

(v) maximum penetration rates of ova were obtained when the pre-incubation and insemination densities of spermatozoa were between 1-100/ml; and (vi) the capacity of spermatozoa to bind to or penetrate ova after approximately 30 hours in culture was significantly diminished (P<0.01).

Subsequently spermatozoa from 15 healthy men of proven fertility, and 15 men with longstanding subfertility but normal spermiograms, were evaluated for their ability to bind to and penetrate zona-free hamster ova. Of the 476 ova inseminated with spermatozoa from the fertile men > 5 sperm per ova consistently bound to the vitelline membrane and 284 ova (59.7%) had swollen sperm heads or pronuclei (still with tails attached) in the ooplasm. The range of individual penetration rates was 23.5 - 88.9%. Of the 586 ova tested with spermatozoa from the infertile subjects only 11 (1.9%) showed any evidence of penetration (range of individual penetration rates 0 - 6.7% and binding to the vitelline membrane was poor (0 or < 5%). Spermatozoa from a further 9 infertile men who had abnormal spermiograms also gave poor penetration rates (4/300 ova, 1.3%). It is concluded that this optimised bioassay has a useful role as an additional test to the classic spermogram, but that its routine use is best reserved for selected cases of unexplained infertility.

HIGH PRESSURE LIQUID CHROMATOGRAPHY: A NEW APPROACH FOR THE PURIFICATION OF INHIBIN FROM OVINE FOLLICULAR FLUID

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Medical Research Centre, Prince Henry's Hospital, Melbourne, Vic. 3004.  

Inhibin is a protein hormone produced by the gonad to suppress pituitary FSH secretion. Several workers have used conventional separation techniques such as salt precipitation, gel filtration chromatography and for Reverse-Phase HPLC in attempts to isolate inhibin (1,2). However, since these conventional chromatographic techniques have not yielded a satisfactory inhibin purification, we investigated the potential application of a novel system, High Pressure Liquid Chromatography (HPLC). For these studies, inhibin activity was measured using a pituitary cell culture bioassay based on suppression of pituitary FSH cellular content (3).

Ovine follicular fluid was initially chromatographed on a 112S gel permeation column ( Waters), in 0.05 M sodium phosphate, pH 7.0. Under these conditions the inhibin bioactivity coeluted with the major protein peak at an void volume (~80,000 daltons) suggesting that the system could not successfully separate inhibin from its bound complexes in follicular fluid (2).

Improved resolution of inhibin was obtained using Reverse-Phase partition chromatography. Initial experiments designed to determine the interaction of inhibin with octadecasilyl-silica (ODS-silica) were performed using C18 Sep-Pak columns. Ovine follicular fluid was acid extracted (4) and chromatographed on a C18 Sep-Pak cartridge (Waters) using a 0.15 TFA/acetonitrile (ACN) step gradient ranging from 0-80% ACN (10% increments). A peak of inhibin bioactivity was eluted in the 30-50% ACN component of the step gradient, corresponding to a 3-fold increase over the specific activity of the adsorbed material. The observed interaction of inhibin with the C18-silica support was further investigated using analytical Reverse-Phase HPLC (C18-silica, Perkin Elmer), eluted with multiple linear gradients ranging from 0-52% ACN in 0.1% TFA (20-35% ACN; 38-42% 12; 42-495% 5; 49-52% 10%). Under these conditions three distinct inhibin bioactive fractions were resolved, eluting as one sharp and two broad protein peaks (at 40%, 45% and 49% ACN). These peaks corresponded respectively to 18%, 47% and 35% of the total eluted inhibin activity. Bioassay results from two runs showed that at least 40% of the activity applied to the column was recovered in the eluted fractions.

These results indicate that Reverse-Phase HPLC may be of potential use in the isolation of follicular fluid inhibin. Furthermore, on the basis of their different interactions with the hydrophobic C18-silica, there appear to be three forms of inhibin in ovine follicular fluid. The further purification of the inhibin component(s) from each of these fractions is currently in progress.

References:

Supported by the National Health and Medical Research Council of Aust.
SUPPRESSION OF PLASMA FSH IN OVARIECTOMIZED Ewes GIVEN FOLLICULAR FLUID

J.K. Findlay, L. Cummins*, T. O'Shea* and B. Bindon*

Medical Research Centre, Prince Henry's Hospital, Melbourne, Vic. 3004, *Physiology Department, University of New England and **CSIRO Division of Animal Production, Armidale, N.S.W. 2350.

We reported previously that steroid-free extracts of ovine follicular fluid (OFF) injected into entire ewes disrupted the events leading to oestrus and ovulation (1). This may have been due to a suppression in FSH levels since OFF will cause a selective suppression of FSH in an in vitro rat pituitary cell bioassay for inhibin (2,3). In this study, we have examined if a similar preparation of OFF would selectively suppress plasma levels of FSH in 2 strains of chronically ovariectomized Merino ewes in the non-breeding season.

Charcoal-treated ewe plasma (2 ml; control) or 0.5-2.0 ml OFF (inhibin potency: 9.0 ku/ml) was given s.c. at 0900 and 1700 h on each of Days 2 and 3 of the experiment. Jugular blood samples were taken at hourly intervals between 1000 and 1500 h from days 1 to 6, and plasma samples were pooled to form a composite for each sheep for each day and assayed for LH and FSH.

### TABLE 1. Mean (SE) FSH and LH levels (ng/ml) on Days 3 and 4 in ovariectomized ewes given OFF or plasma on Days 2 and 3 (*p*<0.05/group).  

<table>
<thead>
<tr>
<th>Day of Experiment</th>
<th>Total OFF (ml)</th>
<th>Inhibin (kU)</th>
<th>LH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Booroola</td>
<td>AB20</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>295 (60)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18</td>
<td>214 (45)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>36</td>
<td>105 (21)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>72</td>
<td>76 (20)</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0</td>
<td>288 (56)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18</td>
<td>216 (41)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>36</td>
<td>117 (42)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>72</td>
<td>217 (6)</td>
</tr>
</tbody>
</table>

FSH levels in OFF-treated ewes decreased in a dose-dependent manner by Day 3 compared to controls, with maximum suppression (around 14% of controls) observed on Day 4 (Table 1). By Day 6 FSH levels had returned to values similar to controls. There was no apparent difference in the FSH response to OFF between strains. LH levels were suppressed, but at the highest dose of OFF to 63-71% of controls. There were no significant alterations in the levels of LH or FSH following treatment with plasma.

In summary, steroid-free OFF caused a dose-dependent suppression of FSH in ovariectomized ewes. This demonstration may provide an explanation for the disruption of the oestrous cycle in entire ewes given OFF (1).


SUPPRESSION OF INDUCED OVULATION BY INHIBIN

J.J. Cummins and T. O'Shea

Physiology Department, University of New England, Armidale, N.S.W.

As part of a project to investigate ovarian factors which could influence ovulation rate (OR), ovain follicular fluid was injected (s.c) into pregnant mice which were then induced to ovulate with 10 IU of HCG.

Various intervals between a dose of 0.15 ml of bovine follicular fluid (BFF) and HCG given at 1800 h on day 6 of pregnancy were examined by killing groups of 10 mice and determining their OR on the morning of day 6 of pregnancy. The interval and the mean OR for each group was as follows: control (no BFF) = 8.4 ± 0.4, 0 h; 9.2 ± 0.3, 3 h; 7.0 ± 0.3, 6 h; 5.7 ± 0.4, 9 h; 3.8 ± 0.3, 15 h; 0.3 ± 0.1, 24 h. (Suppression observed with 0.15 ml BFF; OR = 5.3 ± 0.5, 3 h; 9.3 ± 0.5, 6 h; 3.3 ± 0.5, 9 h; 1.3 ± 0.5, 15 h.)

Depression of induced ovulation was observed in mice which were given 1.2 ml of inhibin (mean OR 67.1 ± 10.7; 9075 u/ml when assayed against ovine testicular lymph protein (1 ul/mg protein) in a paticular cell culture system described in (2)). Incubation of follicular fluid for 2 hours at 75°C of enzymatic digestion with trypsin destroyed its ability to suppress induced ovulation in the mouse.

Charcoal treated ram seminal plasma was tested in mice as a source of inhibin. Given in large doses it was able to cause a significant (p<0.05) depression in induced ovulation rate. The mean OR observed in groups of 10 mice were as follows: 1 ml plasma = 10.4 ± 0.7, 2 ml plasma = 9.5 ± 1.0, 1 ml seminal plasma = 7.9 ± 1.4, 2 ml seminal plasma = 6.8 ± 1.1.

These results suggest that follicular fluid contains inhibin-like activity which is able to depress induced ovulation rate in the pregnant mouse. A pituitary site of action was indicated by the blockage of GnRH induced ovulation.

DEVELOPMENT OF A NEW SYSTEM CAPABLE OF SUSTAINED RELEASE OF NON-STERoidal ANTI-INFLAMMATory COMPOUNDS

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A system capable of delivering small quantities of prostaglandin antagonists over an extended time period has been developed. Preliminary observations were made to analyze the variables which controlled the release in vitro of such drugs when incorporated into silastic (382 M) polymeric rods. These variables were found to include the initial loading of the drug in the rod, the dimensions of the rod, and the pH of the fluid around the rod. The release period obtained with indomethacin in such rods was about 21 days (d), with a fresh quantity of fluid being placed in contact with the rod each day.

To extend the delivery period, the use of filtering and complexing agents in the rods has been explored. The only agents found to be useful in restricting the quantity of indomethacin released were silica and albumin (see Table 1). Another approach to lowering the release rate from the rods was to cover the rods in a low-permeability vinyl sleeve, except at their ends where diffusion of the drug from the rod would be expected to continue. With such a system, the release period of indomethacin in vitro was extended to approximately 100 days - for comparison with the previous results, approximately 12% of the drug had been delivered from the rods at 10 days (Table 1). Such a system, providing it has similar properties in vivo, could provide a useful route to the local delivery of anti-inflammatory compounds to the uterus.

Table 1. Release of indomethacin in vitro from silastic rods with various additives or a vinyl covering.

<table>
<thead>
<tr>
<th>SECONDARY ADDITIVE</th>
<th>INDOMETHACIN RELEASED (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 d</td>
</tr>
<tr>
<td>None</td>
<td>21.8 ± 3.8</td>
</tr>
<tr>
<td>Sephadex G-25 (4%)</td>
<td>18.8 ± 0.1</td>
</tr>
<tr>
<td>Carboxymethyl-</td>
<td>18.4 ± 0.1</td>
</tr>
<tr>
<td>cellulose (4%)</td>
<td></td>
</tr>
<tr>
<td>Fumed silica</td>
<td>19.6 ± 0.8</td>
</tr>
<tr>
<td>0.05% HCl (7%)</td>
<td>14.0 ± 0.5</td>
</tr>
<tr>
<td>Albumin (0.7%)</td>
<td></td>
</tr>
<tr>
<td>Catalyst 43 mg</td>
<td>8.0 ± 0.5</td>
</tr>
<tr>
<td>Rods covered by</td>
<td></td>
</tr>
<tr>
<td>vinyl sleeve</td>
<td>3.0 ± 0.3</td>
</tr>
</tbody>
</table>

The values above are (mean ± SE) for pairs of silastic rods in phosphate buffer (0.1M, pH 7.1) at 37°C.
HISTOCHEMICAL CHANGES IN THE REPRODUCTIVE TRACTS OF EWES INJECTED WITH PROGESTERONE EARLY IN THE OESTRUS CYCLE

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Department of Physiology, The University of New England, Armidale, N.S.W.

Injection of ewes at oestrus with 80 mg of progesterone results in a subsequent cycle of about 7 days (7 day cycles) with lowered fertility whereas 40 mg of progesterone on days 1-4 gives a cycle of 13 days (13 day cycles) of normal fertility. We have compared with control ewes the activity of lactic dehydrogenase (LDH), acid and alkaline phosphatases (ACP and ALP) and the intensity of staining with oil red 0 (ORO) in such shortened cycles using histochromic techniques.

Ewes were killed at known times in relation to oestrus. Frozen sections were examined to determine the luminal epithelia of the ampulla, isthmus, endometrium, caruncles and cervix, and the superficial and deep glands of the endometrium. Activity was scored on a scale of 0-5.

The only tissues to show marked changes during the oestrus cycle of control ewes were the luminal epithelia of the endometrium (LE) and caruncles (CE), and the superficial glands (SG). These all showed minimum values at day 4 of the cycle. Values were lower in the other tissues and did not change significantly.

In 13 day cycle ewes LDH, ACP and ALP cyclic changes were advanced compared with control cycle ewes. e.g. values at day 4 of 13 day cycles were the same as those at day 8 of a control cycle (Table 1). Oestrous values were similar in control and treated ewes. However, the luteal peak values for ACP, ALP and ORO were lower in treated ewes.

In 7 day cycle ewes oestrous values at oestrus were lower with respect to ACP and ORO in LE and CE. This differs from the unchanged values reported using biochemical measurements (1) because of the greater cellular discrimination of histochemistry.

TABLE 1: Histochemical staining in uterine tissues in control and progesterone treated ewes.

<table>
<thead>
<tr>
<th>Ewes</th>
<th>Acid Phosphatases</th>
<th>Oil Red O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LE</td>
<td>CE</td>
</tr>
<tr>
<td>Control</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>8</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>12</td>
<td>4.3</td>
<td>4.2</td>
</tr>
<tr>
<td>16</td>
<td>4.7</td>
<td>3.7</td>
</tr>
<tr>
<td>0</td>
<td>2.4</td>
<td>3.4</td>
</tr>
<tr>
<td>13 day cycle</td>
<td>1.9</td>
<td>1.7</td>
</tr>
<tr>
<td>8</td>
<td>4.4</td>
<td>3.2</td>
</tr>
<tr>
<td>12</td>
<td>4.4</td>
<td>2.9</td>
</tr>
<tr>
<td>0</td>
<td>4.1</td>
<td>3.6</td>
</tr>
<tr>
<td>7 day cycle</td>
<td>2.6</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Analysis of variance indicated that the addition of progesterone to the cultures significantly increased the incorporation into explants. This is consistent with the findings of other workers (263) who showed that progesterone added to the culture medium causes secretory changes in the human endometrium similar to those seen in post-ovulatory endometrium in vivo. Oestradiol-17β exhibited no significant effect on the secretions of any tissue.

The organ-culture technique is, therefore, a useful tool for the study of the effects of hormones on the secretions of the various mucosal tissues of the female reproductive tract of the sheep.


EFFECT OF OESTRADIOL-17β AND PROGESTERONE ON THE INCORPORATION OF D-[U-14C] GLUCOSE INTO SECRETIONS FROM TISSUE EXPLANTS IN ORGAN-CULTURE

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The composition and volume of secretions of the mucosal tissues of the female reproductive tract are controlled by ovarian steroids. In the present study, an organ-culture method similar to that described by Ellis and Stahl (1) was used to investigate whether or not oestradiol-17β and progesterone would alter the incorporation of precursor materials into glycoproteins secreted by certain mucosal tissues of the reproductive tract of the ewe.

Explants of cervix, oviduct and endometrium from spayed ewes which had been primed with 30 μg of oestradiol-17β 3 days previously were cultured for 72 hours in Trowell's T8 medium. This medium contained 0.5μCi/ml D-[U-14C] glucose (287 μCi/m mole) and either 0.01 μg/ml oestradiol-17β, 0.005 μg/ml progesterone, or both 0.01 μg/ml oestradiol-17β and 0.005 μg/ml progesterone. A control culture was also run containing alcohol at the same rate (13% V/V) as the other media but lacking the hormone component.

Secretions were collected and passed through a sephadex G-200 column to separate the macromolecular material. One ml of the void volume material was added to 10 ml Tricin-K100 and the activity counted. The presence of glycoprotein in this fraction was confirmed by electrophoresis and staining with periodic acid-schiff reagent (PAS). Radioactivity was shown to be contained in the PAS positive material by autoradiography.

Results were assessed by analysis of variance.

TABLE 1: D-[U-14C] Glucose incorporation into macromolecules secreted by mucosal tissue explants in organ-culture. Values represent the means of four ewes and are calculated as disintegrations per minute incorporated into glycoproteins per mg explant.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OVIDUCT</th>
<th>CERVIX</th>
<th>ENDOMETRIUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>138.8</td>
<td>105.0</td>
<td>91.5</td>
</tr>
<tr>
<td>Oestradiol-17β (A)</td>
<td>81.8</td>
<td>51.8</td>
<td>163.0</td>
</tr>
<tr>
<td>Progesterone (B)</td>
<td>395.8</td>
<td>169.7</td>
<td>309.5</td>
</tr>
<tr>
<td>(A) + (B)</td>
<td>489.0</td>
<td>213.5</td>
<td>241.8</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>167.0</td>
<td>68.8</td>
<td>131.6</td>
</tr>
<tr>
<td>LSD 1%</td>
<td>234.2</td>
<td>96.5</td>
<td>186.5</td>
</tr>
</tbody>
</table>

Analysis of variance indicated that the addition of progesterone to the culture medium significantly increased the incorporation into explants. This is consistent with the findings of other workers (263) who showed that progesterone added to the culture medium causes secretory changes in the human endometrium similar to those seen in post-ovulatory endometrium in vivo. Oestradiol-17β exhibited no significant effect on the secretions of any tissue.

The organ-culture technique is, therefore, a useful tool for the study of the effects of hormones on the secretions of the various mucosal tissues of the female reproductive tract of the sheep.

PROSTAGLANDINS IN THE OVINE ENDOMETRIUM AND UTERINE LUMEN

N. Colvin, J. Swaney, B. Doughton & J.K. Findlay
Medical Research Centre, Prince Henry's Hospital, Melbourne, 2004.

Although prostaglandin-F2α (PGF) has been shown to be the uterine luteolytic in the ewe (1), there is evidence that uterine endometrial production of PGF is not suppressed in early pregnancy. The concentration of PGF was higher in pregnant (P) compared with non-pregnant (NP) endometrium on day 15, and in uterine flushing (UF) on days 13, 15 and 17 (2). In this study, we examined the content of PGF in caruncular (C) and intercaruncular (IC) endometrium and the content of PGF and 13, 14-dihydro-15-keto-PGF2α (PGFM) in UF of P and NP ewes on Days 11, 13 and 15. At laparotomy, the uterine lumen of 5-6 P and NP ewes on each day was flushed with 20 ml of Medium 199 containing 1 mg/ml Indomethacin and 50 U/ml penicillin. The tract was then removed and C and IC dissected out. Aliquots of tissue were kept at 4°C in citrate buffer (pH 3.5) until homogenized in methanol (0.6 g/ml). The methanol extract was centrifuged and the tissue re-extracted. The pooled methanol extracts were dried under air and the residue dissolved in assay buffer (0.01 M Tris-HCl, pH 7.4; 1% gelatin). The PGF content was measured by RIA at several dilutions and corrected for methodological losses (80% recovery). The uterine flushings were centrifuged and assayed unextracted for PGFM and PGF by RIA at several dilutions.

TABLE 1. Mean (SE) levels of PGF and PGFM in caruncular (C) and intercaruncular endometrium (IC) and uterine flushings (UF) of pregnant (P) and non-pregnant (NP) ewes (5-6 ewes/group).

<table>
<thead>
<tr>
<th>Day</th>
<th>Status</th>
<th>PGF (ng/IC)</th>
<th>PGFM (ng/IC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>NP</td>
<td>2.3(0.6)</td>
<td>3.2(0.9)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>2.1(0.4)</td>
<td>4.0(0.8)</td>
</tr>
</tbody>
</table>

PGF concentration in C and IC did not differ within days and pregnancy status (t-test, p > 0.05). On Days 13 and 15, PGF concentration in C and IC was higher (p < 0.05) in P compared to NP ewes except IC, Day 15, p = 0.1. PGF concentrations were higher in both C and IC on Days 13 and 15 c.f. Day 11 of P, but not NP tissues (Duncans test, p < 0.05). In UF, PGF and PGFM content was higher on Day 15 than on the previous days in both P and NP ewes (p < 0.05) and 15 (p < 0.1).

This data supports the notion that PG production is not suppressed in pregnancy. Since in vivo production of PGF in endometrial tissues of NP and P ewes is similar (3), we suggest that either cellular binding (3) or altered metabolism of PG occurs in pregnancy.


Supported by the Australian Wool Research Trust Fund.

PROSTAGLANDIN PRODUCTION BY HUMAN DECIDUAL TISSUES

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In order to study the changing characteristics of human endometrial prostaglandin (PG) synthesis during early pregnancy a technique for the culture of decidual tissue in vitro has been developed. Tissue was obtained by curettage from informed consenting women undergoing voluntary termination of pregnancy during the first trimester. A suitable cell suspension was obtained by collagenase digestion (2h with 0.1% collagenase type II Worthington) in Heps-buffered Medium 199. After repeated washing in a culture medium (Medium 199 containing 10% fetal calf serum), cells were diluted in medium to a concentration of 3 x 10⁵ cells/ml and cultured for 1-3 days in the presence of inert microcarriers (Cytodex, Pharmacia) after the method of Smith and Vale (1).

In the present experiments the bead attached cells which are stable and suitable for perfusion or static incubation experiments, were washed repeatedly and incubated in fresh media with or without arachidonic acid (C20:4) for periods of 1-2 hours. Prostaglandin production was assessed by measurement of PG content of incubation media by a validated radioimmunoassay (2).

Preliminary investigations suggest that the cells produce little or no PG from endogenous substrate but that PG synthesis is readily stimulated by the addition of C20:4 (Table 1).

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Control</th>
<th>E2 (10⁻⁵ M)</th>
<th>P (10⁻⁵ M)</th>
<th>E2 (10⁻⁵ M) + P (10⁻⁵ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.06</td>
<td>&lt;0.06</td>
<td>&lt;0.06</td>
<td>&lt;0.06</td>
</tr>
<tr>
<td>48</td>
<td>0.06</td>
<td>1.03</td>
<td>0.98</td>
<td>0.87</td>
</tr>
<tr>
<td>72</td>
<td>0.06</td>
<td>0.59</td>
<td>0.06</td>
<td>0.44</td>
</tr>
</tbody>
</table>

As is shown the capacity of the cells to synthesize PG from C20:4 is increased with time in culture and may be influenced by inclusion of steroid hormones in the culture medium.

Comparisons with non-pregnant secretory endometrium and the effects of embryonic tissues and extracts on PG synthesis are currently being studied.

When ewes are grazed on oestrogenic clover pastures for several years, they become permanently infertile. Affected ewes have several abnormalities, including an increase in the number of stromal cells and gland transactions in the lamina propria of the cervix, and a decrease in the number of folds projecting into the lumen (1). Such a change has not been produced by oestrogen in adult animals of any other species. The present study was carried out to see whether this change in the ewe could be caused only by phyto-oestrogens or whether a similar change could be brought about by oestradiol-17β (E2). Groups of 3 mature Merino ewes which had been ovariolectomized a year previously under general anaesthetic were run on non-oestrogenic pasture, and were implanted subcutaneously with silastic tubing implants (i.d. 3.15mm, o.d. 4.65mm) containing 0, 3, 6 or 12 cm lengths of crystalline E2. The release rate of these implants in vitro at 37°C was estimated to be 38µg E2/cm/24h, using %E2. Ewes with a 12 cm implant had a peripheral plasma concentration of 49±8 (S.E.M.) pgl/ml E2, kindly estimated by G.B. Martin (University of Western Australia) using radioimmunoassay. After 140 days, the ewes were slaughtered and the cervix fixed in formal saline. Transverse histological sections were prepared at 6mm intervals along the cervix, and stained with haematoxylin and eosin.

The ewes treated with the 2 larger amounts of E2 had obvious histological changes, including increased numbers of stromal cells and tubular glands in the lamina propria, and fewer folds projecting into the lumen. The number of gland transactions was increased only towards the uterine end of the cervix, and differences between the groups were not statistically significant (ANOV). Folds were most readily determined by counting the numbers of crypts between them. A crypt was counted if it reached more than half-way to the base of the lamina propria, and if it was also at least half as large as its nearest neighbour. The number of crypts varied between the anterior, middle and posterior cervix (ANOV; P<0.01) and numbers also declined significantly (ANOV; P<0.05) as the dose of E2 increased, mean ± S.E.M. values for the 4 groups being 71±15, 61±2, 34±1 and 28±6.

The results show that a steroidal oestrogen can produce the same unusual histological change in the adult ewe as is seen in clove disease. By using large doses of E2, this effect is produced more quickly than can be obtained in ewes grazing oestrogenic pasture. Lastly, changes in the numbers of crypts may provide a method for measuring the amount of change produced by large or prolonged treatment of the ewe with oestrogen.

ACTION OF PROGESTERONE ON UTERINE STEROID RECEPTOR LEVEL: COMPARISON OF THE SHEEP AND MOUSE

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University of Sydney, Sydney. 2006

In the oestriadiol (E₂) stimulated mouse uterus, there is a marked reduction in cytosol E₂ receptor level 24h after progestrone (P) injection with a concomitant decline in a number of anabolic parameters. In the ewe the lack of such anti-uterotrophic action of P is associated with a modest decline in E₂ receptor level (1). Such results suggested that the anti-oestrogenic activity of P in the rodent uterus may not be directly due to its effect on cytosol E₂ receptor level. Accordingly the effect of P on the level of cytosol and nuclear receptors for E₂ and P within 24h after P injection has been examined in these two species. Ovariectomized (OVX) mice received 0.1 µg E₂ on Days 1 and 2 and 0.1 µg E₂ or 0.1 µg E₂ plus 1.0 µg P on Day 3. Groups of OVX ewes received corresponding doses of 40 µg E₂ and 12 µg P at the same times. Groups of 45 mice and 3 sheep were killed at Oh on Day 3 at 2, 4, 6, 8 or 24h. The Day 3 steroid treatments. Nuclear receptors for E₂ and P were as measured as described by Chen and Leavitt (2).

The results appear also to explain why progesterone shows no anti-oestrogenic activity in the sheep uterus. Thus flushing, even with small volumes of isotonic saline, causes extensive damage to the endometrium of pregnant or progesterone treated animals by leaching, rupturing and removing epithelial cells, and by splitting the luminal basement membrane and passing into and through the connective tissue stroma, leaching and rupturing stromal cells and blood vessels. In contrast relatively large volumes produce little endometrial damage in untreated or oestrogen treated ovariectomised mice in which the uterine lumen is open. Damage increases to a maximum on day 5 of pregnancy when the uterine lumen has entered the second stage of closure (8), and the decidua reaction is already well advanced. I conclude that many 'luminal fluid' proteins originate from luminal and stromal cell contents, intercellular fluid and blood, and that changes in the profiles of such proteins in early pregnancy reflect no more than changes in the extent and type of flushing-induced damage as a consequence of alterations in the physical state of the uterus induced by hormones and blastocysts.

THE RESPONSE IN PLASMA PROGESTERONE TO HYPOPHYSECTOMY OF THE FEMALE TAMMAR

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Division of Wildlife Research, CSIRO, Lyneham, A.C.T. 2602

A transient peak of plasma progesterone is associated with reactivation of the quiescent corpus luteum (CL) of the tammar. The rise occurs between 5 and 8 days after removal of pouch young in both the pregnant and non-pregnant cycle (1). Hearn (1974) (2) demonstrated that hypophysectomy of lactating females induced reactivation of the quiescent CL and dormant blastocyst. These results suggested that the CL becomes autonomous once released from the pituitary-induced inhibition.

The following experiment was designed to determine (a) whether the occurrence of the peak of progesterone is dependent upon the presence of the pituitary and (b) whether plasma progesterone levels in hypophysectomised females differ from levels in intact females.

In early lactational quiescence five females were fully hypophysectomised and a control group of another five females underwent a sham-operation on day 0. All females were lactating and their pouch young were reattached to the teats after the operation. Blood samples were collected from lateral tail veins on days 0, 1, 3 to 7, 14 and 21 for determination of progesterone by radioimmunoassay (3). All animals were autopsied at the time of death or on day 21, and the reproductive tract examined.

Both groups retained their young during the experiment although the young of the hypophysectomised group rapidly lost weight, did not remain constantly attached to the teat and died 7 to 11 days after operation. The young of the control group all remained attached and their body weights had doubled by day 21.

Two hypophysectomised females died at day 7, a third at day 12, and the two other animals survived until day 21. The latter three animals were reactivated and were carrying an enlarged vesicle and late stage foetuses respectively. There was no resumption of development of quiescent CL or blastocysts in the control group.

Four of the five hypophysectomised females showed changes in plasma progesterone typical of the pattern and concentration observed in a normal pregnancy or an oestrous cycle. Three animals showed a peak of progesterone (397, 615, 682 pg/ml) around days 6 to 7 after operation, and levels increased in late gestation. Plasma progesterone in the control females remained at basal concentrations of about 200 pg/ml.

We conclude, therefore, that the CL of the tammar is independent of the pituitary once the pituitary-induced inhibition is removed, since it does not require any luteotropic support for its growth or progesterone production during the reactivated cycle.


THE MAJOR STEROIDS IN OVARIAN AND ADRENAL VENOUS PLASMA OF TRICHOSEURUS VULPECULA

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University of Sydney, N.S.W. 2006 and of Chemistry3,

Karolinska Institute, Stockholm, Sweden.

We are presently studying changes in sex steroid secretion during the oestrous cycle/pregnancy of T. vulpecula. Prior to establishing the radioimmunooassays (RIAs) it was decided to obtain a definitive identification, using gas chromatography/mass spectrometry (GC/MS), of the major steroids produced by the ovary and adrenal of this species.

Mature animals were housed under natural light and the stage of the oestrous cycle and mating were determined from daily vaginal smears. Ovariectomised, adrenal and peripheral blood was collected under pentobarbital anaesthesia. For GC/MS analysis plasma samples were extracted with ether and shipped to Sweden for identification and quantification of the major neutral and phenolic steroids (1). Blood from pro-oestrous animals and from single animals at day 13 of the oestrous cycle/pregnancy was analysed. Testosterone (T) was also measured in the blood of these and other animals by RIA. Adrenal glands were removed for histology.

TABLE 1. Major steroids in ovarian and adrenal venous plasma.

<table>
<thead>
<tr>
<th>T (ng/ml)</th>
<th>Phenolic steroids (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian vein</td>
<td>E2</td>
</tr>
<tr>
<td>Pro-oestrous</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Day 13 preg.</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Adrenal vein</td>
<td>E2</td>
</tr>
<tr>
<td>Pro-oestrous</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Day 13 preg.</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

T = Testosterone; P = Progesterone; E2 = Cortisol; B = Corticosterone; E1 = Oestradiol; E3 = Oestrone; nd = not determined.

Oestradiol was identified as the major phenolic steroid (Table 1) and, as expected, was present in high concentration in pro-oestrous venous plasma. Progesterone was the only progesterin detected in ovarian venous plasma. These findings confirm that the major ovarian sex steroids produced by T. vulpecula are the same as those produced by eutherian mammals. The major neutral steroid from the adrenal was cortisol and of considerable interest was the extremely low level of T in all adrenal vein samples in spite of the histological presence of a special zone. RIA confirmed that the T level was no more than 0.2-0.8 ng/ml in adrenal and peripheral plasma samples respectively. Both results contrast dramatically with previous reports of 223 ng/ml for adrenal (2) and 50 ng/ml for peripheral (3) plasma.

LUTEINISING HORMONE IN THE BRUSH TAIL POSSUM
C. A. Horn
Division of Wildlife Research, CSIRO, Lyneham, A.C.T. 2602

The brush tail possum, Trichosurus vulpecula is a monovular and polyoestrus marsupial with an average oestrous cycle of 26 days and a gestation length of 17.5 days. This study presents the luteinising hormone (LH) concentrations with respect to these cycles in these animals.

LH was measured in possums in various reproductive states using a heterologous radioimmunoassay described by Sutherland et al. (1). Basal levels in adult anoestrus females were low, 0.7 ng/ml, but they responded to an intravenous injection of 10 μg luteinising hormone releasing hormone with a peak LH concentration of 15.4 ng/ml within 30 minutes and a return to basal levels within 90 minutes. Castration of three females and one male resulted in elevated LH levels of 6.3, 3.0, 8.1 ng/ml and 18.4 ng/ml after 20 and 13 days respectively.

In the breeding season the concentration of LH was also low, fluctuating from undetectable (0.35) to 1.0 ng/ml, except during the pre-ovulatory surge. The effect of hypophysectomy on LH levels was not demonstrable given these low basal concentrations. Oestrous cycles were initiated by removal of the suckling young (RPY) in the April/May breeding season and the resulting oestrus determined from daily vaginal smears. Daily blood samples were collected from the lateral tail vein. The pre-ovulatory LH surge occurred 9.2 ± 0.9 days after RPY, ranged in value from 5-25 ng/ml and was of less than 24 hours duration. Copulatory plugs were observed 9.1 ± 0.6 days after RPY and the appearance of leukocytes on day 9.9 ± 0.6 RPY i.e. oestrus by definition, (Pilton and Sharman) (2), thus occurred on day 9 RPY. The timing of the LH surge is therefore roughly coincident with oestrus.

These results correlate with the observation that ovulation occurs, on average, one day after oestrus (Shorey and Hughes (3)) and are comparable with LH data from the tammar wallaby, another monovular and polyoestrus marsupial.

References:

MYOMETRIAL ACTIVITY IN THE TAMMAR WALLABY
G. Shaw
School of Environmental and Life Sciences, Murdoch University, Murdoch, Western Australia 6150.

In the tammar wallaby (Macropus eugenii) removal of a sucking pouch young (RPY) initiates development of the diapausing blastocyst in one of the two anatomically separate uteri. Birth ensues 26-28 days later. Despite the apparent similarity of pregnancy to the oestrous cycle (1), the endometrium of the gravid uterus is heavier and more active in secretion than that in the adjacent non-gravid uterus (2, 3).

To assess if pregnancy would also influence myometrial activity, diapause was terminated in a group of wallabies by RPY. At various stages through pregnancy myometrial activity was assessed either (i) in vitro using isometric tension myograms from uterine strips maintained at 37°C in Krebs-Ringer; or (ii) in vivo with intra uterine pressure recordings made using pentobarbitone-anaesthetised animals.

During the first half of pregnancy both uteri showed only slight, irregular activity. Doses of 500 mU oxytocin-S (i.v.) stimulated little or no response in either uterus.

Spontaneous small, regular contractions (10-20 mm Hg, 3-10 sec⁻¹) were recorded in the second half of pregnancy. Contractions in the non-gravid uterus were invariably more frequent than in the adjacent gravid uterus. Oxytocin sensitivity of the gravid uterus increased towards the end of pregnancy (threshold 20-50 mU i.v.). Injection of 1 μg of cloprostenol induced a prolonged period of increased myometrial activity in animals in late pregnancy. In some preparations Braxton-Hicks contractions were recorded for extended periods following treatment with high doses of oxytocin or cloprostenol.

The changing oxytocin sensitivity of uteri confirms an earlier report in another wallaby (Setonix) (4). The differences between myometrial activity of gravid and non-gravid uteri in each female parallel the endometrial differences. Studies are in progress to establish the roles of the conceptus and of unilateral hormone influences from the ovary in establishing these differences.

References:
THE ROLE OF CYCLIC NUCLEOTIDES AND OF EPIDIDYMAL PROTEINS IN THE INITIATION OF SPERM MOTILITY

Dr. D. Hosking
Oregon Primate Centre, U.S.A.
Invited Lecturer


P.R. Davies and I.C.A. Martin
Department of Veterinary Physiology, University of Sydney, N.S.W. 2006

Studies employing the methods of Amann and Lambiase (1), were conducted to determine the daily sperm production per gram of testicular parenchyma in the dog, by the enumeration of elongated spermatids in homogenates of testes. 

Radioautographs were prepared from smears of homogenates of testis and caput epididymis, and histological sections of testes removed 31-40 days following I/V injection of 0.5 m Ci/Kg of tritiated thymidine (H\textsubscript{3}-T). 

Labelled spermatozoa were present in homogenates of caput epididymis 38 days after injection of H\textsubscript{3}-T, over 2 days prior to the time predicted from the data of Foote et al (2), who estimated the length of the cycle of the seminiferous epithelium (CSE) of the dog to be 13.6 ± 0.7 days, and stated that the most mature cells to incorporate H\textsubscript{3}-T were preleptotene primary spermatocytes (PS) in stage 8 of the CSE. 

Radioautographs were prepared from sections of testes removed 1, 12, 24 and 36 hours after injection of H\textsubscript{3}-T. After 1 hour, 42% of 110 tubules examined in stage 1 of the CSE contained labelled preleptotene PS. By 24 hours, labelled leptotene or zygotene PS were present in 49% of 89 tubules examined in stage 2 of the CSE. 

These data indicate that DNA synthesis by PS in the dog continues until late in stage 1 of the CSE, rather than being completed in stage 8. 

From the radioautographs of testes removed at days 31-38 after injection of H\textsubscript{3}-T, the duration of the CSE was calculated to be 13.7 ± 0.22 days, in close agreement with the figure of Foote et al. 

Kennelly (3), in a study of male coyotes, determined that: 
(a) DNA synthesis by PS occurs within the last 30% of stage 1 of the cycle. 
(b) The duration of the CSE was 13.6 ± 0.15 days. 
(c) Labelled spermatozoa could arrive at the caput epididymis 37.4 days after injection of H\textsubscript{3}-T. 

Matings of these two canids produce fertile hybrids and the above data indicate that the similarities in the kinetics of spermatogenesis in these species may be greater than previously suggested.

REGULATION OF THE SYNTHESIS AND SECRETION OF EPIDIDYMAL PROTEINS

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The epididymis synthesizes and secretes specific proteins, some of which are believed to play a key role in the maturation process that spermatozoa undergo in this organ. Several of these proteins have been purified and can be used as markers of epididymal function (1).

By using radioactive precursors, it has been possible to label secretory proteins, to separate them by gel electrophoresis and to visualize them by fluorography. With [35S]methionine or [14C]amino acids as precursors, it was found that a pronounced regional variation occurred in different portions of epididymis with respect to the pattern of synthesized secretory proteins. Radioactive sugars ([3H]mannose, [3H]galactose, and [14C]glucose) were also incorporated into secretory glycoproteins, and even more pronounced regional variations in secretory profiles were evident.

In confirmation of previous results (2), castration was found to affect the synthesis of relatively few secretory proteins in this androgen-dependent tissue. However, castration did have a profound effect on glycosylation reactions, since the incorporation of radioactive sugars was reduced by up to 90%. On the other hand, tunicamycin, which inhibits formation of the N-glycosidic type of bound oligosaccharide, had relatively little effect on sugar incorporation.

Since most secretory proteins are now believed to be synthesized as larger precursors with a hydrophobic signal sequence which is removed by proteolysis as the polypeptide traverses the endoplasmic reticulum, it was of interest to test the effect of various protease inhibitors on protein secretion by the epididymis. TLCK, TPCK, pepstatin, benzamidine and phenanthroline were all without effect on the profile of secretory proteins. On the other hand, procaine specifically inhibited the secretion of certain secretory proteins. To separate them by gel electrophoresis and to visualize them by fluorography. With [35S]methionine or [14C]amino acids as precursors, it was found that a pronounced regional variation occurred in different portions of epididymis with respect to the pattern of synthesized secretory proteins. Radioactive sugars ([3H]mannose, [3H]galactose, and [14C]glucose) were also incorporated into secretory glycoproteins, and even more pronounced regional variations in secretory profiles were evident.

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In confirmation of previous results (2), castration was found to affect the synthesis of relatively few secretory proteins in this androgen-dependent tissue. However, castration did have a profound effect on glycosylation reactions, since the incorporation of radioactive sugars was reduced by up to 90%. On the other hand, tunicamycin, which inhibits formation of the N-glycosidic type of bound oligosaccharide, had relatively little effect on sugar incorporation.

Since most secretory proteins are now believed to be synthesized as larger precursors with a hydrophobic signal sequence which is removed by proteolysis as the polypeptide traverses the endoplasmic reticulum, it was of interest to test the effect of various protease inhibitors on protein secretion by the epididymis. TLCK, TPCK, pepstatin, benzamidine and phenanthroline were all without effect on the profile of secretory proteins. On the other hand, procaine specifically inhibited the secretion of certain secretory proteins.

The effects of CEF on the metabolism of ram sperm could be mimicked by 0.25 to 40 mg/ml crystalline bovine serum albumin (BSA). Stimulation of oxygen uptake was apparent within two hours and persisted over the 8 hours of the experiment (105.0 ± 26.6 versus 19.9 ± 7.0 μl/10⁸ sperm; n = 4). The stimulus was comparable to that produced by 72.2 ± 21.8 μl/10⁸ sperm glucose (23.3 ± 0.13) and was confirmed by the amount of glucose oxidized in the presence and absence of CEF (0.92 ± 0.13 versus 5.40 ± 1.46 μoles/10⁸ sperm). CEF produced a beneficial effect on the survival of washed ram sperm (total motility score 20.5 ± 2.7 versus 17.3 ± 1.8) but it was somewhat less than glucose (25.3 ± 0.13) and usually only became evident after about 6 hours of incubation.

A high molecular weight fraction (HMWF) obtained by passing CEF through a Sephadex G-25 column, was as effective as CEF in stimulating the oxygen uptake of ram sperm (control 10.9 ± 3.2; CEF 43.4 ± 6.5; HMWF 36.6 ± 4.6 μl/10⁸ sperm/hr; n = 3). The effects of CEF on the metabolism of ram sperm could be mimicked by 0.25 to 40 mg/ml crystalline bovine serum albumin (BSA). Stimulation of oxygen uptake was apparent within two hours and persisted over the 8 hours of the experiment (51.2 ± 9.5 versus 9.1 ± 2.0 μl/10⁸ sperm). As with CEF, stimulation of oxygen uptake by BSA was less than with 10 mM glucose (72.2 ± 21.8 μl/10⁸ sperm/hr; n = 4) and the effects were not additive, i.e. CEF and CEF plus glucose elicited about the same oxygen uptake (105.4 ± 26.6 versus 110.2 ± 29.5 μl/10⁸ sperm; n = 4). This suggested that CEF suppressed the oxidation of glucose and was confirmed by the amount of glucose oxidized in the presence and absence of CEF (0.92 ± 0.13 versus 5.40 ± 1.46 μoles/10⁸ sperm). CEF produced a beneficial effect on the survival of washed ram sperm (total motility score 20.5 ± 2.7 versus 17.3 ± 1.8) but it was somewhat less than glucose (25.3 ± 0.13) and usually only became evident after about 6 hours of incubation.

After passage through a Sephadex G-25 column to remove low molecular weight material, BSA was still effective in stimulating the oxygen uptake of ram sperm over 4 hours (control 6.3 ± 1.1; BSA 10.8 ± 3.5; Column BSA 35.2 ± 1.7 μl/10⁸ sperm; n = 3). After similar treatment, ram blood plasma was also effective (25.3 ± 1.0, n = 3) and ram seminal plasma even more so (54.7 ± 6.8).

Human serum albumin (HSA) was as effective as BSA in stimulating the oxygen uptake of ram sperm. However, defatting human serum albumin decreased its effectiveness (control 4.5 ± 1.3; BSA 22.6 ± 6.1; HSA 26.4 ± 4.4; DF-BSA 9.2 ± 0.97 μl/10⁸ sperm/hr). The motility score of the sperm was similarly affected (control 9.9 ± 1.1; BSA 14.0 ± 1.1; HSA 16.1 ± 1.2; DF-BSA 12.1 ± 0.2).
ALTERTATIONS IN THE FLAGELLAR PLASMA MEMBRANE OF RAM SPERMATOZOA DURING POST-TESTICULAR MATURATION

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The post-testicular phase of mammalian sperm maturation involves a complex series of metabolic events which leads to the initiation of motility and acquisition of fertilizing capacity. It is now recognised that Ca²⁺ is a vital regulator in many of these events. We have studied the control of Ca²⁺ fluxes at the level of the sperm flagellar plasma membranes using both ejaculated and epididymal ram sperm (1).

These studies have now been widened to investigate other structural and functional aspects of the flagellar plasma membrane during post-testicular maturation.

The flagellar plasma membranes of caput, caudal and ejaculated ram sperm were prepared from purified flagella using hypotonic lysis and sucrose density gradient centrifugation (2). The protein components of the three different membrane fractions were analysed by SDS-polyacrylamide gel electrophoresis. The caput and caudal plasma membranes have considerable similarity in protein composition. Both contain a large number (>30) of protein species with molecular weights (M.W.) ranging from 10,000 to 200,000. In comparison the flagellar plasma membranes from ejaculated sperm are characterized by containing apparently fewer protein species (approximately 20) of which a 18,000 and 120,000 M.W. species predominate. Only the 18,000 M.W. species plus a minor 30,000 M.W. species are glycoproteins, as indicated by staining with periodate-schiff's reagent or labelling with [3H]-Concanavalin in A.

Morphological features of the flagellar plasma membranes were investigated using freeze-fracture electron microscopy. In these studies, samples were also pretreated with filipin to selectively identify sterol containing regions (3). In caput sperm the sterol distribution of the plasma membrane was found to be confined to discrete areas of the flagella, whereas caudal and ejaculated sperm had a more uniform sterol distribution. The [Ca²⁺ + Mg²⁺]-ATPase of the flagellar plasma membrane varied from 1.50 ± 0.9 umoles ATP produced/mg protein/hr to 1.38 ± 0.78 umoles ATP produced/mg protein/hr for ejaculated and caudal sperm, respectively. No Ca²⁺-ATPase activity could be detected in the membranes from caput sperm. We suggest that these alterations in enzymic activity are related to changes in membrane composition and organization, all of which are required for the full development of sperm motility. (This work supported by the M.R.C. of N.Z.)

PLASMA MEMBRANE CHANGES INDUCED IN EJACULATED RAM SPERM FOLLOWING SELECTIVE REMOVAL OF SEMINAL PLASMA

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It has been reported that an efficient method of separating either ram or bull sperm from semen is achieved by centrifugation through a Ficoll-containing medium (1). Although this procedure caused only minimal mechanical damage to the sperm, it was noted that for bull sperm in particular, motility was lost. We have now investigated the changes in membrane-located Ca\(^{2+}\) transporting systems in ejaculated ram sperm, following washing in Ficoll.

Ram semen was diluted with washing medium (68 mM sodium citrate, 13 mM glycine, 1.66 mM glucose, 171 mM glycerol, 12 mM sodium sulphacetamide, 1250 units/ml penicillin, and 1250 units/ml streptomycin, pH 7.4) and washed twice in Ficoll (1). The sperm are then resuspended in washing medium and stored for up to 3 days at ambient temperature. These washed sperm, which are devoid of motility, are referred to as inactivated sperm. Sperm (inactivated or fresh) were then washed twice by centrifugation in a buffered salts medium (30 mM Tris HCl, 103 mM NaCl, 12.5 mM KH\(_2\)PO\(_4\), 12.5 mM K2HP0\(_4\), 3 mM MgCl\(_2\), 0.4 mM EDTA, pH 7.4) before assaying for Ca\(^{2+}\) transport using an electrochemical technique (3).

Freshly ejaculated ram sperm are motile and have the capacity to control Ca\(^{2+}\) fluxes across the plasma membrane. These sperm maintain a low free Ca\(^{2+}\) level within the cytoplasm, which restricts influx of Ca\(^{2+}\) into the mitochondria. Ejaculated sperm, when subjected to cold shock (3), lose motility. This can be monitored by the large increase of Ca\(^{2+}\) accumulation into the mitochondrial compartment and its subsequent release by a low concentration, 3 \(\mu\)M of the ionophore A23187 (2).

It was found that Ficoll-inactivated ram sperm are similar to untreated ejaculated sperm in that they are both essentially impermeable to Ca\(^{2+}\). However, the inactivated sperm are quite different in their sensitivity to cold shock. Inactivated sperm which have been exposed to cold shock (0\(^0\) for 10 min) did not accumulate Ca\(^{2+}\) into the mitochondria. This could be explained by either an increased resilience of the plasma membrane to physical trauma, or a loss of mitochondrial activity. The latter alternative was excluded by using filament (3) to selectively disrupt the plasma membrane of the inactivated ram sperm. In this case it was found that the inactivated sperm are still capable of mitochondrial Ca\(^{2+}\) accumulation. This strongly suggests that the plasma membrane of ram sperm has been modified during Ficoll washing by selective removal of either membrane components, or seminal plasma components.


CHROMOSOMAL AND REPRODUCTIVE STUDIES OF BULLS EVALUATED FOR ARTIFICIAL BREEDING

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In cattle reduced fertility may be associated with chromosomal aberrations (1). The relationship between fertility and both karyotype (including chromosomal morphology and number per cell) and semen quality was assessed in a group of 14 bulls selected for artificial breeding.

Bulls were classified according to their 60-90 day non-return rates into high, >60% (mean 63 ± 5.60, n=9) and low, <60% (mean 46 ± S.E.5.8, n=7) fertility groups. Lymphocyte cultures were prepared by a standard whole blood technique and stained with Giemsa (2). Chromosomes, in at least 20 cells from each bull, were counted and cells were grouped according to their chromosome number - <60, 60, >60, or polyploid. Sperm was evaluated microscopically (3) and scrotal contents of all bulls were assessed by palpation.

| TABLE I. Chromosomal counts (Mean ± S.E.) for high and low fertility group bulls |
|-----------------------------|-----------------------------|
| Fertility                  | Cell Distribution by Chromosomal Count |
|                            | Total Sperm  | Live Sperm | Morphological Abnormalities |
| Low                        | 12 ± 2.4     | 83 ± 1.9   | 1 ± 0.3                     |
| High                       | 10 ± 2.3     | 88 ± 1.9   | 0.5 ± 0.4                   |

All bulls had testicles of normal size and consistency. High fertility bulls had greater (P < 0.01) numbers of spermatozoa per ejaculate than low fertility bulls, but % live normal and % morphological abnormalities were similar (P > 0.05). No morphological abnormalities in chromosomes were observed and chromosomal counts were similar (P > 0.05) for both groups of bulls.

These results indicate that the standard Giemsa stained (quantitative) karyotype is of little use in differentiating fertility, unless some easily detected important chromosomal abnormality is present. Banded (G-, C-, T-) karyotype (1) however may be more useful in identification of chromosomal aberrations associated with reproductive performance.

Many studies have been conducted to show that cAMP and phosphodiesterase inhibitors can stimulate and maintain the motility of bovine testicular sperm (1), epidymal sperm (2,3) and ejaculated semen (4). The study reported here examined the response of chilled and frozen bull semen to a phosphodiesterase inhibitor, isobutylmethylxanthine (IMX). Experiments were replicated using 4 and 6 bulls for chilled and frozen semen respectively. For both studies, two levels of IMX (0.25mM and 0.50mM) were used and were added at initial chilling or freezing. In the chilled semen study, semen was diluted with IMX-tris extender containing 5 or 20% (v/v) egg yolk, cooled to 5°C and stored at this temperature for 72 hr. The percentage of motile sperm was evaluated immediately after semen had been warmed to 37°C and again after incubation for 5 hr. In the study involving spermatozoa frozen to -196°C the effects of the addition of 20% (v/v) dialysed egg yolk and 6.4% (v/v) glycerol were also examined immediately after thawing and after incubation for 6 hr.

Table 1 shows that the presence of IMX improved the percentage of motile sperm during incubation of both previously chilled and deep-frozen semen. It was also shown in Experiment 1 that survival of spermatozoa was significantly higher in diluents with 20% egg yolk above 0.25mM IMX than in those with 5%. Both experiments indicated that levels of IMX gave little additional benefit in stimulating and maintaining the percentage of motile sperm. The effect of the addition of dialysed egg yolk during incubation of the egg yolk could have resulted in cyclase activity. However, in this experiment, the presence of IMX+ did not have a significant effect.

ACROSOME REACTIONS IN NITILE HUMAN SPERMATOZOA AT LOW AND HIGH pH

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The effects of pH on the rate of acrosome reactions of human sperm cultured in vitro were studied using suspensions enriched with motile cells by a modification of the 'sperm-rise' technique (1). Seven semen samples were centrifuged to remove seminal plasma, and the pellet of sperm was resuspended in 0.5 ml of PBS containing 5% Ficoll 400. 0.2 ml aliquots of this were then carefully layered under 5 ml of complete culture medium (see below). The use of Ficoll produced a sharp interface through which mostly only motile spermatozoa penetrated. After 1-1 1/2 hours at 37°C, the top 4.5 ml was removed, and cultured for 3-5 hours more. Acrosome reactions were monitored by electron microscopy of fixed cells, using both thin sections and surface replicas.

The mean percentage of progressively motile spermatozoa after centrifugation was 29.6 (range 3.9-59.2): that in the culture medium after 'sperm-rise' was 80.7 (65.8-97.5), and there was a significant (P<0.01) correlation between these figures for individual samples. The mean concentration of progressively motile sperm in the final cultures was 1.74 x 10^6/ml (range 0.1-7.5). This technique enabled almost all of the motile spermatozoa in an ejaculate to be isolated in a highly purified and contamination-free manner. There was some evidence that spermatozoa that were initially immotile or poorly motile may have spontaneously reactivated, as in a number of cases (5/21) the numbers of progressively motile spermatozoa recovered after 'sperm-rise' exceeded the numbers estimated for the original ejaculates. The culture medium was BM containing 3% BSA. Sperm were cultured at low pH (7.1-7.35-7.25 mM NaHCO₃) under 5% CO₂ in air, and at high pH (7.6-8.2) mM NaHCO₃ under air alone; motility remained virtually unchanged during the period of culture. Acrosomes were classified by electron microscopy as intact, swollen, normally reacted or abnormally reacted. To count as normally reacted, the inner acrosomal membrane, equatorial segment and midpiece plasma membrane all needed to be intact. At high pH after 4 hours, 17-29% of spermatozoa showed acrosomal swelling, 0-3.5% showed normal acrosome reactions, and 2-5% had abnormal reactions or were obviously dead. At low pH, even after 6 hours of culture, only 3% of spermatozoa showed acrosomal swelling, and 1.1% showed normal acrosome reactions. It thus appears that in human spermatozoa, as in other species that have been studied, acrosome reactions are accelerated at high pH.

Spermatozoa with swelling acrosomes showed patchy decondensation of the acrosomal contents except in the region of the equatorial segment. Final release of the contents appeared to result from the breakdown of the outer acrosomal membrane and plasma membrane. Many spermatozoa showed acrosomal swelling without any obvious change to the plasma membrane, but whether this is a feature of the normal acrosome reaction remains to be determined. Preliminary results also indicate that hyaluronidase is released from spermatozoa during culture, and this is being investigated as a means of monitoring capacitation.


SOME CHEMICAL PARAMETERS OF GOAT SEMEN

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University of Sydney, N.S.W. 2006

As there is little published information on the biochemistry of goat semen, the concentration of seven constituents has been examined during the breeding season. The constituents examined were fructose, glucose, lactic acid, glycerylphosphorylcholine (GPC), glycerophosphate (GP), glycerol and riboflavin. The latter was of particular interest in that it was thought that high concentrations of riboflavin in semen could account for the yellow colour of some ejaculates.

One hundred and twelve ejaculates were collected by artificial insemination from 11 Angora bucks, once or twice weekly, between late April and July. Volume and pH of semen were recorded, and the concentration of sperm determined after centrifugation of the ejaculates. Seminal plasma was obtained for chemical analysis by centrifugation of the chilled semen (0°C) immediately after collection. Glucose, fructose and lactate were assayed enzymically. GPC was isolated on a TLC-cellulose plate with ethanol: NH₄OH: water saturated phenol: water (6:3:21:1, by vol.) as solvent, and determined by the inorganic phosphate method. During GPC isolation, another spot (Rf 0.25) was consistently seen on spraying with ammonium molybdate. It was identified as L-α-glycerolphosphate by chromatography and quantitated by enzyme assay. Mean values ± S.D. of semen parameters are given.

The ejaculate volume was 0.8 ± 0.3 ml, pH 7.01 ± 0.34 and sperm concentration 3.33 ± 0.48 x 10⁹/ml.

The concentrations of fructose (875 ± 97) and lactic acid (73 ± 17) (mg/100 ml) in buck seminal plasma are sufficiently high to be important substrates for maintenance of the motility and fertilising capacity of the sperm. Only trace amounts of glucose were present in semen.

The GPC concentration of seminal plasma (809 ± 154 mg/100 ml) was correlated with whole semen sperm concentration (P < 0.001) indicating that GPC is of epididymal origin. Goat sperm presumably are unable to utilize GPC as a substrate and its metabolizable derivatives (GP, 3.3 ± 1.1) and free glycerol (1.76 ± 1.02), were not present at significant concentrations (mg/100ml) to be significant as energy sources for goat sperm.

Yellow semen was consistently produced by 8 bucks; riboflavin was extracted with ethanol and determined by high pressure liquid chromatography. In yellow, light yellow and white ejaculates, the seminal plasma riboflavin (μg/ml) and fructose (mg/100ml) concentrations were 5.38 ± 2.69 and 1009 ± 105, 3.09 ± 0.85 and 775 ± 221, 1.73 ± 0.86 and 222 ± 191 respectively. The riboflavin and fructose concentrations in yellow were higher than in white ejaculates (P < 0.01). Fructose is known to be secreted by the vesicular glands of the ram and bull. The high concentrations of riboflavin and fructose in yellow seminal plasma suggests, therefore, that the colour is due to riboflavin and that the origin is the vesicular glands.
A bacterial involvement in human infertility is postulated on the basis of differences in the reported incidences of antispermatozoal antibodies (1), and the correlation between asymptomatic bacterial infection and reduced fertility in males (2) and females (3). The precise mechanism(s) is unresolved although spermicidal (4), and/or antigenic cross-reactivity (5) have been suggested. Modification of the host genital tract immune system by bacteria is also a possibility.

The inhibition of lymphocyte blast transformation by seminal plasma (SP) was assayed after absorption of SP with Group A Streptococcus. Group A Streptococcus was cultured in Todd-Hewitt broth, washed and suspended in RPMI. SP, diluted with RPMI and added to bacterial pellets was incubated at 37°C with shaking for 1 hr, recovered for analysis, or reabsorbed. The supernatant was serially diluted and assayed for inhibition of 3H-thymidine uptake by lymphocytes using sub-optimal concentrations of PHA-P.

**Fig. 1. Inhibition of PHA-P stimulated lymphocytes by 2.8% SP.**

1. SP Control
2. SP x 1hr incubation at 37°C
3. SP x absorbed
4. SP x absorbed
5. RPMI 2 x absorbed

Absorption of SP with 2 pellets reduced inhibition by at least 75%. No significant reduction in inhibition occurred by proteolysis. Tolerance of sperm antigenicity in females (and perhaps males) may reside in sperm being able to bind immunosuppressive factors (6). Removal of this factor would expose sperm to the female immune system and the consequential generation of an immune response. As a number of bacteria are implicated in infertility, the mechanism may be generalised.

References:


Exogenous hormones can produce male behaviour in wethers (1) and oestrous ewes (2) and such treated animals can induce sexual activity in anoestrous ewes. The efficacy of one testosterone (T) preparation has been tested under Australian conditions but no information is available on its rate of disappearance from tissues (residue problem).

The aim of the present experiment was to compare Merino wethers and ewes injected 3 times at weekly intervals with 100 mg testosterone propionate (TP, Despig - Varco, Sharp and Dohme) and wethers injected with 100 mg T from testosterone cyclopentyl propionate (TCP, Bercoat-Cookson) for their efficiency as "teasers" in inducing ovulation and behaviour of castrated rams in anoestrous Merino ewes. Three sheep from each treatment were joined with flocks of 100 ewes for 14 days following the last injection late in October. The incidence of ovulation was examined on day 6 of teasing. Treated wethers were joined with the combined flocks after the teasing period and service records obtained on days 7 and 13. Blood samples were collected by jugular venipuncture from 5 sheep in each treatment group, 5 untreated ewes and 3 vasectomized rams on the last day of T treatment and at intervals thereafter until 84 days later. Sera were analysed for T by radioimmunoassay (Diagnostic Products 125 I kit).

Blood samples were collected by jugular venipuncture from 5 sheep in each treatment group, 5 untreated ewes and 3 vasectomized rams on the last day of T treatment and at intervals thereafter until 84 days later. Sera were analysed for T by radioimmunoassay (Diagnostic Products 125 I kit).

Table 1. Ewes ovulating and in oestrus after teasing and the concentrations of T in sera (mean ± S.D.). Different letters denote significant differences within columns.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ewes ovulated (%)</th>
<th>Ewes served (%)</th>
<th>Testosterone (nmol/l)</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ewes</td>
<td>4</td>
<td>5</td>
<td>7.4 ± 7.6a</td>
<td>0.6 ± 0.2a</td>
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<tr>
<td>Vasectomized rams</td>
<td>67</td>
<td>33</td>
<td>20.9 ± 21.9ab</td>
<td>9.3 ± 11.9b</td>
</tr>
<tr>
<td>TP wethers</td>
<td>52</td>
<td>30</td>
<td>38.4 ± 12.8bc</td>
<td>9.3 ± 14.5b</td>
</tr>
<tr>
<td>TP ewes</td>
<td>44</td>
<td>33</td>
<td>38.3 ± 21.8bc</td>
<td>5.9 ± 4.7b</td>
</tr>
<tr>
<td>TCP wethers</td>
<td>70</td>
<td>36</td>
<td>62.8 ± 21.2c</td>
<td>17.1 ± 29.7b</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Testosterone treated sheep were as effective in inducing ovulation and oestrus as the control vasectomized rams. Serum T concentrations declined rapidly in treated sheep and by day 14 post-treatment were not significantly different to T concentration of the vasectomized rams. This indicates that the 2 T products tested are suitable for the induction of male sexual behaviour without accompanying T residue problems.


In a second experiment, groups of 7 castrates were treated with E at 100 or 900 µg daily plus D at 7.5 or 15 mg daily, while 3 reference groups received T at 15 mg or E at 100 or 900 µg daily, respectively. By 4 weeks, the service scores for the reference groups were 7.8 ± 0.7, 2.4 ± 0.4 and 2.8 ± 0.5, respectively, while the scores for those given E + D were as tabulated below.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>T</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose/day</td>
<td>100µg</td>
<td>7.5mg</td>
<td>900µg</td>
</tr>
<tr>
<td>Service score</td>
<td>2.6 ± 0.5</td>
<td>2.7 ± 0.4</td>
<td>9.1 ± 0.7</td>
</tr>
</tbody>
</table>

The present findings show that, in castrated rams, oestradiol is unable to restore normal levels of complete sexual behaviour unless acting with above-critical levels of a non-aromatizable androgen such as dihydrotestosterone. At such levels dihydrotestosterone

(a) potentiates the effect of otherwise sub-optimal levels of oestradiol,
(b) eliminates refractoriness to otherwise excessive levels of oestradiol.

During the response to electrical stimulation of the autonomic nervous system

Most practical method of administering GnRH for reproductive studies in untrained field-reared bulls is by single-dose intramuscular administration. Information is lacking on the effect of dosage of GnRH on LH and testosterone responses when this route of administration is used. Five zebu x British crossbred bulls 17 months of age and uniform in weight (320 ± 3 kg) were used in a Latin square design to study responses to five dose levels of GnRH: 125, 250, 500, 1000, and 2000 ng GnRH/kg live weight (LWT). All bulls also received a dose of 62.5 ng/kg during an extra period at the end of this study. GnRH (Lutenal, 0.2 mg/ml, generously supplied by Schering-St parcel after injection as a single dose intramuscularly. Plasma samples were collected at 30 minutes from 0 to 3 hours and at 1 hour intervals from 3 to 7 hours post-injection for radioimmunoassay of LH and testosterone. LH and testosterone responses were measured as maximum peak heights or as areas under response curves (Table 1).

### Table 1. Mean (+ S.E.) LH and testosterone responses to GnRH

| Dose of GnRH (ng/kg LWT) | LH | Testosterone |
|--------------------------|-------------------|
|                          | Peak ht. (ng/ml) | Area Units |
|                          | Peak ht. (ng/ml) | Area Units |
| 62.5                     | 2.1 ± 0.5        | 103 ± 20   | 7.7 ± 1.8 | 364 ± 83 |
| 125                      | 7.0 ± 2.4        | 255 ± 95   | 6.6 ± 1.8 | 415 ± 99 |
| 250                      | 12.0 ± 3.2       | 373 ± 94   | 7.5 ± 1.5 | 431 ± 73 |
| 500                      | 12.8 ± 2.5       | 380 ± 43   | 6.3 ± 1.3 | 454 ± 77 |
| 1000                     | 28.3 ± 6.9       | 1064 ± 348 | 7.7 ± 2.3 | 515 ± 105 |
| 2000                     | 25.7 ± 6.2       | 1274 ± 426 | 7.6 ± 1.1 | 602 ± 77 |

Increasing the dosage of GnRH increased the time to reach the peak LH response, the height of the response, the area under response curve, and the duration of the response. The maximum LH peak height was reached by the second highest dose (1000 ng/kg). Areas under the response curves continued to rise through the whole range of doses studied. The log-dose response curve for LH area had a shallow initial component (b = 315) followed by a steeper final component (b = 1485), thus lending support to the two pool theory of LH secretion. In contrast to LH, testosterone responses reached the same peak heights for all doses of GnRH, the only effect of increased dosage being to increase the duration of response. Thus, there were small increases in the area under testosterone response curves with increases in GnRH.

These results have usefulness for determining appropriate dosage of GnRH for studies of relationships between hormone responses and reproductive performance in bulls.

COMPENSATORY HYPERTROPHY OF THE BOAR TESTIS FOLLOWING HEMICASTRATION

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Previous studies (1) have shown compensatory testicular hypertrophy in the rat after hemicastration. The quantitative nature of compensatory hypertrophy in the boar testis was studied in Large White x Landrace males which were hemicastrated at 1, 2, 3, 4, and 5 months of age. The remaining testis was removed 8 weeks later, fixed in Susa, sectioned at 7 μ and stained with haematoxylin and eosin. Quantitative morphological methods were used to determine the length of the seminiferous tubules (1), cross sectional area of the tubular cytoplasm (2) and number of germ and Sertoli cells (4). The ratio of germ and Sertoli cell numbers estimated the Sertoli cell occupancy. Treatment and age effects are shown in Table 1.

Table 1. The effects of hemicastration at different ages on the remaining testis of boars.

<table>
<thead>
<tr>
<th>ITEM CASTRATION</th>
<th>+1</th>
<th>+2</th>
<th>+3</th>
<th>+4</th>
<th>+5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis weight</td>
<td>15.41</td>
<td>44.40</td>
<td>108.06</td>
<td>176.70</td>
<td>231.84</td>
</tr>
<tr>
<td>(g)</td>
<td>30.98</td>
<td>81.96</td>
<td>146.86</td>
<td>279.17</td>
<td>254.13</td>
</tr>
<tr>
<td>Testis wt/100kg</td>
<td>45.83</td>
<td>86.25</td>
<td>154.13</td>
<td>247.49</td>
<td>218.39</td>
</tr>
<tr>
<td>body wt (g)</td>
<td>114.14</td>
<td>170.93</td>
<td>238.52</td>
<td>363.52</td>
<td>267.03</td>
</tr>
<tr>
<td>Tubule length/testis</td>
<td>3.39</td>
<td>4.47</td>
<td>5.53</td>
<td>6.23</td>
<td>6.34</td>
</tr>
<tr>
<td>(μ)</td>
<td>4.69</td>
<td>6.02</td>
<td>7.02</td>
<td>9.23</td>
<td>7.98</td>
</tr>
<tr>
<td>Tubule cytoplasm</td>
<td>3.26</td>
<td>9.28</td>
<td>19.22</td>
<td>22.26</td>
<td>27.97</td>
</tr>
<tr>
<td>(10⁻² μ²)</td>
<td>4.02</td>
<td>12.84</td>
<td>20.18</td>
<td>26.02</td>
<td>30.64</td>
</tr>
<tr>
<td>Germ cell/testis</td>
<td>2.78</td>
<td>26.59</td>
<td>121.36</td>
<td>201.76</td>
<td>379.56</td>
</tr>
<tr>
<td>Sertoli cell/testis</td>
<td>6.59</td>
<td>84.83</td>
<td>233.70</td>
<td>491.47</td>
<td>424.39</td>
</tr>
<tr>
<td>Sertoli cell</td>
<td>9.65</td>
<td>7.06</td>
<td>6.25</td>
<td>11.25</td>
<td>13.23</td>
</tr>
<tr>
<td>(10⁻²)</td>
<td>21.49</td>
<td>9.52</td>
<td>7.79</td>
<td>7.22</td>
<td>15.28</td>
</tr>
<tr>
<td>Sertoli cell</td>
<td>0.35</td>
<td>3.39</td>
<td>30.46</td>
<td>28.67</td>
<td>29.64</td>
</tr>
<tr>
<td>occupancy</td>
<td>0.34</td>
<td>9.96</td>
<td>28.22</td>
<td>21.63</td>
<td>32.57</td>
</tr>
</tbody>
</table>

Significant increases in both raw and normalized testis weight following hemicastration were associated with increases in total length and cytoplasmic area of the seminiferous tubules. There was no difference in the occupancy of Sertoli cells by germ cells as hemicastration resulted in increased Sertoli cell and associated germ cell numbers which were the main factors causing compensatory hypertrophy.


TESTICULAR FUNCTION IN THE MARSUPIAL MOUSE Antechinus minimus maritimus

B.A. Wilson and A.R. Bourne
Biological Sciences, Deakin University, Victoria 3127

Male 'die-off' is a feature of reproduction in most species of Antechinus. Physiological studies of this phenomenon have concentrated on the stress syndrome aspect (1). There is comparatively little information concerning the changes in the testis which precede this event. The present study examines the seasonal change in testicular function in a rare species of marsupial mouse, Antechinus minimus (maritimus).

Animals used in this study were collected by live-trapping from Anglesea and Dartmoor. A minimal number of animals were sacrificed (by an overdose of anaesthetic), and their reproductive organs removed and weighed. Samples of testicular tissue were taken for histological examination and measurement of seminiferous tubule diameter. Biosynthesis of steroids were investigated in vitro by incubating testicular tissue with 14C-progesterone. Conversion products were isolated and identified by chromatographic and microchemical techniques.

Trapping data indicated that male 'die-off' occurred in the Anglesea population in early July. Adult males could not be captured after this date.

Testicular weight and tubule diameter were maximal in May (0.31 ± 0.01g, 0.40 ± 0.01 mm, mean ± s.d.). A rapid regression of the testis then occurred. In July the corresponding values were 0.10 ± 0.02g and 0.24 ± 0.03mm. In contrast the size of the epididymis, prostate, and Cowpers glands was maximal in July.

The major conversion products of biosynthesis were 17α-hydroxyprogesterone, androstenedione, and testosterone. An unidentified polar compound was also isolated. Preliminary evidence indicated that testosterone biosynthesis was low in all months except July.

A. minimus has an earlier breeding season than other species of Antechinus. But in general A. minimus has a reproductive cycle similar to A. stuartii. Regression of the testis occurs prior to mating, which is followed by the 'die-off' of all the males in the population. Histological evidence and testicular weight changes indicate that spermatogenesis starts in March and ends in May.

It appears from in vitro biosynthesis that the spectrum of steroids produced by A. minimus is similar to that of eutherian mammals. The results of this study suggest that in A. minimus the spermatogenic and androgenic cycles are not entirely synchronous.

GLUCOSE METABOLISM IN CULTURED SERTOLI CELLS

M. Cross and A.W. Blackshaw
Department of Physiology and Pharmacology, University of Queensland, St. Lucia, 4067, Brisbane.

Follicle stimulating hormone (FSH) stimulates Sertoli cells in culture to secrete androgen binding protein (ABP) and plasminogen activator, and to aromatize testosterone to oestradiol. The action of FSH is mediated through changes in cyclic nucleotide levels and in the present experiments glucose utilization and related enzyme activities have been used as metabolic correlates of protein synthesis.

Sertoli cells isolated from 18-22 day old Wistar rats were maintained for up to 8 days in Eagles Minimum Essential Medium (1). The activities of hexokinase (HK), phosphofructokinase (PFK), pyruvate kinase (PK), lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6PDH) were measured in freeze-dried samples of cells (Table 2) for up to 96 hr after a single addition of hormones FSH (1 μg/ml), luteinizing hormone (LH), testosterone (T, 1 μg/ml) and cyclic AMP (0.1 mM). Glucose uptake, lactate production and ABP secretion were measured simultaneously in the medium (Table 1) as indicators of Sertoli cell function.

The response to FSH only is given in the tables and shows that over the whole 96 hrs there was an increase in glucose uptake and lactate production, whereas stimulation of ABP synthesis and secretion did not occur until 24-48 hr (Table 1).

LDH (Table 2) and G6PDH activities were increased above zero hour controls by FSH treatment. LDH activity was different by 24 hours after FSH addition (p < 0.01). HK and PK activities were maintained at zero-time levels by FSH while control levels fell to a lower plateau. PFK showed no change in activity with hormone treatment.

LH and T had no effect on glucose uptake, lactate production, LDH and G6PDH activities compared to controls whilst c-AMP stimulated glucose uptake, lactate production and ABP secretion.

TABLE 1. ABP production (cpm x 10^3 DHT/μg DNA)

<table>
<thead>
<tr>
<th></th>
<th>Zero</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
<th>96 hr</th>
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<tbody>
<tr>
<td>Glucose</td>
<td>95.5</td>
<td>189.6</td>
<td>194.3</td>
<td>354.8</td>
<td>404.5</td>
</tr>
<tr>
<td>FSH</td>
<td>283.1</td>
<td>421.7</td>
<td>560.3</td>
<td>661.1</td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>554</td>
<td>1767</td>
<td>2406</td>
<td>3252</td>
<td>3436</td>
</tr>
<tr>
<td>FSH</td>
<td>902</td>
<td>3913</td>
<td>4833</td>
<td>7091</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2. Enzyme activities 10^-3 mole KDH

<table>
<thead>
<tr>
<th></th>
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<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
<th>96 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK</td>
<td>398</td>
<td>538.5</td>
<td>338.1</td>
<td>159.5</td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>249.2</td>
<td>360.1</td>
<td>369.4</td>
<td>409.1</td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td>6622</td>
<td>5238</td>
<td>5670</td>
<td>5222</td>
<td>6009</td>
</tr>
<tr>
<td>FSH</td>
<td>7059</td>
<td>7857</td>
<td>7857</td>
<td>7857</td>
<td>7857</td>
</tr>
<tr>
<td>G6PD</td>
<td>315.8</td>
<td>262.4</td>
<td>192.6</td>
<td>340.8</td>
<td>296.5</td>
</tr>
<tr>
<td>FSH</td>
<td>303.9</td>
<td>439.7</td>
<td>469.5</td>
<td>538.9</td>
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</table>

REFERENCES

LEVDIG CELL HYPERPLASIA AND GONADOTROPIN RESPONSIVENESS FOLLOWING CRYPTORCHIDISM IN THE MOUSE.

S.M.L.C. MANDIS, J.B. KERR, D.M. ROBERTSON AND D.M. DE KRESTSER
Department of Anatomy, Monash University, Clayton, Vic. 3168.

In rats experimentally induced bilateral cryptorchidism (crypt.) results in: a) dysfunctions of the Sertoli cell and cessation of spermatogenesis, b) Leydig cell (Lc) hypertrophy1 with an increase in gonadotrophin-induced testosterone production in vitro. The aim of this study was to see if a similar response occurs in mouse Lcs. Lc size (μm³) was determined at 7, 14, 21, 28 and 38 days after bilateral crypt. in adult (2 month) Swiss mice. Mechanically-separated crude Lc suspensions were fixed in 2.5% glutaraldehyde in cacodylate, embedded in Epon-araldite and thin sections were stained with toluidine blue. The mean cross-sectional area of Lcs from each group (n = 6) was determined by a Leitz image analyser. The Lc size decreased significantly from 252±280 μm³ (mean ± SD) for control groups to 237±71* (7 days), 196±69** (14 days), 166±74* (28 days) and 136±44** μm³ (38 days). In separate experiments testes from 28 day crypt and control mice were fixed by whole body perfusion using a Leits image analyser. The Lc size decreased significantly from 252±280 μm³ (mean ± SD) for control groups to 237±71* (7 days), 196±69** (14 days), 166±74* (28 days) and 136±44** μm³ (38 days).

TABLE 1. Characterization of the normal and cryptorchid mouse Lc.

<table>
<thead>
<tr>
<th>Lc area (μm²)</th>
<th>Volume density of Lc (%)</th>
<th>Testicular Lc volume (μm³ x 10⁷)</th>
<th>Lc No./testis x 10¹⁰</th>
<th>Basal T production</th>
<th>HCG stimulated T production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>252 ± 30 (300)</td>
<td>9.1 ± 0.8</td>
<td>9.7 ± 1.1</td>
<td>6.3</td>
<td>23.4 ± 3.0**</td>
<td></td>
</tr>
<tr>
<td>28 day crypt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>325 ± 33 (300)</td>
<td>9.1 ± 0.8</td>
<td>9.7 ± 1.1</td>
<td>6.3</td>
<td>23.4 ± 3.0**</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.005, compared to corresponding controls.

COMPARISON OF THE IN VITRO BIOLOGICAL ACTIVITY AND ANTERIOR PITUITARY AND TESTICULAR CELL RECEPTOR AFFINITY OF LHRH AND ITS ANALOGUES.

M.P. Hedger, D.M. Robertson and D.M. de Kretser. Department of Anatomy, Monash University, Clayton, Victoria 3168.

It has been shown that luteinizing hormone-releasing hormone (LHRH) and several LHRH analogues bind specifically and with high affinity to testicular interstitial cells. To investigate further the specificity of these receptors, additional analogues were examined for their in vitro biological activity and their relative affinities to receptors in anterior pituitary and testicular interstitial cell preparations.

Biological activity in vitro for LHRH and its analogues were determined by stimulation of LH-release from anterior pituitary cells in monolayer culture. Radioreceptor assays employing pituitary homogenates and intact interstitial cells prepared by collagenase dispersion were used to measure binding affinities (Table 1). Tissue from adult male Sprague-Dawley rats were used throughout the study. The tracer used in the radioreceptor assay was [D-Ser(tBu)6]LHRH which was iodinated by the lactoperoxidase method (specific activity 530±Ci/mg).

Results from duplicate assays (Table 1) indicate that a close correspondence in relative in vitro biological and binding activities was observed with the LHRH agonists except for [D-Lys6]LHRH, assayed with the pituitary homogenate receptor system which gave a higher relative activity.

TABLE 1 Binding affinity and in vitro biological activity of LHRH and some of its structural analogues.

<table>
<thead>
<tr>
<th>Analogue</th>
<th>LHRH</th>
<th>Pituitary</th>
<th>Testis</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHRH</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>[D-Ser(tBu)6]LHRH-NEt</td>
<td>1.00</td>
<td>1.01</td>
<td>1.00</td>
</tr>
<tr>
<td>[D-Leu6,des-Gly NH210]LHRH-NEt</td>
<td>0.88</td>
<td>1.66</td>
<td>0.60</td>
</tr>
<tr>
<td>[D-Ala6,des-Gly NH210]LHRH-NEt</td>
<td>0.10</td>
<td>2.99</td>
<td>0.33</td>
</tr>
<tr>
<td>[D-Lys6,des-Gly NH210]LHRH-TAC</td>
<td>0.04</td>
<td>1.89</td>
<td>0.53</td>
</tr>
<tr>
<td>LHRH (native peptide)</td>
<td>0.01</td>
<td>71.7</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>ED50</th>
<th>R.A.</th>
<th>ED50</th>
<th>R.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ac-Ala1,PC1-D-Phe3,D-Trp3)LHRH</td>
<td>0.08</td>
<td>1.47</td>
<td>0.68</td>
<td>1.16</td>
</tr>
<tr>
<td>(Ac-Pro1,D-Phe4,D-Trp3)LHRH</td>
<td>0.5</td>
<td>0.95</td>
<td>1.06</td>
<td>1.29</td>
</tr>
</tbody>
</table>

*ED50 is the concentration of LHRH/analogue (nM) required to displace 50% of the tracer.
**R.A. = relative activity.

IDENTIFICATION OF HCG ANTIBODIES AFTER CHRONIC DAILY HCG TREATMENT TO ADULT MALE RATS


Previous data from this laboratory have demonstrated that daily hCG treatment to adult male rats for one week results in a loss of LH/hCG receptors and an enhanced production of testosterone (T) in vitro and in vivo (1). After 2 or 3 weeks of such treatment, the administered hCG was apparently no longer biologically active, since both LH/hCG receptor numbers and plasma T levels had returned to control values. The aim of the present study was to determine whether or not antibodies to hCG were responsible for this hormone insensitivity by neutralizing the exogenously administered hCG.

Characterization of the hCG binding protein in plasma was determined by gel filtration (Sephacyr S200) of pools of plasma from control and hCG treated rats. An aliquot of each gel filtration fraction was incubated with [125I]hCG overnight at 4°C, and the [125I]hCG binding protein complex precipitated with either anti-rat IgG serum or polyethylene glycol (PEG). The binding protein complex was located in the IgG region of the chromatogram and precipitated with either anti-rat IgG serum or PEG, demonstrating the immunoglobulin nature of the protein. Detectable levels of plasma hCG binding sites were observed after 2 weeks of hCG treatment, and the Kd of the binding protein estimated to be 7.3 ± 4.8 x 10^-11M (Mean ± S.D., n=4) at 4°C. An LH in vitro bioassay was used to measure non-protein bound biologically active LH/hCG in plasma. After one week of daily hCG treatment the circulating levels of biologically active LH/hCG were elevated compared to controls, but not different from controls after 2 or 3 weeks of hCG treatment; which indicated that the exogenously administered hormone had been neutralized by antibodies in vivo. Finally, the gammaglobulin fraction of plasma from rats treated for 2 or 3 weeks with hCG was shown to neutralize biologically active LH/hCG in vitro.

These data demonstrate the presence of hCG antibodies in sufficient concentrations to explain the restoration of plasma T and LH/hCG receptor numbers to control levels after chronic hCG treatment.

THE CORPUS LUTEUM OF THE BRUSH POSSUM DOES NOT REQUIRE LUTEOTROPHIC SUPPORT

G.H. Tyndale-Biscoe and L. Hinds

Division of Wildlife Research, CSIRO, Lyneham, A.C.T. 2602

In the tammar the corpus luteum (CL) can grow and support pregnancy after hypophysectomy (1) and it is devoid of receptors for LH (2).

In the possum the CL secretes greater amounts of progesterone than the tammar’s from days 4 to 12 post-oestrus (3) and the 17 day pregnancy fails if this is prevented by luteectomy before day 10 (4). The CL of this species possesses abundant LH receptors (2), so may require a luteotropic stimulus to support CL growth and the maintenance of pregnancy.

Seventeen females were hypophysectomised on days 1, 4, 8 or 12 post-oestrus and three sham control operations were performed on day 1.

Adrenals, thyroids, ovaries and CL were weighed and stained with haematoxylin and eosin. The sella turcica was serially sectioned to establish the completion of hypophysectomy.

TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Hypox (failed)</th>
<th>Hypox (sham)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Wt. (kg)</td>
<td>2.3±0.4</td>
<td>2.2±0.4</td>
<td>2.1±0.3</td>
</tr>
<tr>
<td>Thyroids</td>
<td>60±48</td>
<td>350±64</td>
<td>139±34</td>
</tr>
<tr>
<td>Adrenals</td>
<td>200±174</td>
<td>149±10</td>
<td>157±17</td>
</tr>
<tr>
<td>Ovary</td>
<td>65±29</td>
<td>177±65</td>
<td>209±112</td>
</tr>
<tr>
<td>CL</td>
<td>49±10</td>
<td>47±17</td>
<td>36±11</td>
</tr>
</tbody>
</table>

All the pregnant animals carried full term foetuses at autopsy on day 18 (Table 2). However, only the sham control females and one of the failed hypophysectomised females gave birth to a live young; in the others the term foetus was found dead in the uterus, cervix or vaginal canal. In another hypophysectomised female killed on day 15 the CL was alive and at the normal stage of development.

Seventeen females were hypophysectomised on days 1, 4, 8 or 12 post-oestrus and three sham control operations were performed on day 1.

In a second experiment, 12 Booroola and 11 control ewes were bled daily over two consecutive oestrous cycles and then weekly throughout the ensuing pregnancy. Oestrus was detected by vasectomized rams fitted with marking crayons and these were replaced by entire rams after the first oestrous cycle. Ovulation rates were determined by endoscopy during the mid-luteal phase of each oestrous cycle. Progesterone levels in jugular venous plasma were determined by radioimmunoassay.

TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Not Pregnant</th>
<th>Term Foetus</th>
<th>Live Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hypox (failed)</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hypox (sham)</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

The plasma and corpus luteum of ruminants (cows and sheep) contain a albumin and transcortin (1,2,3). Pregnenolone, 5α-pregnandiolone, androgens for binding to the testicular steroid. whereas deoxycorticosterone and cortisol do not (1). The protein is distinguishable from other components and on a column of hydroxylapatite (1,2). This study set out to gestational activity, similar to that found in ruminants, and to determine if this protein was ovarial in origin.

Ovarian plasma was collected by a venous outflow technique (4) from a pregnant-rat-binding activity (K = 9.2 ± 1.7 x 10^4 1/nmol) which, like that of cows and sheep, was resolvable into two components on hydroxylapatite. The first component progesterone (K = 9.2 x 10^4 ± 6.6 x 10^4 1/nmol) and was not adsorbed by hydroxylapatite. The second component, bound eluted from hydroxylapatite at a phosphate ion concentration of 0.04 M, was not adsorbed by hydroxylapatite. This component was collected from the dorsal aorta of sacrificed rats and from rats 16 days pregnant. These plasma contains specific for progesterone as well (K = 100 ± 3 x 10^5 1/nmol) for the ovary of pregnant rats (K = 100 ± 3 x 10^5 1/nmol) for the ovary of pregnant rats (K = 100 ± 3 x 10^5 1/nmol).

Concentrated 20α-hydroxyprogesterone (458), 5α-pregnanolone (402), androstosterone (257) with progesterone (set at 100), 17α-hydroxyprogesterone, 17α-hydroxyprogesterone, and deoxycorticosterone, and androstenedione, cortisol, oestradiol, and cholesterol did not. The progesterone-binding component was present in peripheral plasma (29%) and pregnenolone (25%) completed with progesterone (set at 100) and was not solvable into progesterone (K = 4.1 ± 0.8 x 10^4 1/nmol). It was still present in the dorsal aorta of pregnant rats and from rats 16 days pregnant. These plasma contains specific for progesterone as well (K = 100 ± 3 x 10^5 1/nmol).

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Ovarian plasma was collected by a venous outflow technique (4) from a pregnant-rat-binding activity (K = 9.2 ± 1.7 x 10^4 1/nmol) which, like that of cows and sheep, was resolvable into two components on hydroxylapatite. The first component progesterone (K = 9.2 x 10^4 ± 6.6 x 10^4 1/nmol) and was not adsorbed by hydroxylapatite. The second component, bound eluted from hydroxylapatite at a phosphate ion concentration of 0.04 M, was not adsorbed by hydroxylapatite. This component was collected from the dorsal aorta of sacrificed rats and from rats 16 days pregnant. These plasma contains specific for progesterone as well (K = 100 ± 3 x 10^5 1/nmol) for the ovary of pregnant rats (K = 100 ± 3 x 10^5 1/nmol) for the ovary of pregnant rats (K = 100 ± 3 x 10^5 1/nmol).

Concentrated 20α-hydroxyprogesterone (458), 5α-pregnanolone (402), androstosterone (257) with progesterone (set at 100), 17α-hydroxyprogesterone, 17α-hydroxyprogesterone, and deoxycorticosterone, and androstenedione, cortisol, oestradiol, and cholesterol did not. The progesterone-binding component was present in peripheral plasma (29%) and pregnenolone (25%) completed with progesterone (set at 100) and was not solvable into progesterone (K = 4.1 ± 0.8 x 10^4 1/nmol). It was still present in the dorsal aorta of pregnant rats and from rats 16 days pregnant. These plasma contains specific for progesterone as well (K = 100 ± 3 x 10^5 1/nmol).

Concentrated 20α-hydroxyprogesterone (458), 5α-pregnanolone (402), androstosterone (257) with progesterone (set at 100), 17α-hydroxyprogesterone, 17α-hydroxyprogesterone, and deoxycorticosterone, and androstenedione, cortisol, oestradiol, and cholesterol did not. The progesterone-binding component was present in peripheral plasma (29%) and pregnenolone (25%) completed with progesterone (set at 100) and was not solvable into progesterone (K = 4.1 ± 0.8 x 10^4 1/nmol). It was still present in the dorsal aorta of pregnant rats and from rats 16 days pregnant. These plasma contains specific for progesterone as well (K = 100 ± 3 x 10^5 1/nmol).
PLASMA CONCENTRATIONS OF PROGESTERONE AND OESTRADIOL ABOUT OESTRUS IN RED DEER HINDS

B.W. Kelly, K.P. McNatty and G.H. Moore
Invermay Agricultural Research Centre, Mosgiel and Wallaceville Animal Research Centre, Upper Hutt, New Zealand.

In recent years red deer in New Zealand have been investigated for their potential as a farmed animal (1), and more than 104,000 are held in captivity. Red deer are seasonal breeders, with mating commencing in autumn. While much is known about the social and sexual activity of red deer, hormonal changes in the hind during the breeding season have not been documented. Peripheral blood samples were collected once daily from 10 hinds during the period of mating. In five of these hinds mating marks were able to be confirmed either by the presence of spermatozoa in a vaginal swab or by swelling and/or calving about 233 days later. Ovulation rate to this mating, as recorded by laparoscopy, showed that all hinds had a single corpus luteum visible on the ovarian surface. The mean and range of plasma concentrations for progesterone and oestradiol during the nine days about mating for these five hinds are summarised in Table 1.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Day about mating (day 0=mating mark recorded)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-4</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>mean</td>
</tr>
<tr>
<td></td>
<td>range</td>
</tr>
<tr>
<td>Oestradiol (pg/ml)</td>
<td>mean</td>
</tr>
<tr>
<td></td>
<td>range</td>
</tr>
</tbody>
</table>

The pattern of oestradiol concentrations about mating is similar to that recorded in other polyoestrous ungulates, with peak levels near the time of mating. However, the concentration is four to ten-fold higher than that recorded in the cow or ewe, and indeed the upper range of values recorded is more comparable with that recorded in primates (2). One of the functions of these high levels of oestradiol may be to overcome the antagonistic action of progesterone with regard to behavioural oestrus, since elevated progesterone concentrations (> 1 ng/ml) can occur within a day of mating. The source and function of the high progesterone concentrations about mating are obscure. They may be related to the accessory corpus luteum phenomenon in red deer hinds (3), an event that requires elucidation in studies on hormonal changes during the oestrous cycle.

The successful in vitro fertilization and embryo transfer in the stimulated ovulatory cycle (1) has led, under some circumstances, to the need to store human preimplantation embryos. This need arises when a number of embryos develop from a single collection of oocytes or when problems prevent embryo transfer in the cycle of oocyte recovery.

Human embryos resulting from in vitro fertilization and culture were frozen to -30°C (rapid thawing) or to -55°C to -80°C (slow thawing) in order to assess their suitability for frozen preservation. The embryos used in this study were from patients from whom two or more embryos developed from a single collection of oocytes following follicle stimulation with clomiphene citrate and the timing of oocyte recovery with hCG.

A total of 14 human embryos were frozen at three developmental stages. Some embryos were fixed immediately after thawing and processed for electron microscopy (EM) while others were cultured by methods previously described (2) prior to fixation for EM. Two embryos which appeared morphologically normal after thawing were transferred immediately (see Table 1).

Table 1. Treatment of human embryos after thawing

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. embryos</th>
<th>Slow Fast</th>
<th>Post thaw treatment</th>
<th>Accl Fx 12 hr. Culture</th>
<th>Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-cell</td>
<td>4</td>
<td>1 2</td>
<td>3 1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>8-cell</td>
<td>5</td>
<td>3 5</td>
<td>2 1</td>
<td>2 1</td>
<td>1</td>
</tr>
<tr>
<td>Morula</td>
<td>5</td>
<td>5</td>
<td>2 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>E.Blast.</td>
<td>5</td>
<td>5</td>
<td>2 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>14</td>
<td>9 5</td>
<td>8 4</td>
<td>2 2</td>
<td>2</td>
</tr>
</tbody>
</table>

Human embryos frozen at the morula to early blastocyst stage showed a variable proportion of cells appearing morphologically normal after thawing. One morula frozen to -90°C showed excellent structural cryopreservation while another frozen to -80°C developed in post-thaw culture to form an ultrastructurally abnormal blastocyst. No human embryos frozen at the 4-cell stage survived after either rapid or slow thawing, although the embryos failed to continue development when cultured after thawing and all showed severe damage to cell membranes and organelles on ultrastructural analysis. Human embryos frozen at the 8-cell stage and thawed rapidly did not survive, they morphologically resembled the frozen 4-cell embryos and also failed to continue development in culture. One 8-cell embryo frozen to -90°C and thawed rapidly appeared normal with all blastomeres intact. This embryo was transferred without any subsequent pregnancy.

Our results indicate that there may be a stage dependent sensitivity to cryoinjury in human embryos, as exists in embryos of the cow and sheep (3). 4-cell human embryos fail to survive after fast or slow thawing, but some survival can be achieved when embryos of 8-cell to early blastocyst stage are frozen.

References:

INCORPORATION OF 14C-GLUCOSE INTO INNER CELL MASSES ISOLATED FROM MOUSE BLASTOCYSTS BY IMMUNOSURGERY

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School of Veterinary Studies, Murdoch University, W.A. 6150

The two cell types, inner cell mass (ICM) and trophoblastic cells formed during differentiation of the embryo show differential synthesis of glycogen (1). The present experiment has been carried out to study the metabolism of glucose by ICM isolated from mouse blastocysts.

Mouse blastocysts were collected at 13.00 - 14.00 hr on day 4 of pregnancy (day 1 = detection of plug) and zona pellucida was removed using pronase. The ICMs were isolated immunosurgically from one half of the embryos. Following isolation, the ICMs were washed and cultured for 24 hr in 0.28mM, 14C-glucose (ICM1). The remaining half of the embryos, were cultured for 24 hr in 14C-glucose and the ICMs isolated at the completion of culture (ICM2). The ICMs obtained from the two groups of embryos were then fractionated into their major biochemical components. For comparison with the incorporation of isotope into ICMs, intact blastocysts at the same stage of development were cultured for 5 hr and fractionated as above.

TABLE 1. Incorporation of 14C-glucose into various biochemical fractions of ICMs and intact blastocysts. Mean of 3 replicates ± S.E.M.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Glucose carbon incorporated into (pg atoms/embryo)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid</td>
</tr>
<tr>
<td></td>
<td>soluble</td>
</tr>
<tr>
<td>ICM1</td>
<td>4.342±0.08</td>
</tr>
<tr>
<td>ICM2</td>
<td>0.81±0.10</td>
</tr>
<tr>
<td>Blastocyst</td>
<td>3.36±5.50</td>
</tr>
</tbody>
</table>

Very little glucose carbon was incorporated into all the biochemical fractions of the ICM, when compared to incorporation into whole blastocysts. ICMs isolated prior to culture incorporated more isotope into all fractions especially acid-soluble glycogen than ICM isolated from cultured blastocysts.

The results indicate that as the embryo differentiates the trophoblastic cells are much more metabolically active than those of the inner cell mass. The greater accumulation of acid soluble glycogen by ICMs isolated prior to culture could be due to their contamination with trophoblastic cells when immunosurgery is carried out at this early stage of differentiation. During 24 hr in culture the differentiation of the embryo is complete and therefore pure ICM could be recovered at this stage and give a more accurate estimate of incorporation of glucose carbon into this tissue.

The finding that inner cell masses incorporate little glucose into acid-soluble glycogen is in agreement with earlier autoradiographic studies (1).

References:
AMINO ACID TRANSPORT DURING PREIMPLANTATION DEVELOPMENT

P.L. Kaye, H.B. Pratt, M.H. Johnson, R.S. Church and G.A. Schultz
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During development from egg to blastocyst—a process which may occur in vitro in the absence of amino nitrogen source—the protein content of the mouse embryo changes little. However, a number of observations have shown the existence of specific mechanisms for uptake of amino acids (1). We have examined methionine transport by mouse embryos during development and find specific systems of uptake and exchange which diversify as development proceeds.

Eggs and embryos were collected from superovulated mice as described (2). The accumulation of 35S-methionine from medium containing various concentrations of methionine and/or other amino acids was measured by scintillation spectrometry. Amino acid pools were determined by automatic amino acid analysis (2).

The rate of net uptake of 35S-methionine by eggs, fertilized one-cell and 2-cell embryos was similar, slowing to zero after 1 hr in 100 μM methionine. Later developmental stages showed greater uptake rates so that blastocysts still accumulated 35S-methionine after 2 hr despite achieving an internal concentration of almost 15 μM. 35S-methionine uptake by blastocysts was inhibited by a greater variety of amino acids than that of eggs. Methionine uptake became partially dependent on Na by the compacted morula stage. Efflux of pre-accumulated 35S-methionine was shown to be by exchange. This exchange did not occur in media lacking amino acids. However, when methionine at levels as low as 10 μM was added, a rapid equilibration took place. The rate and extent of equilibration was lower in blastocysts than in eggs and was concentration dependent. This may be related to morphology since embryos which were prevented from expansion by cytochalasin D treatment or blastocysts collapsed by cytochalasin D showed equilibration to levels comparable to those of late morulae. A bi-directional transport system for methionine was observed at all developmental stages. Such a system would enable the early embryo to maintain an internal pool of methionine.

The results confirm that the mechanisms of amino acid transport become increasingly complex as development proceeds. The reduced exchange by blastocysts and its dependence on the formation of permeability barriers suggest the development of a topographical asymmetry of methionine transport systems. Perhaps the blastocyst accumulates amino acids through trophoblast cells to the blastocyst fluid from which they may move freely to embryonal cells as required, but are restricted from returning to the environment, so providing the implanting embryo with a storage pool of amino acids.


PREGNANCY ASSOCIATED PROTEINS IN THE MARE

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Department of Veterinary Physiology and Department of Obstetrics and Gynaecology, University of Sydney

An antiserum raised against pregnant mare serum was absorbed with stallion serum. This antiserum then gave 2 precipitates in a crossed immunoelectrophoresis system using pregnant mare serum as the antigen. The 2 precipitates showed different electrophoretic characteristics with one being located in the a2 region and the other in the β1 region.

Tandem crossed immunoelectrophoresis using pregnant mare serum and a commercial preparation of PMSG showed identity between the a2 mobile precipitate and an antigen in the commercial PMSG preparation. The absorbed antiserum used in these studies also inhibited the biological action of the commercial PMSG preparation and pregnant mare serum when tested in mouse uterine and ovarian weight assays.

Affinity chromatography has confirmed that the antigen in pregnant mare serum identified as PMSG is a glycoprotein and further has demonstrated that this is a heterogeneous substance. Biological tests showed that the heterogeneous fractions have a different biological potency.

The β1 mobile protein appears to be a pregnancy associated protein which has not been previously described in the mare.
RESIDUAL EFFECTS OF LOW NUTRITION DURING EARLY PREGNANCY IN THE EWE

R.A. Parr, A.H. Williams and I.P. Campbell

Department of Agriculture, Animal Research Institute, Werribee, Australia.

Embryonic growth and development is retarded when pregnant ewes lose liveweight during the first five weeks of gestation (1). Whether this retardation is permanent even when adequate nutrition is subsequently available, has not been determined. Studies have been conducted to look at foetal size and weight, and foetal and maternal blood glucose at Day 90 of gestation in ewes which were fed either 50% or 150% of a maintenance ration (0.5M or 1.5M) from mating until Day 35 post coitum (p.c.). Merino ewes (n=100) were joined to one Border Leicester ram and if inseminated, each ewe was individually penned on Day 1 p.c. and thereafter fed the respective ration. During this period and at Day 90 p.c. plasma samples were collected for glucose analysis. On Day 35 p.c. embryos were removed from 60 ewes and the remainder (n=40) were returned to pasture until Day 90 p.c. when each single foetus was removed for measurement.

Mean liveweight changes between Day 1 and 35 p.c. were +4.9kg and +2.0kg. in the 0.5 and 1.5M groups respectively. There were no liveweight differences between the two groups at Day 90 p.c. There were no differences in foetal size between sexes within nutrition groups thus these sets of data were pooled to compare foetal size between nutrition groups.

Table 1: Mean (+s.e.m.) embryo and foetal fresh weight and size

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NUTRITION</th>
<th>RATE</th>
<th>FRESH WT.</th>
<th>CROWN RUMP</th>
<th>CHIN-CROWN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 35 p.c.</td>
<td>(g)</td>
<td>(mm)</td>
<td>(mm)</td>
<td>(mm)</td>
<td>(mm)</td>
</tr>
<tr>
<td>0.5M</td>
<td>1.86±0.05(23)*</td>
<td>29.3±0.17</td>
<td>512.1±11.00(12)</td>
<td>7.99±0.09</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td>0.5M</td>
<td>1.65±0.04(20)</td>
<td>28.3±0.34</td>
<td>467.6±17.11(13)</td>
<td>7.64±0.11</td>
<td>P&lt;0.025</td>
</tr>
<tr>
<td>SIGNIFICANCE</td>
<td>N.S.</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Footnotes in parenthesis are total number of embryos/foetuses in each group.

The differences in foetal size and weight between treatments were still evident at Day 90 p.c. Mean plasma glucose concentrations were reduced in the 0.5M nutrition group during the 35 day treatment period (56.08±0.58 vs 61.07±0.64mg/100ml, P<0.001). At Day 90 p.c. this difference was reflected in maternal peripheral (56.08±0.58 vs 61.07±0.64mg/100ml), umbilical venous (27.73±2.55 vs 32.23±3.50mg/100ml) and umbilical arterial plasmas (19.4±2.55 vs 28.7±4.44mg/100ml) from the 0.5M and 1.5M nutrition group ewes and foetuses respectively.

It is possible that the long term influence of this retardation could be expressed in terms of later productivity. This will depend on the animal's capacity for compensatory growth and development during the remainder of pregnancy and post-natal life.

The use of the high fecundity Booroola on commercial farms in New Zealand (1) has led to a need to clarify the factors which contribute to lower growth rates of multiple reared lambs. There have been no reports studying lambs older than 4 weeks or of lambs from large litters (3) where cross-sucking and intra-litter competition may become of importance to lamb milk intake and hence growth rate.

The sucking behaviour of lambs from two ewe flocks (n=101,121) each consisting of approximately equal numbers of Romney and Booroola x Romney first cross ewes were observed for a 6 hour period at 3.6,9 and 12 weeks of age and the frequency of sucking and incidence of cross-sucking was recorded. Lambs were weighed after each period of observation. The results of each of the two flocks did not differ significantly and so have been combined (Table 1).

Table 1: Sucking behaviour of lambs from Romney and Booroola x Romney first-cross ewes for the 4 observation periods.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Romney</th>
<th>Booroola x Romney</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of lambs</td>
<td>Single</td>
<td>Twin</td>
</tr>
<tr>
<td>Frequency of sucking/6 h</td>
<td>1.15</td>
<td>0.92</td>
</tr>
<tr>
<td>(range)</td>
<td>0.94</td>
<td>0.78</td>
</tr>
<tr>
<td>% Cross-sucking</td>
<td>1.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Mean lambs sucking/6 h</td>
<td>63.3</td>
<td>67.1</td>
</tr>
<tr>
<td>Mean lambs sucking only once in total of 24 h observation</td>
<td>8.5</td>
<td>8.9</td>
</tr>
<tr>
<td>Mean growth rate to 12 weeks (g/day)</td>
<td>235</td>
<td>214</td>
</tr>
</tbody>
</table>

Mean sucking frequency of single and twin lambs varied with breed (P<0.05). Booroola singles sucking less frequently, and twins more frequently than Romney lambs with similar birth rank. Triplets sucked less often than lambs of other birth ranks (P<0.05). Cross sucking frequency was lower.

Sucking frequency observed in this study was low in comparison with other reports, e.g., single reared lambs had a sucking frequency about one third that reported elsewhere (2). Nevertheless growth rates of lambs were high, and within rearing rank growth rate was not associated with sucking frequency.

The percentage of lambs (40%) were observed not to suck during any one observation period, and over the total of 24 h observation 4.0 - 20.4% of lambs within rearing ranks sucked only once. This suggests that many lambs, particularly triplets, may be self-weaning at an early age. Further studies are needed to clarify this pattern of sucking frequency, as it may well be altered by such factors as lambing management and stocking rate.
One significant way of increasing sow productivity is to decrease the length of the lactational anoestrus. A successful procedure for breeding sows during lactation has been demonstrated by Hausler et al. (1980), and similar work has been repeated here in Australia.

A field trial was conducted in a large commercial piggery where weaning occurred at 4 weeks. One hundred and five sows were naturally mated on the first morning of their first oestrus after weaning their piglets. Only mature boars were used for groups IV and V.

One hundred and five sows were allocated to five groups. Groups I, II and III were given 1500 i.u. PMSG (Pregnant Mare Serum Gonadotrophin) subcutaneously at 10, 15 or 20 days post-partum respectively. Ninety-six hours later they were given 1000 i.u. HCG (Human Chorionic Gonadotrophin) intramuscularly, and then inseminated at 24 and 36 to 42 hours post-HCG without detection of oestrus. Fresh boar semen was collected and examined microscopically for motility and concentration. Beltsville diluent was added to aliquots of 5 x 10⁹ sperm per insemination. On some days semen for group V was provided by III sows collected from young boars of unproven fertility because no mature boars were available. Groups IV and V acted as untreated controls, with group IV sows being artificially inseminated and group V sows naturally mated on the first and second mornings of their first oestrus after weaning their piglets. Only natural oestrus were used for groups IV and V.

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LH RELEASE AND LUTEAL FUNCTION IN POST-PARTUM EwES AFTER THE PULSATILE ADMINISTRATION OF LH-RH

P.J. Wright, *P.E. Geytenbeek, †J.J. Clarke and ‡J.K. Findlay
Department of Veterinary Clinical Sciences, University of Melbourne, Werribee; *Waite Agricultural Research Institute, Adelaide; †Medical Research Centre, Prince Henry's Hospital, Melbourne.

Ovarian acyclicity in post-partum ewes may result from failure of development of ovarian follicles due to inadequate secretion of LH, which in turn reflects inadequate release of LH-RH (1). Ayclic Merino ewes 26-30 days post partum (n=14) were treated with LH-RH in a regimen (100 ng LH-RH i.v. each h for 46 h commencing at time 0 h) designed to stimulate LH release similar to that associated with pre-ovulatory follicular development in cyclic ewes (2). The patterns of LH release and of subsequent progesterone secretion were assessed and compared with those associated with follicular development, ovulation and luteal function in normal cyclic ewes (n=10), that had been treated with cloprostenol (125 pg i.m. at -12 h) during mid dioestrus. Blood samples for LH determination were taken via jugular venous cannulae each 3 h from 0 to 72 h; in addition, samples were also taken each 20 min during two periods each of 3 h commencing at 0 h (Period 1) and at 21 h (Period 2). Subsequent luteal function was assessed from plasma progesterone levels in blood samples taken by jugular venepuncture each 2-3 days for the following 4 weeks.

During Periods 1 and 2, relative to the cyclic ewes the post-partum ewes had higher mean (+ s.e.m.) plasma LH levels (7.8 ± 1.1 and 0.3 ± 0.4 vs 3.8 ± 1.3 and 3.5 ± 0.5 ng/ml; P < 0.01), and LH pulses of greater frequency (3.0 ± 2.4 ± 0.3 and 2.4 ± 0.2 pulses/3 h; P < 0.01) and amplitude (7.4 ± 0.8 and 3.8 ± 0.5 vs 4.4 ± 0.7 and 3.4 ± 0.9 ng/ml; P < 0.01). A plasma LH surge occurred in 12/14 post-partum ewes commencing at 31 ± 1.9 h. All the cyclic ewes showed an LH surge commencing at 60 ± 2.8 h after cloprostenol treatment. The post-partum and the cyclic ewes had LH surges of similar (P > 0.05) duration (15 ± 0.0 ± 0.8 and 16.1 ± 1.5 h) and with similar (P > 0.05) peak values (197 ± 37 and 142 ± 24 ng/ml). Subsequently in the post-partum ewes plasma progesterone was undetectable (<0.1 ml/ml; n=6) or present at low levels (1.3 ± 0.2 ml/ml; n=6) for short periods (<9 days). In contrast, the cyclic ewes had mean plasma progesterone levels of 2.4 ± 0.2 ng/ml for >12 days.

These results show that the pituitary of acyclic post-partum ewes has the capacity to respond to LH-RH over an extended period of time. Normal ovarian responsiveness to LH in terms of oestrogen production can be inferred from the occurrence of LH surges similar to those seen in normal cyclic ewes. We suggest that the inadequate luteal function in the post-partum ewes was due to an inappropriate frequency, amplitude and duration of LH pulses prior to the LH surge.


ARTIFICIAL INDUCTION OF LACTATION: THE IMPORTANCE OF GLUCOSE

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In ewes induced to lactate artificially it appears that glucose supply to the mammary gland is rate limiting for milk production for several days after milking commences (2). The present study was conducted to examine whether infusions of glucose given at the commencement of lactation would increase milk production in hormone-treated ewes.

Ten nulliparous crossbred ewes were maintained in metabolism cages and fed a pelleted ration containing lucerne chaff and barley. Lactation was induced artificially by developing mammary glands over 60 days with subcutaneous injections of oestrogen plus progesterone then initiating milk secretion with intravenous injections of oxytocin given over 5 days (trigger phase) as described previously (1). Regular hand milking commenced on the first day of the trigger phase.

All ewes were fed sufficient energy to meet their requirements for maintenance until the commencement of the trigger phase when feed intake was increased to provide sufficient energy to sustain a milk yield of 500 g/d (4). Throughout the trigger phase 5 ewes were given continuous intravenous infusions of glucose at a rate estimated to increase glucose irreversible loss to the same as ewes secreting 500 g/d milk. The remaining 5 ewes were given continuous intravenous infusions of saline during the trigger phase.

Indwelling catheters were fitted in both jugular veins of each ewe 5 days before the trigger phase commenced. Blood samples collected before and for 10 days after commencement of the trigger phase were assayed for plasma glucose and insulin. Insulin and glucose concentrations were measured by isotope dilution (3) in 3 ewes from each group on the last day of the trigger phase. Milk yields were recorded daily.

Milk yields of ewes infused with glucose were significantly higher (P<0.05) than those of control ewes throughout the first 10 days of lactation. During the first 2-3 days of the trigger phase plasma insulin and glucose concentrations were significantly higher (P<0.05) for glucose-infused than control ewes. Thereafter values were similar for both groups of ewes. On the last day of the trigger phase glucose irreversible loss was significantly higher (P<0.05) than for glucose-infused control ewes, but glucose pool size was similar in both groups.

It seems reasonable to conclude that glucose supply is rate limiting for milk production during the first few days of artificially-induced lactations.

In the bovine blastocyst, the (Jersey) cows. Thus at Canberra the *indicus* and at Gatton it was blastocysts and embryos were transferred to *Bos indicus* (Brahman) cows inseminated 7 days earlier with *Bos indicus* semen. At Gatton, Queensland, *Bos indicus* (Brahman) embryos were transferred to previously inseminated *Bos taurus* (Jersey) cows. Thus at Canberra the *indicus* embryo was ipsilateral to the corpus luteum and at Gatton it was contralateral.

From 109 transfers to inseminated cows, 38 twin pregnancies were diagnosed at 7-8 weeks (Table 1). Of these 13 produced twin calves, 4 produced one calf and 11 failed to produce a calf at term. Eight pairs were healthy and at 6-7 months gestation are considered to be carrying twins. Of the complete failures, 2 cows died after pregnancy test and 11 experienced resorption or abortion before 8 months of gestation. Almost invariably the *indicus* member of twins would not have survived without intensive care. The data in Table 2 indicate that in twin pregnancies, the time of parturition was determined by the foetus with the shortest expected duration of gestation even when this was in the contralateral horn of the uterus, and suggest that poor viability of the *indicus* twin was due to relative prematurity at birth.

The durations of gestation and plasma progesterone levels immediately after parturition indicate that a foetus in the uterine horn contralateral to the corpus luteum can induce luteolysis and a normal parturition. Clearly there is a mechanism of foetal induction of luteolysis not dependent on the local unilateral route taken by PGF2a. Successful use of embryo transfer in twinning programmes may require the use of genotypes whose calves have similar expected durations of gestation.

**TABLE 1. Establishment and survival of mixed species pregnancies in *Bos indicus* and *Bos taurus* cows.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Donor</th>
<th>Embryos</th>
<th>Pregnancies (7-8 wks)</th>
<th>Calves at term to twin preg.</th>
<th>Twin to twin preg.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bos indicus</em></td>
<td>49</td>
<td>31</td>
<td>19</td>
<td>7</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td><em>Bos taurus</em></td>
<td>60</td>
<td>19</td>
<td>19</td>
<td>6</td>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>

**TABLE 2. The effect of genotype of dam and foetus on duration of pregnancy.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Gestations</th>
<th>Duration Days (S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bos indicus</em></td>
<td>7</td>
<td>282.4 (2.3)</td>
</tr>
<tr>
<td><em>Bos indicus</em></td>
<td>6</td>
<td>295.3 (1.0)</td>
</tr>
<tr>
<td><em>Bos taurus</em></td>
<td>3*</td>
<td>286.0 (2.6)</td>
</tr>
<tr>
<td><em>Bos indicus</em></td>
<td>6</td>
<td>283.2 (2.9)</td>
</tr>
<tr>
<td><em>Bos taurus</em></td>
<td>9</td>
<td>282.3 (1.1)</td>
</tr>
<tr>
<td><em>Bos indicus</em></td>
<td>2*</td>
<td>292</td>
</tr>
</tbody>
</table>

* Contralateral pregnancies
A SERUM FACTOR IN PREGNANT PIGS DETECTED BY A ROSETTE INHIBITION TEST

CSIRO, Division of Animal Production, Prospect, NSW, Australia

It has been reported that a rosette inhibition test (RIT) can be used to detect the appearance of an early pregnancy factor in serum within 6 h of fertilization in mice (1) within 1 h in sheep (2) and at 4 weeks in humans (3). We have developed a rosette test in pigs and have looked for the appearance of a similar factor in serum during pregnancy which influences this test.

Pig peripheral lymphocytes form 20% to 40% spontaneous rosettes with sheep red blood cells. The rosette formation can be inhibited by incubating lymphocytes with anti-pig lymphocyte serum (ALS) in the presence of complement. By using different dilutions of ALS (1/1000 to 1/8000 or higher) a dose response of rosette numbers against ALS concentration can be obtained.

A group of 11 sows were mated and serum samples were obtained prior to mating and at weekly intervals after mating. The influence on the RIT of prior incubation of pig lymphocytes with the sera was investigated. Subsequent examination of the 11 sows at 9 weeks gestation by an ultrasound method showed that 5 of the pigs were pregnant. In all animals the sera collected 1 and 2 weeks after mating had no effect on the RIT. In 2 of the pregnant animals sera obtained 3 and 4 weeks after mating influenced the RIT. That is, at either or both the 1/2000 and 1/4000 dilutions of ALS, the number of rosettes obtained was below 75% of that obtained with a pre-mating serum tested at the same concentration of ALS. In the other 2 pregnant animals only serum obtained at 4 weeks influenced the RIT while in the remaining animal sera obtained at either 3 or 4 weeks had no effect on the RIT. None of the sera collected from the 6 non-pregnant animals during the 4 weeks after mating influenced the RIT.

Since no effect on the RIT was detected in sera obtained from pregnant animals 1 or 2 weeks after mating, it is unlikely that the same serum factor as described in mice and sheep is responsible, but another factor similar to that detected in the human system is present.

Both the type of rosettes and the response were similar to those observed in the human system.


RATE OF BLOOD FLOW TO THE CORPORA LUTEA OF PREGNANCY AND TO THOSE OF PREVIOUS CYCLES THROUGHOUT PREGNANCY IN THE RAT

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The biological significance of the high rate of blood flow to the mammalian ovary remains uncertain. In the rat, there appears to be a large physiological reserve in that total ovarian blood flow can be substantially reduced without affecting progesterone secretion and progesterone secretion increased without affecting flow (4). Furthermore, near term when progesterone secretion rates are declining, total ovarian flow actually increases (2).

In the present study, radioactive microspheres were used to examine the rate and distribution of blood flow to the various tissues of the ovary throughout pregnancy. By using different dilutions of ALS (1/1000 to 1/8000 or higher) a dose response of rosette numbers against ALS concentration can be obtained.

Six or seven Albino Wistar rats were examined on each of Days 4, 7, 10, 13, 16 and 22 of gestation (Day 1 is the day sperm were found in a vaginal smear; rats in this colony normally litter on Day 23 of gestation). Rats were anaesthetized with sodium pentobarbitone, microspheres (15um diameter, Pharmacia Chemicals) were injected into the inferior caval vein and injected with radioactive microspheres. Ovarian tissues were dissected out, corpora lutea of previous cycles were separated from those of pregnancy and both groups were weighed to the nearest 0.1 mg and their radioactivities measured to estimate blood flows.

Individual CL of pregnancy increased rapidly in weight from Day 10 (1.5 ± 0.1 mg) to Day 16 (4.5 ± 0.1 mg) and then levelled off to Day 22 (4.2 ± 0.2 mg). Of particular significance was the finding that over Days 16 to 22 when progesterone secretion declines rapidly (2) CL blood flow actually increased from 973 ± 33 ml.min−1.100g−1 to 1336 ± 70 ml.min−1.100g−1. Thus, there was no evidence that a redistribution of ovarian blood flow initiated luteolysis.

Individual CL of previous cycles also increased in weight between Days 4 (0.5 ± 0.06 mg) and 16 (1.6 ± 0.3 mg) but then declined markedly to Day 22 (0.5 ± 0.06 mg). The CL of previous cycles received a high rate of blood flow which increased throughout pregnancy from 220 ± 49 ml.min−1.100g−1 at Day 4 to 2515 ± 601 ml.min−1.100g−1 at Day 22. Together, these findings suggest that CL of previous cycles might be more biologically significant than hitherto appreciated. Present work is directed at their histological characteristics.

DIFFERENTIATION OF PREGNANCY AND NON-PREGNANCY IN PIGS FROM PLASMA OESTRONE SULPHATE LEVELS

R.I. Cox, Y.S. Pan, M.S.P. Wong and R.M. Hoskinson

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Oestrone sulphate (E1S) concentrations rise substantially in the peripheral blood of the pregnant sow in the third and fourth week of gestation. They are 1.28 ± 0.24 ng/ml plasma (mean ± s.e.) 22 days after mating and reach maximal levels of 5.86 ± 1.2 ng/ml on day 29, whereas non-pregnant sows have levels below 0.1 ng/ml (1). The steroid derives from oestrone synthesised by the blastocyst. Measurement of E1S may be useful for pregnancy diagnosis, or even more significantly for detecting those animals which are not pregnant some 3-4 weeks after mating. The accuracy of an E1S test in such diagnosis was evaluated on 1,213 pigs in this study.

Sows or gilts were mated between February and May, 1980. Blood samples were taken from animals between day 23 and 31 after mating from the right external jugular vein. E1S was measured directly in the plasma by radioimmunoassay (1). Oestrus was detected using a boar and a riding test.

Based on the E1S data for normal pregnancy previously reported (1) four assessments were made and compared subsequently with the percentage of pigs farrowing in 1,213 pigs.

<table>
<thead>
<tr>
<th>E1S (ng/ml)</th>
<th>Assessment of E1S Level</th>
<th>Number of Pigs in Group</th>
<th>% of Group Farrowing</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.1</td>
<td>non-pregnant range</td>
<td>163</td>
<td>1</td>
</tr>
<tr>
<td>0.1-0.3</td>
<td>grossly low for pregnancy</td>
<td>39</td>
<td>10</td>
</tr>
<tr>
<td>0.31-0.70</td>
<td>low for pregnancy</td>
<td>48</td>
<td>69</td>
</tr>
<tr>
<td>&gt;0.71</td>
<td>normal pregnancy levels</td>
<td>963</td>
<td>94</td>
</tr>
</tbody>
</table>

None of these animals had shown oestrus after mating and by the time the blood sample was taken. The predictive assessment of the E1S levels relates well to the percentage of sows farrowing and the definition of the non-pregnant group is particularly good. Only a small percentage of sows with E1S between 0.1 and 0.3 ng/ml maintained pregnancy.

Diagnosis of pregnancy in pigs after 30 days gestation with ultrasonic equipment can be of a similar accuracy to the E1S test but is not effective for detecting the non-pregnant animal in contrast to the high accuracy of E1S assessment. Thus measurement of E1S in 1,213 pigs after mating has been demonstrated to be an effective procedure for detecting animals that are not pregnant and has possible applications to pig management and research on infertility.


HPLC DETERMINATION OF SPECIFIC RADIOACTIVITY OF LACTATE IN PLASMA

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The supply and utilization of glucose and lactate can be studied in the chronically catheterized fetus and ewe by infusing 14C-labelled substrates and determining their specific activity in plasma. 14C-labelled metabolites have been separated by ion-exchange (1) or paper (2) chromatography or by derivitization and extraction from plasma (3) and radioactive content determined. Plasma concentrations were then measured by enzymatic analysis. This approach is time consuming and requires relatively large sample volumes. These experiments were designed to develop a more rapid method for determining the specific radioactivity of plasma lactate.

Reverse phase high pressure liquid chromatography (HPLC) was used to achieve separation. Macromolecular and hydrophobic components were removed from plasma before HPLC by TCA precipitation and washing the supernatant though a C18 SEP/PAK cartridge at pH 2.7. TCA was removed by extraction with diethyl ether. The aqueous phase was recovered and lyophilized.

Plasma extracts (dissolved in 100 µl of 0.01M phosphate buffer of pH as mobile phases) and lactate standards were chromatographed at 2 ml/min on a 4.6 x 250 mm C18, 10µm, column (Waters) with a mobile phase of 0.01M phosphate buffer of pH range 2.3, to 3.5. 1-5µl samples were injected. Eluent absorbance was monitored at 190 nm or 220 nm. As little as 0.5 µg lactate was readily detected under these conditions.

Fractions were collected for determination of 14C content where 14C-lactate and/or 14C-glucose had been added to plasma prior to extraction. At pH 2.7, a peak of similar retention to that of the lactate standard was completely resolved from neighbouring peaks in plasma extracts. Addition of lactate to plasma extracts resulted in an increase in peak area with no change in retention.

None of these animals had shown oestrus after mating and by the time the blood sample was taken. The predictive assessment of the E1S levels relates well to the percentage of sows farrowing and the definition of the non-pregnant group is particularly good. Only a small percentage of sows with E1S between 0.1 and 0.3 ng/ml maintained pregnancy.

Diagnosis of pregnancy in pigs after 30 days gestation with ultrasonic equipment can be of a similar accuracy to the E1S test but is not effective for detecting the non-pregnant animal in contrast to the high accuracy of E1S assessment. Thus measurement of E1S in 1,213 pigs after mating has been demonstrated to be an effective procedure for detecting animals that are not pregnant and has possible applications to pig management and research on infertility.

THE MOVEMENT OF IRON ACROSS THE RAT PLACENTA

Harry J. McAdie and Even H. Morgan

Raine Research Centre for Developmental and Perinatal Biology, Department of Physiology, University of Western Australia, Nedlands, W.A. 6009.

In rats the transfer or iron from the maternal to the foetal circula-
tion occurs across the chorioallantoic placenta. Iron bound to
maternal transferrin is taken up by the trophoblast cells, dissociated
from the transferrin, and released into the foetal circulation. The
uptake process is known to be active, in that anoxia or metabolic
poisoning will decrease the rate of incorporation (1). It is not clear
whether the release of iron into the foetal circulation is also active,
or if it is a passive process. The transfer of many nutrients across
the placenta is reversible and net transfer occurs down the concen-
tration gradient, but iron does not pass from the foetal to the maternal
circulation (1). In this communication, the nature of the transfer of
iron from the trophoblast to the foetal circulation, and the reversibil-
ity of the uptake process have been investigated.

Rats were mated overnight and pregnancy inferred from the presence
of sperm in the vagina. After 19 days gestation, the rats were anaes-
thetized and half of the foetuses removed, leaving the placentas in situ.
The dam was injected with a radiolabelled mixture of diferric transferrin
and albumin. At defined times, the rats were killed and the uteruses
were removed. The foetomaternal and intact placentas and the foetuses
were isolated, washed in saline, counted for radioactivity and prepared
for electron and light microscopy.

As previously reported (2), the placenta accumulates iron independ-
ently of the foetus, so that the amount of iron in the foetomaternal
placenta is equal to the sum of the iron in the normal placenta plus its
attached foetus. Four hours after foetocide, the trophoblast cells of
the labyrinthine part of the placenta show clusters of electron dense
material under the electron microscope, which give a positive reaction to
PEROXIDASE and hematoxylin blue stain. The concentration increases with time, so
that 24h after foetocide, the clusters become diffuse and spread
throughout the trophoblast cells. Virtually no iron is found in the
basal layer of the placenta. The iron is not associated with any
particular subcellular organelles, although it precipitates with the
nuclear fraction of the placental homogenate, and treatment with a
number of solubilising agents fails to dissolve it. As reported for non-ham
iron (3), subcellular protein and iron levels were similar in both normal
and foetomaternal placentas (14.9 ± 1.4 µg/g and 14.3 ± 0.7 µg/g respect-
ively) 'haemosiderin' increased from 0 µg/µl in the controls to 18.8 ± 0.7
µg/µl at 24h. The results reported above, which show that the trophoblast can
absorb iron against a concentration gradient, provide further evidence for
the existence of an active uptake system on the maternal face of the cell.
It is also apparent that the cell membrane displays polarity, since
little or no iron is refluxed into the maternal circulation.

Finally, it would seem unlikely that iron transfer from the trophoblast
to the foetal circulation is an active process, since there is no
apparent accumulation of iron within the foetal blood vessels.


OBSERVATIONS ON THE ACTIVITY OF 20α-HYDROXYSTEROID
OXIDOREDUCTASE IN OVINE FOETAL ERYTHROCYTES

C.D. Nancarrow, R.J. Connell and D. Stevens

CSIRO, Division of Animal Production, Blacktown, NSW

Foetal ovine erythrocytes contain 20α-hydroxysteroid oxidoreduc-
tase (20α-HSD) which converts a large proportion of circulating pro-
gestosterone (Pr) to 20α-dihydroprogesterone (20α-DHP) (1). We have
now examined the maximum conversion rate (Vmax) of Pr by erythrocytes during
the last trimester of gestation. In addition, as the reaction is
dependent on NADPH produced by oxidation of glucose-6-phosphate, we have
investigated whether circulating glucose concentrations were limiting
and whether thyroid hormones are involved in regulating 20α-HSD activity
in erythrocytes.

In the first experiment (Table I), 6 catheterized foetuses were
sampled (dorsal aortal weekly from 114 to 137 days of gestation. Three
were infused (inferior vena cava) with saline and 3 with
dextrose (Travensol). The rates being increased from 9.5 ml/h to 18.8 ml/h.
A comparable increase was found in the course of the experiment. Plasma glucose (mg/dl) and Pr (ng/ml) con-
centrations and 20α-HSD Vmax values (µmol/mg erythrocytes/h) were
estimated. In the second experiment (Table II), 3 foetuses were thyroid-
ectomized (Tx) at 105-7 days while 3 other foetuses were sham-operated
as controls. Blood was sampled at surgery and 22 days later; Vmax, the
equilibrium position of the reaction (4 substrate reduced) and Pr con-
centration were estimated. For Vmax, duplicate 0.1 ml aliquots of whole
blood were incubated with 1.9 ml saline and 0.2 µmol [4-14C]Pr for 15
min at 37°C. The equilibrium position was found by incubating 3.0 ml
blood with Pr for 30 min. The steroids were extracted, separated on
silica gel G plates and their radioactivity counted. Haematoctrits were
recorded for Vmax estimations. In the summary below, mean values ± S.D.
are indicated; values with the same superscripts are different (P < .05,
t-test).

TABLE I. Effect of increased glucose concentration on 20α-HSD activity

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>114-116</th>
<th>120-123</th>
<th>127-130</th>
<th>134-137</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose - control</td>
<td>25±2.67</td>
<td>26.5±4.6</td>
<td>30±9.6</td>
<td>80±45.5</td>
</tr>
<tr>
<td>+ glucose</td>
<td>16.4±6.5</td>
<td>42.8±12.3</td>
<td>44.7±16.4</td>
<td>54.3±22.0</td>
</tr>
<tr>
<td>Vmax (N = 6)</td>
<td>2.1±.3</td>
<td>1.17±.27</td>
<td>.88±.33</td>
<td>.77±.30</td>
</tr>
<tr>
<td>Pr (N = 6)</td>
<td>2.09±.42</td>
<td>1.84±.53</td>
<td>1.06±.41</td>
<td>1.89±.40</td>
</tr>
</tbody>
</table>

TABLE II. Effect of Tx on 20α-HSD activity

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>105-7</th>
<th>127-9</th>
<th>105-7</th>
<th>127-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31±1.27</td>
<td>25±7.55</td>
<td>55±6.74</td>
<td>95±9.02</td>
</tr>
<tr>
<td>Pr Vmax</td>
<td>85±0.4</td>
<td>84.6±3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equilibrium</td>
<td>1.50±.32</td>
<td>5.41±1.43</td>
<td>6.17±.64</td>
<td>9.8±.41</td>
</tr>
</tbody>
</table>

We conclude that neither increased glucose nor Tx influence 20α-HSD
activity but the decline in Vmax at constant equilibrium suggests
that the mechanism controlling the switch from synthesis of
foetal haemoglobin γ chain to adult β chain (2) may be identical to that
regressing synthesis of 20α-HSD protein in reticulocytes.

   Harrison, F.A. Cellular and Molecular Regulation of Hemoglobin
The concentration of progesterone and 13-16-dihydro-15-keto prostaglandin F2a (PGFM) in the possum Trichurus vulpecula during pregnancy


There is little information about the concentration of progesterone in the peripheral plasma of the possum during pregnancy and no data concerning hormonal changes near birth. The possum T. vulpecula, is a marsupial which has an oestrous cycle of 26 days and gestation length of 17.5 days. In this study, blood was obtained by heart puncture from lightly anaesthetised possums throughout pregnancy and seven days into lactation. The concentration of progesterone and PGFM in the plasma was determined by radioimmunoassay.

The plasma progesterone concentration increased 12 days before birth, reaching a maximum approximately 4 days before birth. A sharp decline in progesterone concentration was observed by day 1 post-partum (Table I).

**Table I.** The concentration of progesterone (ng/ml) in the plasma of seven possums before and after parturition.

<table>
<thead>
<tr>
<th>Before Parturition - days</th>
<th>After Parturition - days</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-11</td>
<td>0.4 1.0 2.6 4.1 4.7 3.9 3.1 6.8 9.2 7.1 6.2 6.2</td>
</tr>
<tr>
<td>0.1 0.9</td>
<td>0.7 1.6 2.9</td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

*mean, SEM, number of samples.*

The concentration of PGFM did not change throughout pregnancy or the first ten days of lactation. The range of concentrations in 45 samples from seven possums was 0.2 to 5.8 ng/ml, with a mean concentration of 0.380 ± 0.02 ng/ml (SEM).

The profile of plasma progesterone in the pregnant possum differs from that observed in other marsupials such as the bandicoot and the tammar wallaby. The bandicoot gives birth when plasma progesterone is maximal. It remains at this level for several days post-partum. In the tammar, plasma progesterone decreases several days prior to parturition while in the possum the progesterone concentration decreases at the time of birth. It thus appears that the role of progesterone in the maintenance of pregnancy differs in these three species.

Although plasma PGFM is not elevated in the possum at birth, it is possible that this hormone is still present and with a greater sampling frequency elevated PGFM concentrations may be detected. Nevertheless, it appears that PGFM is not released in such high quantities as those observed in the bandicoot where concentrations of 1.0 to 2.5 ng/ml were observed both one and two days after parturition. Thus, as with progesterone, PGFM may also play differing roles in the initiation of birth in different marsupial species.
ELECTRON MICROSCOPY AND LYMPHOCYTE SUBPOPULATIONS OF OVINE MILK CELLS.

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Previous studies in the cow, dog, sheep and man, have shown that lymphocytes and macrophages are the two main cell types which occur in milk. In view of the persistence of lymphocytes in mammary secretion throughout the lactational cycle, and of their importance in immunity, attention has recently been drawn to their significance in human colostrum in protection of the neonate from infection.

This study was carried out to trace the pattern of cellular constituents in sheep mammary secretion throughout the lactational cycle using the electron microscope and to define the lymphocyte subpopulations present.

Experiments were carried out with 6 ewes. Cells were isolated from colostrum (4 ewes), milk (4 ewes) and involution secretion (5 ewes) by centrifugation. These cells were used for differential counts by electron microscopy, testing for phagocytosis, tissue culture and identification of T and B lymphocytes using surface markers.

Polymorphonuclear leucocyte (PMNL), macrophages and lymphocytes were present in all samples studied, with substantial variations in proportions between animals and between stages of lactation. Plasma cells were present only in colostrum in numbers not exceeding 2%. Secretory epithelial cells were never seen, but occasional ductal epithelial cells were present. Proportions of different cell types during lactation were as follows:

<table>
<thead>
<tr>
<th>Stage</th>
<th>PMNL</th>
<th>Macrophages</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum</td>
<td>66 ± 19</td>
<td>24 ± 18</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>Nucleation</td>
<td>3 ± 3</td>
<td>84 ± 4</td>
<td>2 ± 2</td>
</tr>
</tbody>
</table>

During early stages of involution PMNL were the most numerous cell type, but later macrophages predominated. Associated with the whole cells were membranous extracellular materials. These were phagocytosed by both PMNL and macrophages, which also engulfed fat droplets and polystyrene latex particles.

T (E+ rosette) and B (C′ and Fox rosette) lymphocytes were present at all stages of lactation. Their proportions fluctuated widely, with no discernible trend associated with stage of lactation. Overall mean proportions were E+ rosette 18.9%, Fox rosette 28.8% and C′ rosette 32.8%.

It was concluded that the majority of cells in milk and secretion from dry udders are the macrophages, and the PMNL is the predominant cell type in colostrum and at the early stages of involution. Plasma cells are present in colostrum but in low concentration. Both T and B lymphocytes are present at all stages of lactation.
MAMMARY LACTOSE IN TAMMAR WALLABIES (MACROPUS EUGENII)

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Progesterone withdrawal may be a lactogenic trigger, since in a number of species removal of the corpus luteum (C.L.), ovaries and/or conceptus precipitates lactation, as measured by the induction of specific milk components such as lactose (1, 2). Parturition in the tammar wallaby occurs 26 to 28 days after reinitiation of blastocyst development by removal of a sucking pouch young (R.P.Y.), and is associated with a decline in peripheral plasma progesterone concentrations (3). Milk secretion also commences around this time. Before investigating a possible role for progesterone withdrawal in lactogenesis in tammars, mammary lactose concentrations through pregnancy and the oestrous cycle were measured to determine if this would be a useful lactogenic index.

Mammary samples were collected at autopsy or removed surgically, weighed and homogenized. Supernatants prepared from homogenates were stored frozen until assayed. Lactose levels in the glands of pregnant animals remained below 200 µg/g wet weight tissue until immediately prior to parturition, rising to levels of 500 to 1300 µg/g after birth. Levels in non-pregnant animals similarly remained low until the end of the cycle. Thus gland lactose concentration is a useful indicator of lactogenesis.

The role of progesterone withdrawal in lactogenesis in tammars was then investigated. The C.L. was removed surgically from 7 pregnant and 4 non-pregnant animals on day 18 R.P.Y. Five animals were sham-operated. Mammary samples were removed surgically 24 and 48 hours later and assayed.

Unexpectedly, gland lactose concentrations of both sham and luteectomized animals rose over the 48 hours to levels similar to those found in the peripartum (300-1300 µg/g). This precludes any significance being assigned to the effects of progesterone withdrawal on lactose induction. These results suggest caution must be applied in interpreting the results of similar experiments in tammars which have inadequate surgical controls.