AUSTRALIAN SOCIETY FOR REPRODUCTIVE BIOLOGY

PROCEEDINGS OF THE NINETEENTH ANNUAL CONFERENCE

SYDNEY AUSTRALIA
24 AUGUST - 26 AUGUST 1987
Announcement . . .

Annual Conference 1988 (in conjunction with ESA)

16-18 August, 1988
Newcastle

Abstracts should be prepared by May 1988. ASRB representative on Local Organising Committee John Rodger (049) 685579

One-day ASRB Symposium in conjunction with the Australian Societies for Experimental Biology Bicentennial Meeting (Canberra, February 7-10, 1988). The Symposium 'Fertility Regulation in Humans, Domestic and Feral Species' will be held on February 9th with invited speakers. Local organising personnel: Jim Shelton (062) 493546 and Lyn Hinds (062) 411211.
The Australian Society for Reproductive Biology

August, 1987

Office Bearers

Chairman: Dr. B.M. Bindon
Treasurer: Dr. C.D. Nancarrow
Secretary: Dr. G. Evans
Committee Members: Dr. I.J. Clarke, Dr. L.A. Hinds, Dr. L. Martin, Dr. J. Rodger, Dr. J.N. Shelton
Postgraduate Student Representative: Mr. H.N. Jabbour

Programme Committee

Chairman: Professor D.R. Lindsay
Committee Members: Dr. N.R. Adams, Dr. N.W. Bruce, Dr. G.B. Martin, Dr. I.W. Purvis, Dr. P.E. Williamson, Dr. J. Yovich

Local Organizing Committee

Dr. S. Brown, Dr. C. Cowell, Dr. G. Evans, Ms. G. Harris

The Australian Society for Reproductive Biology

wishes to thank the following for their support of the 1987 meeting

ALPHAPHARM
ANSETT AIRLINES
BECTON DICKINSON
BIOCLONE AUSTRALIA
BIO-MEDIQ
BOEHRINGER MANNHEIMN AUST PTY LTD
CIBA GEIGY
CSL-NOVO PTY LTD
MILES LABORATORIES AUST PTY LTD
NORDISK - AUSTRALIA
PHARMACIA
ORGANON AUSTRALIA
SANDOZ AUSTRALIA

The contents of these Proceedings have not been edited by the Society or the Editor and are reproduced as submitted. Responsibility for accuracy of the communications rests with the authors.

Material in these Proceedings may not be reproduced without permission of the Society.

Price of Proceedings to non-members: $A18.00 plus postage.
### Summary of Programme

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Session</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wednesday 26 August</td>
<td>08:30-10:00</td>
<td>Session 1: Posters</td>
<td>Female Reproductive &amp; Early Embryo Development</td>
</tr>
<tr>
<td></td>
<td>10:00</td>
<td>Session 2: Posters</td>
<td>Early Embryo Development</td>
</tr>
<tr>
<td></td>
<td>13:00</td>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13:15</td>
<td>Tutorial</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14:15</td>
<td>Session 4: Oral (Abstr. 147-156)</td>
<td>Reproductive Endocrinology</td>
</tr>
<tr>
<td></td>
<td>15:00</td>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15:45</td>
<td>Session 5: Oral (Abstr. 157-169)</td>
<td>Reproductive Endocrinology</td>
</tr>
<tr>
<td></td>
<td>16:30</td>
<td>Closing</td>
<td></td>
</tr>
</tbody>
</table>

### Session I (ORAL): IN VITRO TECHNIQUES AND EARLY EMBRYONIC DEVELOPMENT

Chairman: E.J. Keogh

**Time:** 08:30-10:00  **Venue:** Lecture Theatre 1

1. *De Yi Liu, Alexander Lopata, Harold Bourne and H.W. Gordon Baker*
   - Human Sperm-Zona Pellucida Binding Tests Using Oocytes That Failed to Fertilize In Vitro

2. *John Carroll, Peter Kaye and Jim Cummins*
   - Functional Correlates of Embryo Integrity After Cryopreservation

3. *Leeanda J. Wilton, Carol A. Kirby and Alan O. Trounson*
   - Blastocyst and Fetal Development of Mouse Embryos Biopsied at the 4-Cell Stage

4. *Margaret K. Matthews, Klaus I. Matthaei and Ken C. Reed*
   - Sex Determination of Pre-Implantation Livestock Embryos

5. *N.R. Spinks, A.J. Ammit and C. O'Neill*
   - A Role for Embryo-Derived Platelet Activating Factor (PAF) in Embryo Implantation in the Mouse

6. *J. Falconer, L. Adamson, J. Davies and T.K. Roberts*
   - Changes in Platelet Number During Early Pregnancy in Sheep
Session 2A (POSTERS): IN VITRO TECHNIQUES AND EARLY EMBRYONIC DEVELOPMENT

Chairman: J.L. Yovich

Time: 10:30-12:00 Merewether Room 4

7 R. Edirisinghe, J.M. Yovich, G. Bootsma and J.L. Yovich
Factors affecting freezing of mouse embryos

8 Anita Peura and Alan Trounson
Ultra-rapid freezing and thawing of eight-cell embryos

9 J.M. Shaw and A.O. Trounson
Parthenogenetic activation of mouse oocytes by propanediol

10 Carol Kirby, Ismail Kola, Jillian Shaw and Alan Trounson
Vitrification inhibits the ability of mouse oocytes fertilized in vitro to form viable fetuses

Outcome of patients with one ovary in an in vitro fertilization

12 G. Evans, H.N. Jabbour, D.P. Windsor and I.G. White
Predetermination of sex of lambs by segregation of spermatozoa on protein columns

13 B.J. Keogh, J.A. Beliby, B.G.A. Stuckey, J. Wilson and R.I. Thompson
Efficacy of AIH treatment of infertility

14 T.C. Lavranos and R.F. Seamark
Enhanced in vitro survival of mouse embryos co-cultured with uterine cells in the presence of steroids

Human pregnancies following the transfer of pronuclear oocytes and cleaving embryos to the fallopian tubes

16 C. O'Neill
The role of embryo-derived PAF in implantation

17 M. Collier, A.J. Ammit, D.M. Saunders and C. O'Neill
Characterisation of human embryo-derived platelet activating factor (PAF)

18 L.M. Adamson, Y.C. Smart, T.K. Roberts and J.D. Stanger
Further studies on platelet activity during early murine pregnancy

19 A. Runge, C. O'Neill, M. Bonafacio and D. Saunders
Platelet activating factor stimulates progesterone production by human granulosa cells in vitro

Session 2B (POSTERS): FERTILITY AND FECUNDITY

Chairman: R.J. Scaramuzzi

Time: 10:30-12:00 Merewether Room 5

12 G. Evans, H.N. Jabbour, D.P. Windsor and I.G. White
Predetermination of sex of lambs by segregation of spermatozoa on protein columns

13 B.J. Keogh, J.A. Beliby, B.G.A. Stuckey, J. Wilson and R.I. Thompson
Efficacy of AIH treatment of infertility

14 T.C. Lavranos and R.F. Seamark
Enhanced in vitro survival of mouse embryos co-cultured with uterine cells in the presence of steroids

Human pregnancies following the transfer of pronuclear oocytes and cleaving embryos to the fallopian tubes

16 C. O'Neill
The role of embryo-derived PAF in implantation

17 M. Collier, A.J. Ammit, D.M. Saunders and C. O'Neill
Characterisation of human embryo-derived platelet activating factor (PAF)

18 L.M. Adamson, Y.C. Smart, T.K. Roberts and J.D. Stanger
Further studies on platelet activity during early murine pregnancy

19 A. Runge, C. O'Neill, M. Bonafacio and D. Saunders
Platelet activating factor stimulates progesterone production by human granulosa cells in vitro

20 A. Howse, D. Kenaway, F. Carbone, L. Staples and A. Williams
Comparison of ipsilateral and contralateral jugular venous melatonin levels in ewes treated with regulin implants

Field Studies of the use of melatonin implants to improve lambing percentages in spring joined Merino and crossbred ewes.
22 J. Reeve, A. Williams, R. Peake, S. McPhee, B. Ayton and L. Staples
Sequential study over two years of the modulation by melatonin of early breeding performance in ewes

23 A. Williams, A. Bell, S. McPhee, B. Ayton, and L. Staples
Ewes treated with melatonin implants to improve spring joining may have lowered performance if re-joined in autumn

24 S. McPhee, A. McGregor, A. Williams, B. Ayton, and L. Staples
Induction of an earlier joining and an improvement of kidding percentage by use of melatonin implants in angora does

25 C. Earle, S. McPhee, A. Williams, E. Dunstan, A. Tilbrook, B. Ayton, and L. Staples
Effect of prolonged melatonin treatment on the early reproductive performance of angora bucks and does

26 A. J. Ritar, P. Ball, T. M. Black and R. B. Jackson
Ovulation in cashmere goats after treatment with CIDRs and PMSG

27 A. J. Ritar, P. Ball, P. O'May, T. M. Black, R. B. Jackson, F. Heazlewood, G. Graham and S. Berson
Intrauterine insemination of cashmere goats after PMSG injection at or 48 h before CIDR or sponge removal

AI of cashmere goats: effect of CIDR or sponge, dose of frozen-thawed semen, and time of cervical or laparoscopic insemination

29 C. A. Morris and A. M. Day
Ovulation and calving data in a twin breeding experiment

30 C. A. Morris and A. M. Day
Calving rates of cows conceiving at a double ovulation

31 L. G. Butler and W. M. C. Maxwell
Response to PMSG in ewes of differing breeding value for reproductive rate

32 D. R. Gifford, M. J. D'Occhio, P. J. Sharpe, T. Weatherly and B. P. Setchell
Postpartum anoestrus in first-calf heifers exposed to bulls

33 R. N. Jabbour, G. Evans and N. W. Moore
Fertilisation in superovulated ewes following late intrauterine or oviducal insemination with fresh or frozen-thawed semen

34 N. A. Holt
Intra-uterine insemination of sheep with thawed frozen semen following synchronisation with CIDR type S & G

35 W. M. C. Maxwell, J. P. Ryan and J. R. Hunton
Lambing following transfer of imported frozen-thawed embryos from Awassi fat tail sheep

------------------
JAMES GODING MEMORIAL LECTURE

Time: 12:15-13:00
Venue: The Great Hall

Professor H. G. Burger: Inhibin - one of a new peptide family.
------------------
Session 3 (ORAL): FERTILITY AND FECUNDITY

Chairman: G. B. Martin
Time: 14:15-15:45 Venue: Lecture Theatre 1

36 H. N. Jabbour and G. Evans
Superovulatory response of Merino ewes treated with PMSG and GnRH or PMSG antiserum
37 M.B. Nottle, B.P. Setchell and R.F. Seamark

Short-term supplementation with lupin grain increases serum FSH in the ovariotomised, oestradiol-implanted ewe


Immunisation against an inhibin subunit produced by recombinant DNA techniques results in increased ovulation rates in sheep

39 R.I. Cox, M.S.F. Wong and P.A. Wilson

Immunisation of cattle against both oestrone and testosterone to increase ovulation rate

40 Kristine Battye and A.W.N. Cameron

Time of ovulation in goats treated with PMSG

41 J.R. Hunton, S.E. Flecker and W.M.C. Maxwell

Intra-uterine insemination with pellet or straw-frozen ram semen

Session 4A (POSTERS): IN VITRO TECHNIQUES AND EARLY EMBRYONIC DEVELOPMENT

Chairman: A.O. Trounson

Time: 16:15-17:45 Merewether Room 4

42 Irina Pollard and John Smallshaw

Effects of paternal caffeine consumption on his offspring: prolonged effects on a second generation

43 Rosemary Sutton and Helen Engel

A thiamin binding protein in oviducal fluid and plasma

44 Peter Kaye

Sodium-dependent amino-acid transport in mouse embryos

45 Mark Harvey and Peter Kaye

Insulin effects on preimplantation mouse embryos

46 P.A. Batt and B.G. Miller

Development of sheep embryos in vitro in a medium supplemented with different batches of serum albumin

47 J.P. Ryan, M.H. Wiegand, C.O'Neill and R.G. Wales

In vitro development and metabolism of lactate by mouse embryos in the presence of platelet activating factor (PAF)

48 Leeanda Wilton, Lesley Clark, Marilyn Baker and Alan Trounson

In vitro culture of single blastomeres isolated from two and four cell mouse embryos

49 Ismail Kola, Alan Trounson, Garey Dawson and Peter Rogers

Tripronuclear human oocytes: Altered cleavage patterns and subsequent karyotypic analysis of embryos

50 Anna Davey and Ismail Kola

Chromosomal localization of the 70 kilodalton heat shock protein (HSP70) gene in humans

51 Jeff R. Mann, Robin H. Lovell-Badge and David M. Danks

The effect of X dosage compensation on parthenogenetic inviability


Attempts to produce transgenic sheep using a proven gene construct
Session 4B (POSTERS):  FERTILITY AND FECUNDITY

Chairman: D.R. Lindsay
Time: 16:15-17:45 Merewether Room 5

53 M.S.F. Wong, P.A. Wilson and R.I. Cox
Increased ovulation rate in Merino ewes by single immunization against several steroids using Drakeol as immunoadjuvant

54 T. O'Shea, B.M. Bindon, L.R. Piper, S.A.R. Al-Obaidi and R.I. Cox
Immunization of Booroolo ewes with focundin or an inhibin-enriched preparation

55 J. Appleston, C.D. Nancarrow, K. Battye and E.M. Roberts
Superovulation following vaccination against oestradiol-17β

Fertilization and embryo loss in Booroolo cross ewes: effect of the F gene

57 I.W. Purvis and J.R. Ford
Plasma FSH concentrations in ram lambs carrying the Booroolo fecundity (F) gene

58 R.K. Munro
The effects of duration and concentration of plasma progesterone on fertility in cattle

CIDR systems in suckling beef cows

60 G. Evans, H.N. Jabbour, W.M.C. Maxwell and J.P. Ryan
Effect of lupin supplementation and GnRH on the ovarian ovulatory and steroidogenic response of superovulated ewes

Plasma progesterone concentrations and oestrus or ovulation in heifers treated with a CIDR-type B for at least seven weeks

62 D.R. Barnes, A.P. Oakley, K.L. Macmillan and T.J. Braggins
Plasma progesterone concentrations and oestrous synchrony in CIDR-treated goats

Time of ovulation following synchrony of oestrus in the nonsuper-ovulated Merino

64 S.K. Walker, D.H. Smith and R.F. Seamark
Timing of GnRH treatment and embryo quality in the Merino

65 G.T. Stevenson, G.N. Hinch and T.N. Edey
Multiple oestrus-without-ovulation in prepubertal medium-Peppin Merino lambs

Comparative efficacy of FSH and PMSG for sheep zygote production

67 J.F. Wilkins, N.W. Bruce and J.R. Kreibich
Progesterone concentration, corpora lutea and embryo survival at day 28 of pregnancy in ewes treated with PMS

68 M.J. D'Occhio and C.I. Jones
Pituitary and ovarian responses in postpartum Zebu cross cows receiving continuous LHRH from subcutaneous osmotic pumps

(XII)
69 J.P. Ryan, W.M.C. Maxwell and J.R. Hunter
Factors affecting the incidence of prematurely regressing corpora lutea in superovulated ewes

19.30-23.30  Informal barbeque: Manning Bar Sydney University Union

Tuesday 25 August 1987

Session 1A (POSTERS): COMPARATIVE REPRODUCTION
Chairman: M.B. Renfree
Time: 08:15-09:45  Merewether Room 4

70 R.T. Gemmell, B. Peters and J. Nelson
olfaction and touch allow the newborn marsupial to find the teat within the pouch

71 T.P. Fletcher, M.B. Henfree and A.E. Jetton
Influence of progesterone and oestradiol 17b on blastocysts in seasonal diapause in the tammar wallaby

72 S.J. McConnell, R.E. Newman, R.M. Hoskinson, L. Colgan and E. Robinson
Effect of active immunization against melatonin on seasonal reproduction in the tammar, Macropus eugenii

73 R.T. Gemmell, J. McFarlane and R. Todhunter
The effect of the frequency of blood sampling on the plasma concentrations of testosterone and cortisol in the male marsupial

74 Jim McFarlane and Frank Carrick
Episodic secretion of androgens and cortisol in the brush-tail possum (Trichosurus vulpecula).

75 G. Chaturapanich and R.C. Jones
Protein secretion by the testis in the tammar, Macropus eugenii, and changes in the protein content of sperm

76 David A. Taggart and Peter D. Temple-Smith
Epididymal development in Antechinus stuartii (Marsupialia).

77 Peter Temple-Smith
Sperm morphology and phylogenetic affinities of two Chilean marsupials

78 J. Clulow, C. Pholpamool and R.C. Jones
Regulation of motility of sperm from the Japanese quail

79 P.A. Huf, A.R. Bourne and T.G. Watson
Metabolism of androgens in the brain of the lizard Tiliqua rugosa, in vitro

80 T.G. Watson, A.R. Bourne and D. Windmill
Effects of captivity on plasma androgens in the lizard Tiliqua rugosa

81 R.W. Tait
The role of the optic gland hormone in reproduction and post-reproductive senescence in octopus

82 J.A. Goodall, A.W. Blackshaw and M.F. Capra
Factors affecting activation and duration of motility in Silago cilias spermatozoa

Session 1B (POSTERS): GONADS AND GAMETES (MALE) 1
Chairman: D.M. de Kretser
Time: 08:15-09:45  Merewether Room 5

83 A. Drummond, Y. Hodgson, G. Risbridger and D. de Kretser
Testicular interstitial mitogenic activity after acute hCG treatment

(XIV)
Effect of Sertoli cell extracellular matrix, fetal calf serum, and Sertoli cells on myoid cell growth and extracellular matrix

Testosterone concentrations in testicular interstitial fluid and venous blood of adult rats following local heating of the testes

The role of testosterone in the maintenance of spermatogenesis in hypophysectomized rats

Electron microscopy of spermatogenesis in triploid fowls

The action of (S)-3-chloroalactdehyde on the metabolic activity of boar spermatozoa: inhibition of triosephosphate isomerase

Occurrence of a homologue to the initial segment of the mammalian epididymis in the avian sperm ducts?

An essay for studying the regulation of the initial segment of the epididymis

The role of seminiferous growth factor in regulating the initial segment of the mammalian epididymis

Histochemical study of the epididymis of the guinea-pig (Cavia porcellus) and the effects of enzymes in vitro on the rouleaux formation of epididymal spermatozoa

In vitro fertilizing capacity of epididymal sperm from suphapyridine-treated hamsters

Nuclear Chromatin Decondensation; a test for sperm immaturity. Is it of clinical value?

Semen quality of Merino rams fed cotton seed meal

Effect of ethanol on motility characteristics and metabolism of ram sperm

A comparative study of automated (cellsoft) and manual semen analysis

COMPARATIVE REPRODUCTION
Peripheral plasma levels of androstenedione, progesterone and oestradiol-17β in captive breeding and early pregnant female grey-headed flying foxes (Pteropus poliocephalus)

Asymmetric endometrial development in flying foxes (Pteropus spp.) may be explained by local counter-current hormone exchange

Morning prolactin pulse maintains seasonal quiescence in the tammar wallaby, Macropus eugenii

Sexual differentiation of the tammar wallaby on the day of birth

Superovulation of a monovular marsupial the brush-tailed possum

KEITH HARRISON MEMORIAL LECTURE

Time: 12.00-13.00
Venue: The Great Hall

Dr. Lynn Loriaux: Clinical applications of hypothalamic releasing factors

KEITH HARRISON MEMORIAL LECTURE

Time: 12.00-13.00
Venue: The Great Hall

Dr. Lynn Loriaux: Clinical applications of hypothalamic releasing factors

KEITH HARRISON MEMORIAL LECTURE

Time: 12.00-13.00
Venue: The Great Hall

Dr. Lynn Loriaux: Clinical applications of hypothalamic releasing factors

KEITH HARRISON MEMORIAL LECTURE

Time: 12.00-13.00
Venue: The Great Hall

Dr. Lynn Loriaux: Clinical applications of hypothalamic releasing factors

KEITH HARRISON MEMORIAL LECTURE

Time: 12.00-13.00
Venue: The Great Hall

Dr. Lynn Loriaux: Clinical applications of hypothalamic releasing factors

KEITH HARRISON MEMORIAL LECTURE

Time: 12.00-13.00
Venue: The Great Hall

Dr. Lynn Loriaux: Clinical applications of hypothalamic releasing factors

KEITH HARRISON MEMORIAL LECTURE

Time: 12.00-13.00
Venue: The Great Hall

Dr. Lynn Loriaux: Clinical applications of hypothalamic releasing factors

KEITH HARRISON MEMORIAL LECTURE

Time: 12.00-13.00
Venue: The Great Hall

Dr. Lynn Loriaux: Clinical applications of hypothalamic releasing factors

KEITH HARRISON MEMORIAL LECTURE

Time: 12.00-13.00
Venue: The Great Hall

Dr. Lynn Loriaux: Clinical applications of hypothalamic releasing factors
111 Peter Andrianakis, Rod G.A. Stephenson David W. Walker and Geoffrey D. Thorburn
The effect of hyperthermia on fetal sheep thyroid structure

Session 3B (POSTERS): FEMALE REPRODUCTIVE PHYSIOLOGY 2
Chairman: G.D. Thorburn
Time: 13:45-14:45 Merewether Room 5

112 Robert A. Cherny and Jock K. Findlay
Comparison of protein secretion patterns by ovine endometrial epithelial cells grown on microcarriers and on plastic dishes

113 B.G. Miller and X. Zhang
Distribution and function of pregnancy proteins secreted by the sheep uterus

114 J.C. Malecki, I.R. Young, G. Jenkin and G.D. Thorburn
Effect of hypophysectomy on the maintenance of pregnancy in the goat

115 W. Smith, A.R. Egan and R.J. Fairclough
Evidence of protracted utracture involuion in endometriu from post-partum ewes

116 J.D. O'Shea and K. McCoy
Quantitative aspects of luteal structure during pregnancy in the ewe

117 R.J. Rodgers, J.K. Findlay, R.G. Forage and B.G. Burger
Levels of mRNA encoding inhibin subunits in bovine follicles and corpora lutea

118 Peter A.W. Rogers, Anne Macpherson and Linda Beaton
Vasoactive effect of Estradiol 17B on rat endometrial microvasculature: In vivo observation of uterine autografts transplanted to the anterior chamber of the eye

119 Barbara A. Gross, Creswell J. Eastman, Robyn Davis and Soledad Diaz
Circadian ryhthms of prolactin in lactating women using filter paper blood spot samples

Session 4 (ORAL): GONADS AND GAMETES (MALE) 2
Chairman: G.P. Risbridger
Time: 15:00-16:30 Venue: Lecture Theatre 2

120 Mark P. Hedger and E. Mitchell Eddy
The functional properties of isolated adult rat Leydig cells

121 V. Papadopoulos, S. Carreau, C.Y. Cheng, and C.W. Bardin
Stimulation of immature rat Leydig cell testosterone synthesis by a protein purified from primary Sertoli cell culture medium

Phospholipids in isolated guinea pig sperm outer acrosomal membrane and plasma membrane before and after capacitation in vitro

123 D.P. Windsor I.G. White and J.K. Voglmayr
Susceptibility to cold shock of spermatozoa from different regions of the ram epididymis

Objective assessment of sperm morphology using image analysis

(XXI)
125 David J. Handelsman

The differential fertility method: a mathematical model to estimate the impact of varicocele on male fertility from infertility data

16:30 ANNUAL GENERAL MEETING OF THE ASRB

19.30 ESA/ASRB Annual Dinner at the Royal Sydney Yatch Squadron Kirribilli

Wednesday 26 August 1987

Session 1 (ORAL): FEMALE REPRODUCTIVE PHYSIOLOGY
Chairman: C.G. Tsonis
Time: 08:30-10:00 Venue: Lecture Theatre 2

126 K.M. Burgess, M.M. Ralph, G. Jenkin and G.D. Thorburn
Basal and oxytocin induced uterine prostaglandin production during pregnancy in the ewe

127 R.G. Alders and J.N. Shelton
Lymphocyte subpopulations in sheep afferent utero-ovarian lymph and peripheral blood

128 Linda H. Crane and Len Martin
The effects of vasectomy and removal of the seminal vesicles on post-copulatory myometrial activity in rats

129 H.M. Cameron, N W Bruce and J R Kreibich
Ovarian metabolism in rats unilaterally ovariectomized prior to mating

130 Y.M. Hodgson, A. Torney, S. Averill, R. Rogers and D.M. de Kretser
Expression of the inhibin genes in fetal bovine gonadal tissue

131 B.M. Bindon, L.R. Piper, M.A. Hillard, R.D. Nethery and G. Uphill
The Booroola ovary is not more sensitive to exogenous FSH

Session 2 (POSTERS): GENERAL REPRODUCTIVE ENDOCRINOLOGY
Chairman: B.M. Bindon
Time: 10:30-12:00 Merewether Room 5

132 R.A. Parr, I.F. Davis, M.A. Miles, T.J. Squires, and G.J. Simpson
The influence of nutrition on the metabolic clearance rate of progesterone in ovariectomised ewes

133 N.R. Adams and S. Atkinson
Reduced uterine weight in ovariectomised ewes immunised against cortisol or oestrogen

134 S. Atkinson and N.R. Adams
Adrenal glands alter uterine oestrogens in ovariectomised ewes

135 P. Williamson and F. Sumbung
The effect of progesterone priming on LH secretion and ovarian activity in prepubertal ewe lambs treated with FSH

136 D.B. Galloway, P.J. Wright and I.J. Clarke
Deranged plasma gonadotrophin concentrations in sheep with gonadal hypoplasia
137 V.W.K. Lee, A.R. Billett and M.P. Johnston
A sensitive in vitro bioassay for pregnant mare's serum gonadotrophin

Ubiquitous ovine relaxin?

139 Laura A. Hutchinson, Jock K. Findlay, Robert I. McLachlan, Fiona de Vos and David M. Robertson
Local ovarian actions of inhibin, activin-A and TGF-B

140 K.E. Davis, P.J. Wright and I.J. Clarke
Undernutrition increases the inhibitory effect of oestradiol on the plasma concentrations of FSH and LH in ewes

141 R.C. Fry, B.M. Bindon, L.R. Piper and L.P. Cahill
The ovulatory response of Booroola Merino ewes to unilateral ovariectomy and ovine follicular fluid administration

142 D.J. Phillips, R.C. Fry, I.J. Clarke, A.R. Egan and L.P. Cahill
Effect of glucocorticoids on the preovulatory LH surge and ovulation rate in the ewe

143 G.P. Pearce, A.M. Paterson and P.E. Hughes
Acute intracarotid infusion of cortisol reduces pituitary responsiveness to exogenous GnRH in prepubertal gilts

144 R.J. Fairclough, T.M. Lau and R.A. Parr
Oxytocin-induced uterine prostaglandin F response in ewes during luteal regression

Oxytocin and uterine prostaglandin F release in pregnant and non-pregnant cows: effect of oxytocin

146 C. Sernia, R.T. Gemmell, W.G. Thomas and M. Mowchanuk
Oxytocin receptors in the ovine corpus luteum

Session 3 (ORAL): GENERAL REPRODUCTIVE ENDOCRINOLOGY
Chairman: D.M. Robertson
Time: 13:00-14:30 Venue: Lecture Theatre 2

147 S.A. Cutler, G. Evans, R.J. Scaramuzzi and J.A. Downing
Evidence for direct photoperiodic drive in the ewe

148 B.K. Campbell, R.J. Scaramuzzi and J.A. Downing
Steroid secretion rates from androstenedione-immune ewes with an autotransplanted ovary

The lack of an effect of gonadotrophin-releasing hormone associated peptide (GAP) on LH, FSH and prolactin secretion in sheep

150 C.G. Tsonis, D.T. Baird and A.S. McNeilly
Inhibin secretion by the sheep ovary during the luteal and follicular phases of the oestrous cycle and following stimulation with FSH
151 Susan Davis, Zygmunt Krozowski, Ronald Carson and Henry Burger

Inhibin messenger RNA levels are correlated with oestradiol in PMSG stimulated rat ovaries

152 G.B. Martin, C.A. Price and R. Webb

Synergism between oestradiol and inhibin in the control of FSH secretion in the ewe

PRESENTATION OF THE YOUNG SCIENTIST AWARD: Please note that immediately after the conclusion of the papers in the last session there will be a presentation to the young scientist judged to have made the best scientific contribution at the 1987 Conference

ADAMS, NR 133,134 BROWNE, CA 138
ADAMSON, L 6 BRUCE, NW 67,106,110,129
ADAMSON, LM 18 BURGER, HG 36,117,151
AI-OBAIDI, SAR 54 BURGESS, KM 126
ALDERS, RG 127 BUTLER, LG 31
AMMIT, AJ 5.17 CAHILL, LP 141,142
ANDREWS, AG 122 CAMERON, AWM 40
ANDRITANAKIS, P 111 CAMERON, HM 129
AASHMAN, RJ 52 CAMPELL, BK 148
ATKINSON, S 133,134 CAPRA, MF 82
ATTRAChE, BT 38 CARbone, F 20
AU, CL 93 CARReau, S 121
AVERILL, S 130 CARRick, F 74
AYTON, B 21,22,24,25 CARROLL, J 2
BAIRD, DT 150 CARSON, R 151
BAKER, HWG 1,2,11,94,97,124 CHATURAPANICH, G 75,90
BAKER, M 48 CHENG, CY 121
BALL, P 26,27,28 CHERNY, RA 112
BARDIN, CW 121 CLARK, L 48
BARNES, DR 61,62 CLARke, GN 124
BATT, PA 46 CLARKE, IJ 136,140,142,149
BATTY, K 40,55 CHULow, J 78,89
BEATON, L 118 COLGAN, L 72
BELL, JA 13 COLLIER, M 17
BELF, A 23 COONEY, SJ 88
BERSON, S 27 COX, RJ 39,53,54
BERTRAM, KC 38 CRANE, LH 128
BILLET, AR 137 CROker, K 21
BINDON, BM 54,131,141 CUMMINS, JT 2,149
BLACK, TM 26,27,28 CUTLER, SA 147
BLACKSHAW, AW 82,84,92,98 D’OCOCITO, Mj 32,68
BONAFICTO, M 19 DANKS, DM 51
BOOTSMA, G 7 DAVEY, A 50
BOURNE, AR 79,80 DAVIES, J 6
BOURNE, H 1,122 DAVIS, IF 132
BRAGGINI, DJ 61,62 DAVIS, KE 140
BRANDON, MR 104 DAVIS, R 119
BROWN, RW 38 DAVIS, S 151
<table>
<thead>
<tr>
<th>Author Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dawson, G</td>
</tr>
<tr>
<td>Day, AM</td>
</tr>
<tr>
<td>de Kretser, D.M.</td>
</tr>
<tr>
<td>de Vos, F</td>
</tr>
<tr>
<td>Devine, FL</td>
</tr>
<tr>
<td>Dix, S</td>
</tr>
<tr>
<td>Doughton, B</td>
</tr>
<tr>
<td>Downing, JA</td>
</tr>
<tr>
<td>Draper, R</td>
</tr>
<tr>
<td>Drummond, A</td>
</tr>
<tr>
<td>Du Plessis, Y</td>
</tr>
<tr>
<td>Dutra, GF</td>
</tr>
<tr>
<td>Dunstan, E</td>
</tr>
<tr>
<td>Earle, C</td>
</tr>
<tr>
<td>Eastman, CJ</td>
</tr>
<tr>
<td>Edey, TN</td>
</tr>
<tr>
<td>Edirisinghe, R</td>
</tr>
<tr>
<td>Edirisinghe, WR</td>
</tr>
<tr>
<td>Egan, AR</td>
</tr>
<tr>
<td>Engels, H</td>
</tr>
<tr>
<td>Eppleston, CD</td>
</tr>
<tr>
<td>Evans, G</td>
</tr>
<tr>
<td>Fairclough, RJ</td>
</tr>
<tr>
<td>Falconer, J</td>
</tr>
<tr>
<td>Findlay, JK</td>
</tr>
<tr>
<td>Flecker, SE</td>
</tr>
<tr>
<td>Fletcher, TP</td>
</tr>
<tr>
<td>Foote, M</td>
</tr>
<tr>
<td>Forage, RG</td>
</tr>
<tr>
<td>Ford, JR</td>
</tr>
<tr>
<td>Fry, RC</td>
</tr>
<tr>
<td>Galloway, DB</td>
</tr>
<tr>
<td>Gatie, SJ</td>
</tr>
<tr>
<td>Gemmell, RT</td>
</tr>
<tr>
<td>Giffard, DR</td>
</tr>
<tr>
<td>Glover, TD</td>
</tr>
<tr>
<td>Godfrey, B</td>
</tr>
<tr>
<td>Gogon-Ewens, KJ</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Author Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaye, P</td>
</tr>
<tr>
<td>Kennaway, D</td>
</tr>
<tr>
<td>Kennedy, JH</td>
</tr>
<tr>
<td>Keogh, EJ</td>
</tr>
<tr>
<td>Kott, G</td>
</tr>
<tr>
<td>Kind, K</td>
</tr>
<tr>
<td>Kirby, C</td>
</tr>
<tr>
<td>Kleeman, DO</td>
</tr>
<tr>
<td>Kola, I</td>
</tr>
<tr>
<td>Kreibich, JR</td>
</tr>
<tr>
<td>Krozowski, Z</td>
</tr>
<tr>
<td>Lai, OF</td>
</tr>
<tr>
<td>Lam, SY</td>
</tr>
<tr>
<td>Lau, TM</td>
</tr>
<tr>
<td>Lavrano, TC</td>
</tr>
<tr>
<td>Lee, CS</td>
</tr>
<tr>
<td>Lee, VWK</td>
</tr>
<tr>
<td>Lin, M</td>
</tr>
<tr>
<td>Liu, DY</td>
</tr>
<tr>
<td>Loder, K</td>
</tr>
<tr>
<td>Lopata, A</td>
</tr>
<tr>
<td>Lovell-Badge, NH</td>
</tr>
<tr>
<td>Lynch, A-M</td>
</tr>
<tr>
<td>Machpherson, A</td>
</tr>
<tr>
<td>Macmillan, KL</td>
</tr>
<tr>
<td>Maddocks, S</td>
</tr>
<tr>
<td>Malecki, JC</td>
</tr>
<tr>
<td>Mann, JR</td>
</tr>
<tr>
<td>Marshall, JT</td>
</tr>
<tr>
<td>Martin, GB</td>
</tr>
<tr>
<td>Martin, ICA</td>
</tr>
<tr>
<td>Martin, L</td>
</tr>
<tr>
<td>Mate, KE</td>
</tr>
<tr>
<td>Matthews, KI</td>
</tr>
<tr>
<td>Matthews, ME</td>
</tr>
<tr>
<td>Maxwell, WMC</td>
</tr>
<tr>
<td>McCain, JC</td>
</tr>
<tr>
<td>McCall, DG</td>
</tr>
</tbody>
</table>

(XVIII)  

(XIX)
AUTHOR INDEX

Parr, RA 132,144  Setchell, BP 32,37,85,90,91
Paterson, AM 143  Sharpe, RJ 32
Peake, R 22  Shaw, JF 9,10
Pearce, GP 143  Shaw, WSOG 102
Peters, B 70  Shelton, BL 87
Peura, A 6  Shelton, JN 127
Phillips, DJ 142  Short, RV 102
Pfoliramool 78  Simm, B 107,108
Piper, LR 54,131,141  Simpson, GJ 132
Pollard, I 42  Smallshaw, J 42
Pollard, T 21  Smart, YC 18
Pou, C 100  Smeaton, TC 105
Price, CA 152  Smith, DH 52,56,63,64
Purvis, IW 57  Smith, W 115
Ralph, MM 126  Sowerbutts, SF 85
Raychoudhury, SS 84  Spinks, N 5,89
Reed, KC 4  Squires, TJ 132
Reeve, J 21,22  Stanger, JD 18
Renfree, MB 71,102  Staples, L 20,21,22,23,24,25
Rice, GE 138  Stephenson, RGA 111
Risbridger, G 83  Stevenson, GT 65
Ritar, AJ 21,26,27,28  Stojanoff, A 122
Roberts, EM 55  Stone, GM 90,91
Robert, TK 6,18  Stuckey, BGA 13
Robertson, DM 38,86,139  Sujart, S 90
Robinson, E 72  Sumbaung, F 135
Robinson, JS 109  Sutton, R 43
Rodger, JC 103  Svalbe, ID 124
Rodgers, RJ 117  Swan, MA 96
Rogers, PAW 48,118  Swann, RT 106
Rogers, R 130  Taggart, DA 76
Rowsell, AL 124  Tait, RW 81
Runge, A 19  Tam, PFL 93
Ryan, JP 5,35,47,60,69  Tambi, M 97
Saunders, D 17,19  Taufa, VR 61
Scaramuzzi, RJ 147,148  Temple-Smith, PD 77
Seamark, RF 14,37,52,56,63,64  Thomas, GB 149
Sernia, C 146  Thomas, WG 146

Thompson, RI 13  Wong, MSF 39,53
Thompson, WF 97  Wright, PJ 136,140
Thorburn, GD 111,114,126,138  Young, IR 114
Thorne, MH 87  Yovich, J 7,15
Tilbrook, AJ 25,145  Yovich, JM 7,15
Todhunter, R 73  Yun-tian, S 86
Tolstoshev, P 38  Zhang, X 113
Torney, A 130  Zupp, JL 85
Trouwson, AO 3,8,9,10,48,49
Tsonis, CG 150
Turner, SR 15
Tyndale-Biscoe, CH 101
Uphill, G 131
Vishwanath, R 95,96
Vize, P 52
Voglmayr, JK 123
Wagg, M 21
Wales, RG 47
Walker, DW 111
Walker, SK 52,56,63,64
Walley, JRE 56
Walsh, A 91
Watson, TG 79,80
Weatherly, T 32
Webb, R 152
Webster, M 109
Wells, JRE 52
White, IG 12,25,96,123
Wiegand, MH 47
Wilkins, JF 67
Williams, A 20,21,22,23,24,25
Williamson, P 135
Wilmut, I 66
Wilson, J 13
Wilson, PA 39,53
Wilton, L 3,48
Windmill, D 80
Windsor, DP 12,123

(XXX)
HUMAN SPERM - ZONA PELLUCIDA BINDING TESTS USING OOCYTES THAT FAILED TO FERTILIZE IN VITRO

De Yi Liu, Alexander Lopata, Harold Bourne & H.W. Gordon Baker

University of Melbourne Department of Obstetrics & Gynaecology & Reproductive Biology Unit The Royal Women's Hospital Melbourne

Sperm binding to the zona pellucida (ZP) is an important preliminary step in human fertilization. To develop a test for sperm - ZP binding, human oocytes which failed to fertilize in vitro (no evidence of two pronuclei or cleavage 48 to 60 hours after insemination) were collected and used fresh or after preservation in 1M (NH$_4$)$_2$SO$_4$. The number of sperm bound to the ZP can be counted under the microscope (X 200) by rolling or crushing the oocyte under a cover slip. Oocytes from couples with any oocytes fertilized in IVF had significantly (p < 0.001) more sperm bound to the ZP (23 sem 3, n = 61, 32 patients) than did those from patients in whom all oocytes failed to fertilize (5.9 + 0.9 n = 79, 13 patients). However, the number of sperm bound to the ZP during IVF did not influence the number of sperm which bound subsequently (r = 0.125, NS). Because the number of sperm bound varies widely (0 - > 100/ZP) heterogenous inseminations with mixtures of equal concentrations of normal donor and test sperm differently labelled with fluorescent dyes (10 ug/ml, fluorescein isothiocyanate and 5 ug/ml tetramethylrhodamine B isothiocyanate) were used to control for this variability in the ZP. Washed fresh or salt stored oocytes were incubated at 37°C in 5% CO$_2$ in air with labelled sperm in 1ml Ham's F10 with 10% human sperm for various times then washed to remove loosely adherent sperm and the number of sperm tightly bound to the ZP was counted. Approximately 25% of ZP tested had less than 2 sperm bound and results for these were excluded. Time course studies showed that the number of sperm bound was relatively constant between 0.5 and 4 hours of incubation (14 + 2/ZP but increased at 8h (29 + 8) and 16h (41 + 11). Increasing sperm concentrations between 10$^0$ and 10$^7$ per/ml resulted in increasing numbers of sperm bound. Average sperm numbers were between 5 and 20/ZP with sperm concentrations of 5 - 20 x 10$^7$/ml and incubation times of 2 - 4 hours. Sperm from semen samples which failed to fertilize in IVF had lower ZP binding than did normal fertile sperm.

Conclusion: ZPs from oocytes which failed to fertilize in IVF will be useful for examining some aspects of sperm function.
FUNCTIONAL CORRELATES OF EMBRYO INTEGRITY AFTER CRYOPRESERVATION

**John Carroll, Peter Kaye and Jim Cummins**

Department of Physiology & Pharmacology and Animal Sciences Production, University of Queensland, St. Lucia, 4067

and **Department of Obstetrics & Gynaecology, University of Adelaide, S.A.**

Cryopreservation produces embryos which are morphologically normal and those which are irreversibly damaged. Since a significant proportion of the former group is not viable there must be critical damage not obvious in the normal microscopic examination. We have measured two biochemical functions which are likely to be susceptible to cryodamage: Na-dependent glycine uptake and endocytotic protein accumulation; in order to reveal this "subliminal" damage.

Embryos were collected from superovulated F1 mice in modified M2 medium (1). Some were frozen using 1.5 M dimethylsulphoxide (DMSO) and stored in liquid nitrogen (2). Those remaining, to serve as controls, were kept in M2 at room temperature and assayed as soon as the dehydrated embryos were placed in the programmable freezer. After storage in liquid N₂ overnight the frozen embryos were thawed at 8°/min and DMSO removed by equilibration with increasing concentrations of M2. They were sorted into groups containing embryos which were morphologically normal, > 50% blastomeres intact or < 50% blastomeres intact. Glycine uptake (1) or uptake and metabolism of [¹²⁵I]BSA (3) was measured as described.

Initial assays showed that 0.25 M DMSO, stepwise dehydration in 1.5 M DMSO and stepwise dehydration and rehydration did not alter glycine uptake by fresh 2-cell embryos or morulae. After freezing, 52 (±) 9% of 2-cell embryos and 83 (±) 7% of morulae survived. In 2-cell embryos and morulae which were normal after freezing, glycine uptake was about 90% of that of fresh embryos but this was not a statistically significant difference. In 2-cell embryos with only one cell intact uptake was 40% of that by normal embryos. No uptake occurred when both cells were damaged. Accumulation of [¹²⁵I]BSA was greater (P < 0.05) in normal frozen-thawed 2-cell embryos but not morulae, when compared to controls. In 2-cell embryos the rate of uptake of BSA increased as the number of blastomeres damaged increased.

The results show that 2-cell embryos are more susceptible to cryodamage than morulae and that this is reflected in endocytotic and possibly amino-acid transport functions of apparently normal embryos. Since these functions involve complex cytoskeletal and/or membrane changes, further analysis of this phenomenon may provide insight into the source of cryodamage to embryos.


This research was supported by a grant from MMBRC to PJK.

BLASTOCYST AND FETAL DEVELOPMENT OF MOUSE EMBRYOS BIOPSIED AT THE 4-CELL STAGE

Leenda J. Wilton, Carol A. Kirby and Alan O. Trounson,
Monash University Centre for Early Human Development, Queen Victoria Medical Centre, Melbourne.

Pre-natal genetic diagnoses including amniocentesis, chorionic biopsy and fetal heart puncture are performed at 6-14 weeks of gestation. Positive diagnoses usually result in elective termination of first trimester pregnancies. The development of IVF techniques has provided access to pre-implantation embryos and it has been suggested that if genetic testing could be done on the early embryo in vitro prior to implantation then "normal" embryos could be selected for uterine transfer, hence avoiding many first trimester abortions.

We have previously reported a micromanipulative technique for the biopsy of a single cell (blastomere) from 4 and 8 cell mouse embryos with in vitro embryo survival to the blastocyst stage of 39% and 85% respectively (1). We have concentrated on biopsy of 4 cell mouse embryos now report that modifications to the micromanipulative technique and post-biopsy culture have resulted in a very high embryo survival rate. Furthermore, we have demonstrated that mouse embryos biopsied at the 4 cell stage will form viable fetuses when transferred to recipient mice.

Four cell F1 mouse embryos were cultured in Ca²⁺/Mg²⁺-free medium 2 with EDTA for 1 hour. For biopsy each embryo was held in place by suction through a blunt glass micropipette. A sharpened micropipette was forced through the zona pellucida and a single blastomere gently drawn into the pipette and removed from the embryo. Biopsied embryos were cultured in microdrops of human amniotic fluid for a) 48 hours and scored for blastocele formation or b) 24 hours and transferred to the uteri of pseudopregnant recipient mice which were examined for fetal formation and implantation sites on day 15 of pregnancy. Control embryos were treated identically to experimental embryos but were not biopsied.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>BLASTOCELT</th>
<th>NUMBER EMBRYOS</th>
<th>%IMPLANTATIONS#</th>
<th>% FETUSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAsAL</td>
<td>TRANSFERRED</td>
<td>(MEAN+S.D.)</td>
<td>(MEAN+S.D.)</td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>97.6 (139)</td>
<td>121</td>
<td>59.1 ± 29.6</td>
<td>41.1 ± 28.4</td>
</tr>
<tr>
<td>BIOPSIED</td>
<td>94.4 (143)</td>
<td>121</td>
<td>45.2 ± 23.6</td>
<td>16.5 ± 16.3*</td>
</tr>
<tr>
<td># Implantations includes fetuses and resorptions.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We have markedly improved the success of biopsy of 4 cell mouse embryos as shown by the proportion that reach the blastocyst stage in vitro (Table 1). Biopsied embryos show an apparently normal implantation rate in foster mice but have a reduced ability to develop into fetuses, although the high variability makes interpretation of this result speculative. We are currently trying to optimize embryo micromanipulation, culture and transfer to improve fetal formation after embryo biopsy.

SEX DETERMINATION OF PRE-IMPLANTATION LIVESTOCK EMBRYOS

Margaret E. Matthews, Klaus I. Matthaei and Ken C. Reed
Department of Biochemistry, Faculty of Science
Australian National University
CANBERRA A.C.T. 2601

We have previously reported the isolation of a repeated sequence of DNA (BRY.1; bovine repeat, Y-linked) that is associated exclusively with the Y chromosome in domestic breeds of cattle and sheep (1). Further studies have led to the identification of two additional Y-specific repeats (BRY.2, BRY.3) that are closely linked to BRY.1 in both species.

It is possible to use such Y-specific DNA sequences as 'probes' in standard nucleic acid hybridizations to determine the genetic sex of individual animals. In principle, such analyses can be applied to minute tissue samples comprising no more than a few cells, and hence to small biopsies of embryos prior to their transplantation. Traditional methods of in situ hybridization to interphase chromosomal DNA are appropriate in a laboratory environment but the time, materials, and level of expertise required make it unsatisfactory for field work. These problems are compounded by an inescapable degree of subjectivity in interpreting results from a very small sample of cells. A group of French scientists has recently isolated a Y-specific repeated DNA sequence (different from the BRY repeats) and reported its application to bovine embryo sexing by in situ immuno-cytochemistry (2). The assay procedure required 30 hand yielded definitive data in 57% of biopsies from 7-8 day embryos.

We have developed an alternative in vitro assay that is capable of unambiguously detecting BRY.1 homologues in a single Y chromosome within 3 h. The assay procedure requires neither sophisticated instrumentation nor a high level of technical expertise for its implementation in the field. Preliminary data from commercial field trials will be available within 2 months.

References
In mice early pregnancy has been shown to be accompanied by thrombocytopenia, the extent of which is dependent on the number of fertilized eggs present in the reproductive tract (1). The decrease in platelet count occurs before implantation and can be induced by the transfer of fertilised eggs into the uterus. It is due to increased platelet activation and consumption caused by platelet activating factors from the fertilised egg (2). If present in other animals, particularly women or domestic animals, this thrombocytopenia occurs later in pregnancy.

Peripheral blood samples were collected by venepuncture from the jugular veins of 12 Corriedale ewes during the first 70 days of pregnancy. Day 0 was the day ewes were served by a fertile ram. Pregnancy was confirmed by failure of the ewe to return to service. Platelets were counted using a Coulter Counter. Significance was tested using Wilcoxon’s rank sum test. Results are summarised in the figure below. Each point shown is the mean (×10^9/l) of results for 9-12 samples. The asterisks indicate periods when platelet counts were significantly different from time 0 (*P<0.05, **P<0.01).

Peripheral numbers of platelets rose between 20 and 25 days after mating before declining to a nadir between 40 and 50 days. We conclude that, in contrast to the mouse, in the sheep implantation is accompanied by an increase in circulating platelets. However, a thrombocytopenia occurs later in pregnancy during the period of placentation growth.

ULTRA-RAPID FREEZING AND THAWING OF EIGHT-CELL MOUSE EMBRYOS

Anita Peura and Alan Trounson

Centre for Early Human Development, Monash University, Monash Medical Centre, Clayton Road, Clayton, Victoria.

Mouse 2-cell embryos may be frozen ultra-rapidly by plunging them directly into liquid nitrogen after a brief exposure to high concentrations of dimethyl sulfoxide (DMSO) and sucrose (1). The present experiments were undertaken to determine the survival rate and viability of 8-cell mouse embryos frozen in this way.

Eight-cell mouse embryos were obtained from superovulated immature C57B16xCBA(F1) mice 66-68 hours after HCG injection. Apparently normal uncompacted 8-cell embryos from superovulated mice were pooled and used for the experiments. The embryos were divided into Control (no treatment), Solution Controls (exposed to the solutions but not frozen) and two freezing groups (frozen in 2M or 3.5M DMSO). DMSO was made up in modified Dulbecco's phosphate buffered (PBl) containing 20% fetal calf serum (FCS) and 0.25M sucrose. Embryos were placed in 2M or 3.5M DMSO solutions and sealed in 0.25ml freezing straws for 3 mins and then plunged directly into liquid nitrogen. Embryos were stored for 1 day to 6 weeks in liquid nitrogen and thawed rapidly in a waterbath at 37°C. Embryos were expelled from the freezing straw into the PBl solution containing 0.25M sucrose but no DMSO and incubated in this for 8-9 mins at RT. Embryo survival was recorded, and surviving embryos were either cultured in T6 + 10% FCS for 24 hrs and transferred to foster mothers or cultured for 48 hrs and their stage of development and cell numbers scored.

The results of the study are summarized in Table 1. There was no significant difference in survival and viability between embryos frozen in 3.5M DMSO and their Solution Controls whereas embryos frozen in 2M DMSO had lower viability.

![Table 1](image)

We conclude that high survival and viability may be achieved with 8-cell mouse embryos using this extremely simple and rapid freezing method.


PARTHENOGENETIC ACTIVATION OF MOUSE OCCYTES BY PROPANEDIOL.

Shaw JM, and Trounson AO. Centre for Early Human Development, Monash University, Monash Medical Centre, Clayton, Victoria.

As a part of a study evaluating the safety of oocyte cryopreservation, we exposed unfertilized mouse oocytes to propanediol (PrOH) or dimethylsulfoxide (DMSO) to assess whether these cryoprotectants would cause parthenogenetic activation.

Oocytes were collected from superovulated immature Random Swiss (RS) or C57B16xCBA-F1 (F1) mice at known times after HCG administration. The oocytes were exposed to PrOH (1.5M), DMSO (1.5M), medium (untreated control), or a known parthenogenetic activator, ethanol (EtOH) (1.5M), for 4.5, 9, or 18 min at 1, 8, or 27°C. Following exposure the oocytes were thoroughly washed, and cultured in vitro either for a further 6h and assessed for activation, or for 3 days and assessed for blastocyst formation.

PrOH caused a dramatic increase in the proportion of eggs which degenerated. This effect increased with temperature and with time of exposure. More than 60% of RS oocytes (Table 1) and 19% of F1 oocytes degenerated after 4.5 min exposure to PrOH at 27°C. Among oocytes remaining viable the proportion of eggs which parthenogenetically activated was increased by both EtOH and PrOH, but not DMSO, with the effect tending to be more pronounced at the higher temperatures (Table 1). Work with F1 mice demonstrated that oocytes collected and exposed to EOH or PrOH 14 hr post HCG activated less readily (P<0.01) than oocytes collected and treated at 17-18 hr post HCG. Approximately 5% of oocytes exposed to PrOH or EOH developed to the blastocyst stage in vitro.

![Table 1](image)

We conclude that under the range of conditions tested, DMSO induced significantly less degeneration and parthenogenetic activation than did PrOH, and may be preferred to PrOH as a cryoprotectant.
VITRIFICATION INHIBITS THE ABILITY OF MOUSE OOCYTES FERTILIZED IN VITRO TO FORM Viable FETUSES.

Carol Kirby, Ismail Kola, Jillian Shaw, and Alan Trounson.

Centre for Early Human Development, Monash University, Monash Medical Centre, 172 Lonsdale Street, Melbourne, Australia, 3000.

The ovulated oocyte is in metaphase of the second meiotic division with chromosomes are gathered on the spindle. Magistrini and Szollosi (1) have reported that cooling down of such oocytes causes depolymerization of the spindle. It is thus possible that oocytes frozen at this stage could result in chromosome scatter, and give rise to embryos which develop abnormally. This study evaluates the viability of embryos derived from the in vitro fertilization of vitrified oocytes.

Unfertilized mouse oocytes were vitrified. After thawing the oocytes were fertilized in vitro and cultured to the 2-cell stage before transfer to pseudopregnant (Day 1) recipients. On day 15 the mice were sacrificed, and the number of implantations, resorptions and morphologically normal fetuses evaluated.

Table I: Development of embryos derived from the in vitro fertilization of vitrified oocytes.

<table>
<thead>
<tr>
<th>Group</th>
<th>No of oocytes</th>
<th>% fertilized</th>
<th>% implanted</th>
<th>% resorbed</th>
<th>% foetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>766</td>
<td>87</td>
<td>61% (70)</td>
<td>14% (22)</td>
<td>47% (35)</td>
</tr>
<tr>
<td>Vitrification</td>
<td>932</td>
<td>19</td>
<td>12% (63)</td>
<td>7% (61)</td>
<td>5% (26)</td>
</tr>
<tr>
<td>Solutions (10 mins)</td>
<td>663</td>
<td>26</td>
<td>16% (63)</td>
<td>7% (41)</td>
<td>10% (37)</td>
</tr>
<tr>
<td>Vitrification (5 mins)</td>
<td>767</td>
<td>19</td>
<td>8% (43)</td>
<td>5% (57)</td>
<td>3% (19)</td>
</tr>
<tr>
<td>Solutions (3 mins)</td>
<td>703</td>
<td>37</td>
<td>27% (73)</td>
<td>6% (23)</td>
<td>20% (36)</td>
</tr>
<tr>
<td>Slow-cooling (DMSO)</td>
<td>745</td>
<td>42</td>
<td>23% (53)</td>
<td>11% (45)</td>
<td>13% (49)</td>
</tr>
</tbody>
</table>

a - as a percentage of total oocytes b - as a percentage of fertilized oocytes c - as a percentage of implanted embryos.

These results demonstrate that vitrification of oocytes has a significant effect on the number of oocytes that are fertilized, number of fertilized oocytes that implant and number of embryos that get resorbed. Furthermore the number of preimplantation embryos that were chromosomally aneuploid was also significantly increased in the vitrified groups. We conclude that oocyte vitrification and freezing adversely affects the viability and developmental potential of embryos derived from these oocytes.

PREDETERMINATION OF SEX OF LAMBS BY SEGREGATION OF X & Y SPERMATOZOA ON PROTEIN COLUMNS

G. Evans\(^1\), H.N. Jabbour\(^1\), D.P. Windsor\(^2\) & I.G. White\(^2\)

Departments of Animal Husbandry\(^1\) & of Veterinary Physiology\(^2\)
University of Sydney, NSW 2006.

Sex predetermination has long been a goal of animal research workers. A technique of layering semen on protein columns (1) has been tested in sheep (2). Though the results of the latter trial were encouraging, the number of lambs was small. In this study, we wished to determine the efficacy of the technique on a larger scale.

Semen was collected from four Merino rams, pooled and processed as previously described (2). Briefly, the procedure involved layering 2 ml of semen diluted in Tris-citrate buffer on 6 ml of Tris-citrate buffer containing 6% BSA in a 10 ml test tube. After 2 h at room temperature, the layers were separated, the spermatozoa removed by centrifugation and resuspended in diluent containing egg yolk and glycerol before freezing by the conventional pellet method.

In March 1986 at Marulan, NSW, 395 mature Merino ewes were treated with intravaginal progestagen pessaries (Repromap, Upjohn) for 12 days and 350 IU PMSG (Poligon, Intervet) at pessary withdrawal. They were split into two groups and inseminated into the uterus with the aid of a laparoscope 61-68 hours later. The ewes received 50 x 10\(^6\) motile frozen-thawed spermatozoa obtained from either lower fraction (n=196) or the upper fraction (n=199) of the columns. Each group of ewes lambed naturally in separate paddocks, dead lambs were collected periodically, and live lambs were counted and sexed 13-15 days after expected lambing. Overall, 219 lambs were sexed, 68 of which were dead. The sex ratios of lambs recorded in each group are shown in Table 1.

Table 1. The sex ratio of lambs following insemination with spermatozoa obtained from the lower or upper fraction of protein columns.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Live Lambs</th>
<th>Dead Lambs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower</td>
<td>41(\pm)29g</td>
<td>24(\pm)18g</td>
<td>65(\pm)47g</td>
</tr>
<tr>
<td>Upper</td>
<td>41(\pm)40g</td>
<td>18(\pm)8g</td>
<td>59(\pm)48g</td>
</tr>
</tbody>
</table>

The data were analysed using a Z-test. More males (58%) than females (42%) were produced after insemination with sperm from the lower fraction (P<0.05). There was no significant deviation from a 50:50 male:female sex ratio of lambs produced from the upper fraction.

This experiment resulted in a less pronounced alteration of the sex ratio of lambs than that previously reported (2). However, the higher number of males produced from the lower fraction is in agreement with the results of the previous study and indicates that the technique may have practical application if it can be sufficiently refined.

ENHANCED IN VITRO SURVIVAL OF MOUSE EMBRYOS CO-CULTURED WITH UTERINE CELLS IN THE PRESENCE OF STEROIDS

T.C. Levandoski and R.F. Seemark
Dept. of Obstetrics & Gynaecology, University of Adelaide, S.A. 5000

The aim of the study was to establish an in vitro system to allow investigation of the cellular interactions of the uterus and embryos in the peri-implantation period. 8-cell mouse embryos were obtained from BALB-C/C57 3-4 week old F1 females, and were cultured in media alone, in the presence of a confluent monolayer of mixed uterine cells (co-culture), and in co-culture in the presence of steroids. Uterine cells were obtained by Trypsin-EDTA (0.05% Trypsin:0.02% EDTA in Earle's Balanced Salt Solution without Calcium and Magnesium) digestion of the uterine tubes obtained at the same time as embryo collection. The uterine cells were plated out in 4-well multidish (NUNC, West Germany) at a concentration of 1.3X10^6 cells per well, left at 37°C, 5% CO₂, in air for approximately 24 hours to allow for confluence prior to the addition of 10-20 embryos per well. The culture media was prepared from powdered Minimal Essential Medium (MEM: Eagle with Earle's Salts, with L-glutamine without sodium bicarbonate; Flow laboratories) supplemented with 10% fetal calf serum (CSL, Australia) and penicillin, streptomycin, fungizone as the antibiotic (CSL, Australia). The effects of steroids on the viability of embryos in co-culture were studied by the addition of either or both of the following steroids: (1) Estradiol (E2, 10μg/ml, Steraloids Inc., USA); (2) Progesterone (P, 1μg/ml, Steraloids Inc., USA). Media in wells was not changed over the culture period. The development was scored each day over the 5 day culture and final survival was recorded as the number of embryos which implanted, i.e. showed outgrowth on the plastic dish when cultured in media alone and when co-cultured showed invasive outgrowth on the uterine monolayer.

The results are shown in Table 1. In vitro development of murine embryos cultured in media alone (control) or co-cultured with uterine cells with or without added steroids

<table>
<thead>
<tr>
<th>Number of Embryos</th>
<th>% Blastocyst</th>
<th>% Implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (E2)</td>
<td>69.5</td>
<td>30.2</td>
</tr>
<tr>
<td>Co-culture (E2)</td>
<td>75.9</td>
<td>56.0</td>
</tr>
<tr>
<td>E2 + P</td>
<td>89.9</td>
<td>74.8</td>
</tr>
</tbody>
</table>

* Denotes significant difference from control group (p > 0.01, Chi-square analysis)

Co-culture with uterine monolayers led to a significant enhancement in embryo survival in the absence of steroids. Once steroids were added either alone or in combination, embryo survival significantly increased. This technique provides an in vitro system which allow 86.8% of 8-cell embryos to survive 5 days in culture and is now being employed in the study of cellular interactions of the uterus and the embryo, in particular, the study of protein secretion by both the embryo and uterus during the implantation period. In addition, co-culture is being investigated as a means of improving the survivability of embryos in embryo-manipulation and transfer programs.

HUMAN PREGNANCIES FOLLOWING THE TRANSFER OF PRONUCLEAR OCYTES AND CLEAVING EMBRYOS TO THE FALLOPIAN TUBES

PIVET Medical Centre, 166-168 Cambridge Street, Leederville, Perth, Western Australia 6007.
*Research Fellow, Dept. of Obstetrics & Gynaecology, University of Western Australia.

Pronuclear stage tubal transfer (PROST) is a technique which involves in-vitro fertilisation of oocytes which have usually been recovered by transvaginal ultrasound-directed techniques (1). The following day, pronuclear oocytes are transferred into the fallopian tubes. The technique was developed as an extension of our gamete intrafallopian transfer (GIFT) programme applied for non-tubal infertility cases (2). It has the prognostic value of confirming fertilisation in those couples with oligospermia or asthenospermia and enables fertilisation in cases with antispermatozoal antibodies. PROST has provided useful diagnostic information for the management of couples who have failed to conceive in other treatment programmes such as GIFT and has particular advantages over IVF for those receiving fresh donated oocytes for ovarian failure. Fourteen pregnancies resulted from 52 transfers, providing a pregnancy rate of 27% per transfer. The pregnancy rates were higher than a matched IVF series in the male factor and female antisperm antibody groups and reached statistical significance for the ovum donation group. More recently, cleaving embryos at the 2-cell, 4-cell and 8-cell stages were transferred in the procedure known as TEST (Tubal Embryo Staging Transfer), following the thawing of cryopreserved embryos, with 2 pregnancies from 4 transfers. The preliminary data suggests that both pregnancy rates and fetal wastage will be improved over conventional in-vitro fertilisation and embryo transfer techniques for the described infertility groups.3 At this stage we are uncertain if the favourable results are due to a tubal factor conferring an improved chance of implantation and subsequent embryo development, or the tubal environment simply providing a favourable culture milieu for early embryos which might otherwise be treated unfavourably within the early post-ovulatory uterine environment.

THE ROLE OF EMBRYO-DERIVED PAF IN IMPLANTATION

C O'Neill

Human Reproduction Unit, Royal North Shore Hospital of Sydney, 2065

The production of embryo-derived PAF is essential for the establishment of pregnancy in mice (1). Antagonists of PAF inhibit implantation and exogenous PAF results in the return to normal implantation rates. This study was designed to assess the direct role of PAF on embryos by studying blastocyst outgrowth in vitro in the presence of PAF antagonist.

Two antagonists were used: Iloprost (Schering AG, Berlin) inhibits PAF action on platelets by elevating intracellular cAMP levels and SRI 63-441 (Sandoz Research Institute, East Hanover, NJ) competitively binds to PAF receptors on the plasma membrane.

Embryos were grown from the 2 cell to the expanded blastocyst stage over 72 hr in protein free HET medium, in 10 ul drops under paraffin oil. Blastocysts were transferred to 10 ul drops of minimal essential medium (Flow Labs, Sydney) with 1% FCS (Flow Labs), containing various concentrations of Iloprost or SRI 63-441. After 48 hr the cultures were examined and the number and extent of blastocyst outgrowth were recorded.

Table I: The proportion (%) of mouse blastocysts undergoing normal outgrowth in presence of the PAF antagonists Iloprost and SRI 63-441.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Iloprost (ug/ml)</th>
<th>SRI 63-441 (ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>(n) (x S.E.M) (n)</td>
<td>(n) (x S.E.M) (n)</td>
</tr>
<tr>
<td>1</td>
<td>0 ± 0 35</td>
<td>10 0 ± 0 10</td>
</tr>
<tr>
<td>10</td>
<td>21 ± 12 28</td>
<td>10 19 ± 1 25</td>
</tr>
<tr>
<td>100</td>
<td>28 ± 13 30</td>
<td>10-2 45 ± 11 35</td>
</tr>
<tr>
<td>1000</td>
<td>32 ± 15 25</td>
<td>10-3 71 ± 15 30</td>
</tr>
<tr>
<td>10,000</td>
<td>55 ± 14 25</td>
<td>control 65 ± 14 45</td>
</tr>
<tr>
<td>control</td>
<td>53 ± 17 30</td>
<td></td>
</tr>
</tbody>
</table>

Iloprost and SRI 63-441, which have both been shown to inhibit implantation in vivo are here shown to inhibit in a dose-dependent manner the ability of mouse blastocysts to undergo outgrowth in vitro. Such outgrowth is generally considered to be of equivalent to implantation in vivo. The results suggest that PAF acts on blastocysts, and this is required for implantation. The effect of SRI 63-441 suggests that PAF acts via membrane receptors.

(1) Spinks NR and O'Neill C (1987). Lancet, 1, 106

REFERENCES

FURTHER STUDIES ON PLATELET ACTIVITY DURING EARLY MURINE PREGNANCY

L.M.Adamson, Y.C.Smart, J.D.Stanger and T.K.Roberts
University of Newcastle and Lingard Hospital, Newcastle, N.S.W.

Early pregnancy associated thrombocytopenia (EPAT) has been demonstrated as an initial response to a fertile mating in the murine system. (1,2) Studies using embryo culture media (ECM) suggests that the platelet activity is mediated by an embryo derived soluble factor (EPAT-factor). (2,3) EPAT-factor is thought to be related to a potent phospholipid mediator of platelet activation, PAF-acether. (3,4) We have previously shown that pre-mating injection of PAF-acether causes reduced implantation rate. (4) We concluded from this that PAF-acether inhibits the EPAT event by inducing platelet desensitization.

This paper reports an extension of our observations to pre-mating ECM administration. ECM or control culture media (CM, containing no embryos) was injected into two groups of OS mice (N=12 and N=14 respectively) on three consecutive days prior to mating. ECM samples were collected from cultures in which groups of >10 embryos had grown from the one cell stage to the blastocyst stage (i.e. 5 days). All ECM samples were pooled and injected neat in 400μl volumes. The mice were sacrificed on day 10 post-mating and the number of implantation sites counted. Results (Table 1) showed that pre-mating administration of ECM causes a decline in implantation rate. Findings also suggest that ECM shows different characteristics in induction of platelet desensitization than that demonstrated by PAF-acether. This may indicate an ECM-induced alteration to the period of uterine receptivity to the implanting embryo.

Table 1: Number of animals with zero or > zero implantation sites evident on day 10 after ECM and CM treatment.

<table>
<thead>
<tr>
<th>No. Implantation Sites</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ECM</td>
</tr>
<tr>
<td>&gt; zero</td>
<td>4</td>
</tr>
<tr>
<td>zero</td>
<td>8</td>
</tr>
<tr>
<td>total</td>
<td>12</td>
</tr>
</tbody>
</table>

* X^2 = 10.12, p < 0.005


PLATELET ACTIVATING FACTOR STIMULATES PROGESTERONE PRODUCTION BY HUMAN GRANULOSA CELLS IN VITRO

A.C. Runge, C O'Neill, M Bonifacio, D.M. Saunders

Human Reproduction Unit, Royal North Shore Hospital of Sydney, 2065.

It has been reported for a number of species that the peripheral progesterone (P_4) concentration in the early luteal phase (pre-implantation) is higher in conceptual compared with non-conceptual cycles. A possible explanation would be the production of a pre-implantation embryonic luteotropic stimulus. Since embryo-derived PAF is the only characterized preimplantation embryonic signal, its luteotropic potential was tested in vitro.

Granulosa cells were collected from women undergoing in vitro fertilization. Women had ovarian hyperstimulation with combined human menopausal gonadotrophin and Clomiphene citrate. Based upon peripheral E_2 concentration and ovarian ultrasonography, final ovarian maturation was induced with hCG (1500 iu). Approximately 36 hr later, follicles were aspirated using the ultrasound guided transvaginal approach. Follicular fluids from any one patient were examined and pooled for all follicles which yielded a pre-ovulatory oocyte.

The cells were washed twice by centrifugation with minimal essential medium (MEM) containing 1% FCS and resuspended to a final concentration of 50-100 x 10^6/ml in MEM 10% FCS. 1 ml aliquots were prepared in Costar multi well dishes and cultured overnight. The media was replaced after 24h and a further 24h culture was allowed. If normal monolayer development of the culture had been initiated then cultures were continued for a further 24h in medium with 0.3% BSA as the only protein and containing various concentrations of PAF. Cultured media was collected and progesterone measured by radioimmunoassay. Cells were washed with 0.9% saline and then dissolved in IN NaOH. The protein content of the cells was determined (Bio Rad) and the results expressed as pg/ml P_4/g cell protein. To test that the effects were PAF specific, some cultures were performed in the presence of the PAF antagonist SRI 63-441.

PAF at low doses consistently enhanced the P_4 production by human granulosa cells in culture. The dose response was variable from subject to subject. But a typical quadratic effect was observed. Doses of 0.1 to 10³ µg/ml PAF resulted in 40-80% increase in P_4 production (Control: 11.8 pg/ml P_4/g protein. log PAF/mgl : 20.5 pg/ml P_4/gg protein) while higher doses were not different from control. SRI 63-441 (5 µg/ml) consistently inhibited the enhancement of production by granulosa cells.

These results show that PAF specifically enhances P_4 production by human granulosa cells in vitro. It may provide support for PAF as a preimplantation luteotrophin but it remains to be determined whether PAF, which is relatively labile in blood, exerts the same effect in vivo.
**FIELD STUDIES OF THE USE OF MELATONIN IMPLANTS TO IMPROVE LAMBING PERCENTAGES IN SPRING JOINED MERINO & CROSSEDBRED EWES**

K. Croker\(^1\), E. Dunstan\(^2\), J. Reeve\(^3\), A. Williams\(^4\), S. McPhan\(^5\), B. Ayton\(^6\), M. Wagz\(^7\), T. Pollard\(^8\), J. Parker\(^9\), M. Foote\(^10\), A. Ritar\(^11\), & L. Staples\(^12\), Depts. of Agri. WA\(^1\), SA\(^2\), Vic\(^3\), NSW\(^4\), Tas\(^5\) & Private Consultant\(^6\).

Development of REGULIN s/c implants (Gene Link Aust. Ltd.) for continuous dose of Melatonin to grazing animals has enabled several field studies on the effects of such treatments on reproductive performance at Spring joining. This paper collates data from 12 trials and 23 comparisons conducted during 1985 and 1986 in a collaborative programme to test various treatments in moderately seasonal breeds.

Treatments tested were (1) a sequence of 2 implants at -6 & -2 weeks relative to joining, (2) 1 implant at -4 weeks. (3) 1 implant at -3 weeks, & (4) 2 implants at -4 weeks. Breeds tested were mature Merino (M), maiden Merino (m), mature BLxM (B), mature Polworth (P), mature & maiden Merino x Dorset (D & d). Joining times ranged from early Oct. to late Jan., with most flocks joined in Nov. or Dec.

Mating and conception patterns were assessed by use of harnessed rams (typically 2-3%). Fertility & fecundity were assessed by mid pregnancy sonography.

Over all, treatments improved the response to "ram effect" to give a more precisely-timed and shorter joining period only in those flocks where control responses to ram effect were poor. REGULIN did not affect mating or conception patterns in flocks spontaneously cycling, or in which control response to ram effect was already good.

Fecundity (foetuses/ewe pregnant) was increased by treatment in all trials by a mean of 16% above relevant controls (range 4-33%) and the proportion pregnant was increased by a mean of 3% with an increase apparent in 75% of treated groups (an increase of 41% in fertility in one flock of maiden Merinos was excluded from the mean). These effects combined to give a mean increase in flock performance of 20-4 additional foetuses/100 ewes treated (Table 1).

Table 1. Increased number of foetuses/100 ewes treated coded according to breed and treatment strategy (TREAT.).

<table>
<thead>
<tr>
<th>TREAT.</th>
<th>m</th>
<th>M</th>
<th>B</th>
<th>P</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>+19</td>
<td>+26,+21,+27,+28</td>
<td>+16</td>
<td>+16</td>
<td>+18</td>
</tr>
<tr>
<td>(2)</td>
<td>+59</td>
<td>+12,+9,+26,-3</td>
<td>+4,+17,+12</td>
<td>+24</td>
<td>+8</td>
</tr>
<tr>
<td>(3)</td>
<td>+20</td>
<td>+26,+8</td>
<td>+36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

REGULIN modestly boosted potential lambing percentages in Merino & Crossbred ewes joined prior to the Summer solstice. These results cannot be equated directly with British breed ewes. These studies were sponsored by Gene Link Australia Limited.

**COMPARISON OF IPSILATERAL AND CONTRALATERAL JUGULAR VENOUS MELATONIN LEVELS IN EWES TREATED WITH REGULIN IMPLANTS.**

A. Howse, D. Kennaway\(^1\), F. Carbone\(^2\), L. Staples & A. Williams


Jugular venous (J.V.) concentrations of exogenous melatonin are often compared to the normal J.V. level measured during scotophase to assess the physiological significance of an exogenous dose. In making such comparisons for doses given at the base of the ear (e.g. REGULIN, Gene Link Aust. Ltd.) it is important to consider the anatomical relationships between the site of sampling and the treatment site.

Comparison was made between daylight melatonin levels, measured by direct RIA, in the ipsilateral and contralateral jugular veins after s/c insertion of a REGULIN implant at the base of the ear. Plasma profiles were also assayed following treatment with prototype handmade implants or with implants made by an automated procedure.

Table 1. Mean (+/- SEM) J.V. melatonin levels (pM) from samples obtained on days 1,2,3,6 and 9 after implantation.

<table>
<thead>
<tr>
<th>Venous level</th>
<th>Replicate 1 (3 ewes)</th>
<th>Replicate 2 (2 ewes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipsilateral</td>
<td>4368+/−866 (15 samples) 6188+/−999 (10 samples)</td>
<td>845+/−83 (14 samples) 1303+/−145 (10 samples)</td>
</tr>
<tr>
<td>Contralat.</td>
<td>1000+/−150 (15 samples) 1400+/−200 (10 samples)</td>
<td>1000+/−150 (15 samples) 1400+/−200 (10 samples)</td>
</tr>
</tbody>
</table>

Table 1. Mean (+/- SEM) J.V. melatonin levels (pM) from samples obtained on days 1,2,3,6 and 9 after implantation.

Melatonin levels from the ipsilateral J.V. contained 4.8-5.2 times the contralateral venous level. The implants are close to the junction of several muscles whose venous drainage is into the ipsilateral jugular vein. Since there is very little venous crossover, this explains the elevated concentrations in local samples. The REGULIN implants (18 mg melatonin) maintained peripheral venous concentrations at about the scotophase J.V. level (which is itself about twice the true peripheral level) for approximately 70 days with near zero order kinetics. These implants thus provide a reliable method of continuous melatonin dosage over this period.

This work was sponsored by Gene Link Aust. Ltd.
SEQUENTIAL STUDY OVER TWO YEARS OF THE MODULATION BY MELATONIN OF EARLY BREEDING PERFORMANCE IN EWES.

J. Reeve, A. Williams, R. Peake, S. McPhee, B. Ayton & L. Staples

Dept. Agric. & Rural Affairs, Research Institutes at Rutherglen and Werribee, Vic., 3030

Studies of ovulation rate following the use of REGULIN treatment (Gene Link Aust. Ltd.) to improve early breeding performance suggested that long term effects may occur (1). To assess whether treatments would be effective in a second year or whether residual effects would be seen after treatment in only 1 year, BLxM ewes which had been treated with REGULIN in 1985 (2) were either untreated (T-C, n=50) or treated again (T-T, n=50) before joining on 11th Nov in year 2. Responses in year 2 were compared with the performance of similar ewes which had never been treated (C-C, n=50). Measurements in year 2 included mating patterns and mid pregnancy sonography.

Table 1. Foetuses per ewe in group (F/E), and conception day (CD) of ewes treated with REGULIN.

<table>
<thead>
<tr>
<th>1985 Joining Date Group</th>
<th>C-C</th>
<th>T-C</th>
<th>T-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/E CD</td>
<td>F/E CD</td>
<td>F/E CD</td>
<td></td>
</tr>
<tr>
<td>30 Sept A 0.91 24.7 1.00 24.7 1.08 27.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31 Nov B 1.12 23.8 1.03 22.4 1.00 22.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23 Dec C 1.02 23.8 1.08 23.8 1.14 22.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Feb D 1.02 26.3 1.06 24.9 1.26 23.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 Mar E 1.06 24.2 0.91 24.3 1.22 24.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean 1.03 24.6 1.02 24.0 1.14 23.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Performance of ewes which had been treated in year 1 but not in year 2 was not consistently different from ewes which had never been treated (difference in F/E = -1, range -15 to +9). All ewes responded well to the “ram effect” regardless of prior treatment history.

Performance of ewes which received treatment in both years was increased from control in 4 out of 5 groups (mean increase +11, range -1 to +24). Responses in the second year were lower than those observed in the same ewes in their first year of treatment (2). Overall the study suggests that REGULIN treatment does not cause long term effects in the subsequent season but that repeat treatment gives a modest increase in fecundity in both years.

This study was supported by Gene Link Australia Limited

INDUCTION OF AN EARLIER JOINING AND AN IMPROVEMENT OF KIDDING PERCENTAGE BY USE OF MELATONIN IMPLANTS IN ANGORA DOES

S. McPhee, B. McGregor, A. Williams, B. Ayton and L. Staples
Dept. of Agric. & Rural Affairs, A.R.I. Werribee, Vic. 3030.

Studies in several breeds of sheep (1,2) have successfully optimized treatment strategies for use of "Regulin", a (s.c.) melatonin implant to improve early reproductive performance. This study tested the efficacy of Regulin in Angora goats.

A commercial herd of 207 mature Angora does located at Boort, Victoria (lat. 36° 08' Sth) were held in isolation from bucks or rams and allocated on liveweight (mean 44.1 kg) to control (C) and treated groups (T). The T group received a (s.c.) implant of Regulin 4 weeks prior to the commencement of mating. Does were mated in three replicates of C and T. Each replicate (n = 34 or 35) was mated to a harnessed single sire.

Table 1. Foetuses/doe in group, foetuses/doe pregnant, minimum period over which 80% of does conceived (CP days), mean time from joining to mating and to conception.

<table>
<thead>
<tr>
<th>Group</th>
<th>Foetuses/doe in Group</th>
<th>Foetuses/doe Pregnant</th>
<th>80% CP</th>
<th>Mean Mat Day</th>
<th>Mean Conc Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>104</td>
<td>1.04 a</td>
<td>1.46 a</td>
<td>41 a</td>
<td>38</td>
</tr>
<tr>
<td>T</td>
<td>69</td>
<td>1.45 b</td>
<td>1.64 a</td>
<td>28 a</td>
<td>26</td>
</tr>
</tbody>
</table>

Values with different superscripts differ significantly P<0.01.

One T group (n = 34) was excluded from the tabled results because the buck died prior to day 28. Foetuses/doe in this group and mean conception day after replacing the buck were 1.35 and 49d respectively. The proportion of does not pregnant was less (P<0.01) for T (11.6%) than C (28.6%) and the proportion of does bearing multiples was greater (P<0.01) for T (52.9%) than C (31.4%).

Regulin treatment of Angora does in November for a December joining provided a practical method of obtaining a defined early joining with a substantial increase in foetal percentage and decreased incidence of non pregnant does.

This study was supported by Gene-Link Australia Limited.


EFFECT OF MELATONIN TREATMENT ON THE EARLY REPRODUCTIVE PERFORMANCE OF ANGORA BUCKS AND DOES

C. Earl, S. McPhee, A. Williams, E. Dunstan, A. Tilbrook, B. Ayton and L. Staples

A continuous delivery of melatonin, "Regulin", provides a mechanism to advance the onset of the breeding season and obtain an earlier seasonal peak of ovulation rate in sheep and goats (1,2). Treatments commencing 6, 4 and 3 weeks prior to joining have been shown to induce more reliable early joining and increased fecundity.

Previous trials have not assessed whether it is necessary to also treat males or whether there are any advantages from extending the duration of melatonin treatment into the joining period.

A commercial flock of Angora does located near Keith, SA (lat.36°06'S) were allocated by body weight (mean 32kg) to four groups, (CC) bucks and does not treated, (BC) bucks only treated, (CD) only does treated, (BD) bucks and does treated. Treatment consisted of Regulin implants (Gene Link Australia Ltd) given to bucks or does at 4 weeks prior to joining (1/1/87). Groups were mated separately with 3% harnessed bucks. Oestrus pattern and foetal percentage (by sonography) were recorded (Table 1).

Table 1. Foetuses/doe in group (F/D), Foetuses/doe pregnant (F/DP), % not Pregnant, % bearing multiples, % conceiving by day of mating.

<table>
<thead>
<tr>
<th>Gr</th>
<th>n</th>
<th>F/D</th>
<th>F/DP</th>
<th>% Not Pregnant</th>
<th>% Multiples</th>
<th>% Conc. by day</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>99</td>
<td>0.92c</td>
<td>1.32b</td>
<td>25.3b</td>
<td>27.3ab</td>
<td>59a</td>
</tr>
<tr>
<td>BC</td>
<td>98</td>
<td>0.92b</td>
<td>1.27b</td>
<td>18.4a</td>
<td>62a</td>
<td>72ab</td>
</tr>
<tr>
<td>CD</td>
<td>87</td>
<td>1.30b</td>
<td>1.38b</td>
<td>5.7a</td>
<td>35.6bc</td>
<td>lb</td>
</tr>
<tr>
<td>BD</td>
<td>93</td>
<td>1.37a</td>
<td>1.55a</td>
<td>11.8a</td>
<td>48.4c</td>
<td>lb</td>
</tr>
</tbody>
</table>

Values with different superscripts differ significantly P<0.05.

Extended melatonin treatments of does increased fecundity and decreased the proportion non-pregnant to give an extra 38 kids expected/100 does treated. However, in contrast to previous studies, conception patterns were delayed. Treatments of bucks offered no consistent advantage.

The study was sponsored by Gene Link Australia Limited.

OVULATION IN CASHMERE GOATS AFTER TREATMENT WITH CIDRS AND PMSG
A.J. Ritar, P. Ball, T.M. Black and R.B. Jackson
Department of Agriculture, P.O. Box 180, Launceston South, Tasmania, 7249

Treatment of female goats, sheep and cattle with Controlled Internal Drug Release (CIDR) devices suppresses oestrus and ovulation (1). As with progestagen sponge treatment (2) does may need to be injected with PMSG at or around CIDR removal to ensure that they all ovulate.

The experiment reported here examined time and rate of ovulation in cashmere goats after CIDR removal. Mature does weighing 29-44 kg were treated with Type G CIDRs (AMI Plastic Moulding Co., N.Z.) for 18 days. Does were injected with 200 I.U. or 400 I.U. PMSG either at (0 h) or 48 h before (-48 h) CIDR removal, and one other group received no PMSG. Animals were examined by laparoscopy at five-hourly intervals between 35 and 45 h (-48 h group) or between 45 and 55 h (0 h group) after CIDR removal to determine whether ovulation had occurred. Laparoscopy was repeated the following day (65-75 h after removal) to determine final ovulation rates. Results are presented in Table 1.

TABLE 1. Number of animals having ovulated and the rate of ovulation/does ovulating at each time of laparoscopy.

<table>
<thead>
<tr>
<th>Time of PMSG (h)</th>
<th>Progesterone device</th>
<th>Does ovulating at each time of laparoscopy (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>CIDR</td>
<td>0: 10 40 50 55 Final 40 10 5 (1.0) 2 (1.5) 5 (1.2) 7 (1.6)</td>
</tr>
<tr>
<td></td>
<td>Sponge</td>
<td>0: 10 5 (1.6) 8 (1.6) 10 (1.8) 11 (1.7) 1.4 (1.3) 9 (1.9) 11 (2.6) 21 (1.6)</td>
</tr>
<tr>
<td>0 h</td>
<td>Sponge</td>
<td>- 7 (1.4) 10 (2.2) 21 (1.6) 12 (2.2) 21 (1.6) 12 (2.2) 12 (3.1)</td>
</tr>
</tbody>
</table>

A higher ovulation rate was induced by injecting 400 I.U. than 200 I.U. PMSG (2.87 ± 0.32 v. 1.95 ± 0.13; P<0.02). All does receiving either 200 or 400 I.U. PMSG ovulated whereas only 7/10 does with no PMSG ovulated within the 75 h after CIDR removal. Injection of PMSG at -48 h induced ovulation around 35-40 h and almost all does (21/22) ovulated by 45 h. Injection at 0 h resulted in a delay in ovulation to approximately 10 h later (at 45-50 h) and almost all does (21/22) ovulated by 55 h. A higher proportion of does ovulated by the first laparoscopy for the 400 I.U. than the 200 I.U. PMSG treatment (X² = 6.63; P<0.02). Between does, ovulation occurred over a period of more than 10 h. For both -48 h and 0 h treatments, ovulation within some does (with multiple ovulations) occurred over a 5-10 h period (data not shown).


Supported by Rural Credits Development Fund.

PMSG INJECTION AT OR 48 H BEFORE CIDR OR SPONGE REMOVAL
Department of Agriculture, Launceston South, Tasmania

Injection of PMSG 48h before rather than at intravaginal progestagen removal may improve subsequent fertility to AI. This study examined the fertility following intrauterine insemination with frozen-thawed semen after injection of PMSG 48h before or at removal of CIDRs or sponges.

Two groups of cashmere does (Group 1: weaned 25 days before AI; Group 2: weaned at least 3 months before AI) were treated with either Type 0 CIDRs (AMI Plastic Moulding Co.) or 45 mg chronogest sponges (Intervet) for 19-20 days. PMSG (400 I.U. Proligon, Intervet) was injected at 48h before (-48h) or at (Oh) device removal. Does were sedated with Rumpun and were inseminated shortly after the estimated mean time of ovulation (1, 2); namely, at +40 h for CIDR, -48h to +40h for sponges, -48h, and at +40h for sponges, Oh. Pellet-frozen semen was thawed and deposited into each uterine horn (total volume = 0.12 ml containing approximately 50 x 10^6 motile cells) via the laparoscope. Conception rates to AI are based on Day 64 non-return rates.

Table 1. Fertility following insemination of does treated with CIDRs or sponges and PMSG injected at -48h or Oh.

<table>
<thead>
<tr>
<th>Progesterone Time PMSG</th>
<th>Does inseminated (%) conceived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Device (h) Group 1 Group 2 Overall</td>
<td></td>
</tr>
<tr>
<td>CIDR</td>
<td>26 (50.0) 17 (64.7) 43 (55.8)</td>
</tr>
<tr>
<td>-48 23 (50.9) 18 (66.7) 41 (63.4)</td>
<td></td>
</tr>
<tr>
<td>Sponge</td>
<td>24 (41.7) 17 (76.5) 41 (56.2)</td>
</tr>
<tr>
<td>-48 23 (43.8) 17 (82.4) 40 (60.0)</td>
<td></td>
</tr>
</tbody>
</table>

The results in Table 1 show that the overall conception rates did not differ for CIDRs and sponges (50/84, 59.5% v. 47/84, 56.0% resp.). The 5.7% difference in fertility following treatment with PMSG at -48h and Oh (50/81, 61.7% v. 47/84, 56.0% resp.) was not significant. The group of does weaned only 25 days before insemination (Group 2) had a much lower conception rate than Group 2 does which were weaned at least 3 months beforehand (47/96, 49.0% v. 50/69, 72.8%; X² = 9.155, P<0.005) suggesting a carry-over effect of the previous lactational stress on fertility.

Supported by Rural Credits Development Fund.

INTRAVAGINAL INSÉMINATION OF CASHMERE GOATS AFTER PMSG INJECTION AT OR 48 H BEFORE CIDR OR SPONGE REMOVAL
AI OF CASHMERE GOATS: EFFECT OF CIDR OR SPONGE, DOSE OF FROZEN-THAWED SEMEN, AND TIME OF CERVICAL OR LAPAROSCOPIC INSEMINATION

A.J. Bitar, P. Ball, T. Black, R.B. Jackson, P. O'May, F. Heazlewood, and G. Graham

Department of Agriculture, Launceston, Tasmania

Large-scale commercial application of AI in goats needs to be based on the use of frozen-thawed semen at the controlled estrus and ovulation. Fertility results are presented for goats inseminated with one of several doses of frozen-thawed spermatozoa either into the cervix or via the laparoscope into the uterus before or after the time of ovulation controlled by CIDRs or sponges.

Cashmere does were treated with either Type G CIDRs (ARI Plastic Moulding Co.) or Chronogest sponges (Intervet) for 15-20 days and injected with 200 I.U. PMSG (Folligon, Intervet) at the time of device removal. Pellet-frozen and thawed semen from 5 cashmere bucks was used for insemination at three dose rates of motile spermatozoa either into the cervix (160 v. 120 v. 80 x 10^6; mature does only) or via the laparoscope into the uterus (60 v. 30 v. 15 x 10^6; maiden and mature does). Does were inseminated either before or after the estimated time of ovulation (45 h or 55 h, CIDR; 55 h or 65 h, sponge). Conception rates to AI are based on Day 42-46 non-return rates.

There was no difference in the conception rates after CIDR and sponge treatment (138/502, 61.6% v. 75.0%). Preliminary results with a higher sperm dose rate (280 x 10^6) did not differ, 0.28 vs 0.14; P < 0.01). For the 3 years separately, conception rates were 0.43, 0.48, and 0.44 for the 3 years, respectively. By contrast, conception rates were significantly higher (P < 0.001) for cervical insemination (66.5%) than for laparoscopic insemination (56.7%); P = 0.012.

In 1982, a twin breeding experiment was set up using 69 mature 'twinning' cows (T) with at least 2 previous sets of twins each and 29 control cows (C) balanced as far as possible for breed and age. Ovulation data were all obtained by one veterinarian after ovarian palpation per rectum, during the 8-week mating period in spring/summer each year (1983-85), totalling 1051 records from separate oestrus cycles. Results were classified by pregnancy status at a subsequent palpation; for non-pregnant cows, further ovulation recordings were made in the autumn/winter (1984-85). At each palpation the veterinarian was given no previous cow data.

Table 1. Table 1. Conception rates following cervical and laparoscopic insemination of different doses of motile spermatozoa.

<table>
<thead>
<tr>
<th>Dose (x10^6)</th>
<th>% Conceived</th>
<th>Dose (x10^6)</th>
<th>% Conceived</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>137</td>
<td>43.8</td>
<td>120</td>
</tr>
<tr>
<td>120</td>
<td>133</td>
<td>52.0</td>
<td>120</td>
</tr>
<tr>
<td>180</td>
<td>120</td>
<td>65.7</td>
<td>180</td>
</tr>
</tbody>
</table>

Fertility did not improve with an increase in the sperm dose rate for either cervical or laparoscopic insemination. Conception rates for laparoscopic AI (maiden: 146/211, 68.6%; matures: 250/363, 68.9%) were higher (P < 0.0001) than for cervical AI (maures only: 203/408, 49.8%). Insemination before the estimated mean time of ovulation results in higher fertility than AI 10 h later for CIDRs (173/260, 66.5% v. 136/242, 56.2%); P = 0.05) and for sponges (147/228, 64.5% v. 143/252, 56.7%); P = 0.10).

Commercially acceptable fertility rates are therefore obtainable for both cervical and laparoscopic AI using frozen-thawed semen. Supported by the Rural Credits Development Fund.
Morris and Day (1) have described an experiment to study the rates of ovulation and twinning in cows selected for twinning (T) or in control cows (C). Mean twin calving rates (1984-86) were 0.092 and 0.011 respectively and corresponding ovulation rates (the proportion of double and multiple ovulations) were 0.276 and 0.144. In the 2 herds overall, respectively 0.33 and 0.08 double and multiple ovulations led to twin calvings.

The objective here is to report on the rate of bilateral and unilateral double ovulations recorded in T and C cows conceiving at that double ovulation. Data from the 2- and 3-year-old daughters were also included. These records were augmented by those from 2 large private Milking Shorthorn herds with high twinning rate. Averaged over both private herds, the twinning rate was 0.031, the proportion of double and multiple ovulations was 0.105, and the proportion of doubles that were bilateral was 0.36, similar to the T herd (1).

<table>
<thead>
<tr>
<th>Herd</th>
<th>Calving years</th>
<th>LL</th>
<th>LR</th>
<th>RR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>1984-86</td>
<td>13</td>
<td>19</td>
<td>29</td>
<td>61</td>
</tr>
<tr>
<td>Twin calving</td>
<td></td>
<td>0.08</td>
<td>0.37</td>
<td>0.10</td>
<td>0.18</td>
</tr>
<tr>
<td>Private</td>
<td>1985-86</td>
<td>10</td>
<td>15</td>
<td>24</td>
<td>49</td>
</tr>
<tr>
<td>Twin calving</td>
<td></td>
<td>0.10</td>
<td>0.40</td>
<td>0.13</td>
<td>0.20</td>
</tr>
<tr>
<td>Overall rate</td>
<td></td>
<td>0.09</td>
<td>0.34</td>
<td>0.11</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Includes 2 cows conceiving to multiple ovulations, 1 calving twins.

There was a positive relationship between ovulation rate and twin calving results (Table 1) for cows conceiving at a double ovulation were similar in the experimental and private herds. Overall, ovulation status affected twin calving rate (P<0.01). Proportionally 0.31 of calvings were twins following unilateral double ovulations compared with 0.38 from bilateral double ovulations.

Restricting the data to mature T and C cows, there were 5 twin sets from 12 cows conceiving at a bilateral double ovulation (proportionally 0.42). Of these, the C herd contributed only one double ovulation. From the limited data on unilateral double ovulations, the T herd produced 4 twin sets from 15 pregnancies (proportionally 0.22), compared with zero twin sets from 16 pregnancies in the C herd (P<0.05). Thus, compared with the C herd, the T herd had more double ovulations, mainly due to more bilaterals, with a higher embryonic or foetal survival in pregnant T cows.

There were no significant effects due to index or index x treatment interaction. These data do not support the hypothesis that, within a flock, animals of different breeding value for reproductive rate vary in their response to PMSG.

In many regions of Australia a long postpartum anoestrus contributes significantly to reduced fertility in beef herds, the problem being most severe in primiparous heifers. An effect of the male on reproductive function in female conspecifics has been demonstrated in several species and under a variety of circumstances. The present study therefore sought to determine whether exposure of first-calf heifers to bulls would reduce their extended postpartum anoestrous period. On day 3 postpartum, 2-year-old Angus heifers and their calves were either introduced into a paddock containing vasectomised Angus bulls (bull exposed (BE); bulls : heifers, 1:20), or isolated from bulls to serve as controls (C). Ovulation of ovarian activity was taken as the first occasion when plasma progesterone concentrations were > 1.5 ng/ml in jugular vein samples taken weekly from days 21 to 120 postpartum. The data were analyzed by ANOVA and Chi-squared procedures and results for postpartum anoestrous periods are shown in the Table.

<table>
<thead>
<tr>
<th>Postpartum Anoestrus (days)</th>
<th>Control (n = 50)</th>
<th>Bull exposed (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td>4 (8%)</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>&lt; 40</td>
<td>12 (24%)</td>
<td>22 (46%)</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>20 (40%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>&gt; 80</td>
<td>10 (20%)</td>
<td>3 (6%)</td>
</tr>
</tbody>
</table>

The postpartum anoestrous periods for C and BE heifers were 62 ± 4 and 46 ± 3 (mean ± SEM) days, respectively (P < 0.05). Findings of particular significance were that a greater (P < 0.05) proportion of BE heifers had resumed cyclic ovarian activity by 40 days postpartum compared with C heifers, and that the proportion of heifers which had failed to cycle by 60 days postpartum was less (P < 0.01) for BE than for C heifers. Fertility in cattle is known to increase with successive cycles postpartum. Therefore, an earlier return to cyclic ovarian activity in first-calf heifers could be expected to increase fertilisation rates during subsequent mating. The demonstrated “bull effect” could be readily implemented in industry simply by exposing females to vasectomised bulls from the commencement of calving.

Study supported in part by the J.S. Davies Bequest and the NLRDC.
INTRA UTERINE INSEMINATION OF SHEEP WITH THAWED FROZEN SEMEN FOLLOWING SYNCHRONISATION WITH CIDR TYPE S & G

N. A. Holt
Riverina Artificial Breeders Pty. Ltd., Albury, N.S.W. 2640

Laparoscopic artificial insemination (L.A.I.) is practised in all States, predominantly in Merino sheep. Estimates from operators of the number inseminated in the 1986-87 breeding was 35,000. Oestrus synchronisation is induced using polyurethane sponges impregnated with Flugestone acetate (Chronogest, Intervet Aust.) or Medroxyprogesterone acetate (Repronap, Upjohn Aust.). PMSG is given at sponge withdrawal (400 iu) with L.A.I. performed between 50 and 66 hours, depending on operator preference.

The use of 9% progesterone impregnated silastic elastonomer, 0.33g/device CIDR Type S and CIDR Type G (Alex Harvie Industries, Hamilton, N.Z.) have had little use in Australia. Comparative trials found no difference with synchrony of oestrus and fertility between Chronogest sponges and CIDR Type S (1). Type S and Type G differ in shape and ease of insertion only.

300 CIDR Type S and 300 CIDR Type G were used in two L.A.I. programmes in January in South Eastern Australia using crossbreed and merino sheep. CIDR's were implanted for 12 and 13 days and 400 iu of PMSG (Pregeneol, Heriot Aust.) given at CIDR withdrawal. Ram semen frozen using the method of Salamon (2) was used and all rams had previously attained fertility levels of 70% in L.A.I. L.A.I. was performed from 42 - 50 hours after CIDR withdrawal without reference to the time of onset of oestrus Trial 1 and with reference to onset of oestrus, Trial 2.

<table>
<thead>
<tr>
<th>Trial 1</th>
<th>Implant Type</th>
<th>No. Retained</th>
<th>% Retained</th>
<th>No. Pregnant</th>
<th>% Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIDR S</td>
<td>150</td>
<td>99.3%</td>
<td>115</td>
<td>77%</td>
<td></td>
</tr>
<tr>
<td>CIDR G</td>
<td>150</td>
<td>99.3%</td>
<td>131</td>
<td>88%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trial 2</th>
<th>Implant Type</th>
<th>% Showing Oestrus</th>
<th>% Showing Oestrus</th>
<th>No. Pregnant</th>
<th>% Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIDR S</td>
<td>150</td>
<td>66%</td>
<td>95%</td>
<td>98</td>
<td>66%</td>
</tr>
<tr>
<td>CIDR G</td>
<td>150</td>
<td>65%</td>
<td>96%</td>
<td>107</td>
<td>72%</td>
</tr>
</tbody>
</table>

Satisfactory retention, oestrus synchronisation and fertility was obtained from both CIDR Type S and CIDR Type G. No advantage was obtained from teasing using vasectomised rams.


LAMBING FOLLOWING TRANSFER OF IMPORTED FROZEN-THAWED EMBRYOS FROM AWASSI FAT TAIL SHEEP

W.M.C. Maxwell, J.P. Ryan* and J.R. Hunton+

Department of Agriculture, Adelaide, SA, *Department of Obstetrics & Gynaecology, Royal North Shore Hospital, St Leonards, NSW and +Animal Breeding & Research Institute, Katanning, WA

In 1985 we reported the superovulation, recovery and fertilisation of embryos from Awassi fat tail sheep in Cyprus (1). This paper presents the pregnancy and lambing results following freezing of embryos in Cyprus, importation to Australia, thawing of embryos and transfer to recipients.

Eggs were collected during May 1985 from 139 mixed age Awassi ewes treated with 3 superovulatory regimes as previously described (1). Fertilisation and morphology of recovered eggs were examined and late morula or early blastocyst stage embryos processed for freezing in 1.2 ml ampoules by methods previously described (2). During processing for freezing and thawing, the embryos were washed and treated with citric acid and trypsin according to a protocol provided by the Australian Animal Health and Quarantine Service.

The ampoules, stored in 20 l liquid nitrogen containers, were transported by air from Cyprus to the Commonwealth Quarantine Station on Cocos (Keeling) Islands and stored for 12 months. In May 1986, the ampoules were thawed (2) and the embryos re-washed and treated with citric acid and trypsin. The embryos were then re-examined before transfer (1 to 6 at a time) to Dorset Horn x Merino ewes at day 6 of an induced oestrous cycle. The recipients were subjected to real time ultrasound 40 days after transfer and the number of foetuses recorded. The number of lambs born was recorded at lambing in October 1986.

A total of 311 embryos were frozen-stored. Two hundred and sixty one thawed embryos were transferred to 85 recipient ewes, of which 35 (41.2%) were pregnant with 51 foetuses (19.5% of embryos) 40 days after transfer. Fifty one live lambs were born of which 42 survived to weaning at 3 months of age.

The results of this programme have shown that techniques developed in Australia may be applied to the importation of Middle East fat tail sheep in the form of frozen embryos.

SUPEROVULATORY RESPONSE OF MERINO EWES TREATED WITH PMSG AND GnRH OR PMSG ANTISERUM

B.N. Jabbour & G. Evans
Department of Animal Husbandry, University of Sydney, NSW 2006

PMSG is a relatively cheap hormone which is often used for superovulating animals. However, its use is associated with the development of persistent large follicles, which may adversely affect fertility through excessive oestrogen secretion (1). In an attempt to stimulate a high ovulatory response without development of persistent follicles, a study was designed to compare the effect of GnRH or PMSG antiserum (PMSG/AS) on Merino ewes treated with PMSG.

Forty-eight mature Merino ewes were treated in the breeding season with progesterone sponges (Repronap, Upjohn) for 12 days. They were given 1200 IU of PMSG (Folligon, Intervet) s.c. 48 h before pessary withdrawal (PW) and randomly allocated to 4 groups (n=12) as follows: Gp1, control; Gp2 received 100 µg GnRH (Partagyl, Intervet) i.v. 12 h after PW; Gp3 received 100 µg GnRH i.v. 24 h after PW; Gp4 received 3 ml of ovine PMSG/AS (2) s.c. 24 h after PW. The presence or absence of ovulation points was determined at laparoscopy performed on 6 ewes in Group 2 at 36 h after PW and on 6 ewes in each of Groups 1, 3 and 4 at 48 h after PW. The number of ovulation points (CL) and large unruptured follicles (LF, >5 mm) was determined in all ewes at laparoscopy 72 h after PW.

The presence of fresh ovulation points was observed at the first inspection in 1/6 ewes in Gp1, 5/6 in Gp2, 2/6 in Gp3 and 3/6 in Gp4. All ewes had ovulated when inspected at 72 h. The numbers of CL and LF and total follicular development (TFD = CL + LF) observed at 72 h are recorded in Table 1.

In conclusion, the results provide further evidence to suggest that increases in FSH secretion between days 12-14 of the oestrous cycle (1,2). The present study was undertaken to determine whether similar increases in FSH could be demonstrated in ovarioctomised ewes in which post-castrational rises had been inhibited by oestradiol (E2).

20 mature Merino ewes (43.2±1.0 kg live weight) which had been paired according to live weight were used in the experiment which began in April. 3cm E2 implants reported to produce similar levels to those in intact ewes (3) were inserted subcutaneously at ovarioctomy. Animals were housed indoors in individual pens and fed a maintenance ration of wheaten hay. Supplemented animals received 500g of lupin grain per day for seven days (days 0-6). FSH was measured by radioimmunoassay and data analysed by ANOVA procedures. Mean serum FSH prior to, during and after supplementation are summarised in the following table.

Table 1. The ovarian response of mature Merino ewes treated with 1200 IU PMSG and GnRH or PMSG/AS (x ± s.e.m.)

<table>
<thead>
<tr>
<th>Groups</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSG</td>
<td>Gp12</td>
<td>Gp14</td>
<td>PMSG/AS</td>
<td></td>
</tr>
<tr>
<td>Total Follicular Development</td>
<td>14.6±0.9a</td>
<td>8.7±0.8b</td>
<td>14.3±1.4a</td>
<td></td>
</tr>
<tr>
<td>Corpora Lutea</td>
<td>9.9±0.3a</td>
<td>5.7±0.7b</td>
<td>14.8±1.6c</td>
<td></td>
</tr>
<tr>
<td>Large Unruptured Follicles</td>
<td>4.6±0.4a</td>
<td>3.9±0.4b</td>
<td>1.6±0.5a</td>
<td></td>
</tr>
<tr>
<td>Values with different superscripts within each row differ (P&lt;0.01).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment with GnRH at 12 h advanced the time of ovulation and reduced the TFD and CL. Time of ovulation in ewes in Gps 3 &amp; 4 was unaffected by treatment. Although the TFD was similar in Gps 1, 3 &amp; 4, the numbers of CL were higher in ewes treated with GnRH or PMSG/AS at 24h after PW compared with PMSG-treated controls.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>This study indicates that treatment with GnRH or PMSG/AS at the appropriate time may improve the superovulatory response of ewes to PMSG by increasing the proportion of developing follicles which ovulate. These treatments may also have beneficial effects on fertility of superovulated ewes by limiting peri-ovulatory oestrus secretion.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References:
IMMUNISATION AGAINST AN INHIBIN SUBUNIT PRODUCED BY RECOMBINANT DNA TECHNIQUES RESULTS IN INCREASED OVULATION RATES IN SHEEP


*Medical Research Centre, Prince Henry’s Hospital, Melbourne, Victoria, 3004; Biotechnology Australia Pty Ltd., Roseville, NSW, 2069; **Department of Anatomy, Monash University, Clayton, Victoria, 3168.

Inhibin (NIH) is a gonadal glycoprotein hormone which preferentially suppresses FSH synthesis and release from the pituitary (1). In bovine follicular fluid, NIH exists as a disulphide-linked heterodimer in 58 and 31-32KD molecular weight forms (2). The difference between the two m.w. forms is attributed to cleavage of the amino-terminal 166 amino acids of the A subunit (A, fragment) to leave the carboxy terminal 134 amino acids (Ac subunit) attached to 116 amino acid B subunit (3). The aim of this study was to examine the effect of immunisation of ewes against recombinant bovine Ac INH fusion protein on antibody titre, NIH binding in plasma and ovulation rate.

The design is shown in the Table. A conjugate was made by coupling the fusion protein to keyhole limpet haemocyanin (KLH) using glutaraldehyde. Each animal received four injections of 100-300 µg fusion protein over 93 days. The animals were synchronised using progesteragen sponges and subjected to laparoscopy for the determination of ovulation rates in two consecutive cycles (Days 115 and 135).

**Table: Immunisation of Border Leicester-Merino ewes against recombinant bovine Ac INH: effects on antibody titre and ovulation rate**

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Immunogen</th>
<th>Anti-bac Inhibin Binding (%)</th>
<th>No. of Corpora Lutea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Titre (Abs)</td>
<td>Day 115</td>
<td>Day 135</td>
</tr>
<tr>
<td>A</td>
<td>5</td>
<td>None</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>KLH</td>
<td>0.06±0.02</td>
<td>&lt;1</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>bac</td>
<td>1.03±0.10</td>
<td>19.3±6.8</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>bac-KLH</td>
<td>0.74±0.42</td>
<td>14.7±9.4</td>
</tr>
</tbody>
</table>

The immunised animals had mean ovulation rates for each cycle which were significantly (P < 0.001) above the rates for the controls. Analysis of antisera taken on Day 115 showed significant anti-fusion protein antibodies and iodinated NIH binding capacity in the test but not control groups. Furthermore, antisera to the bac fusion protein in 4 out of 7 ewes neutralised the NIH bioactivity of NIH follicular fluid in an NIH in vitro bioassay.

These data demonstrate that neutralisation of NIH can be effected by immunisation with bovine A, subunit and that such immunisation results in elevated ovulation rates as predicted from the biological role of NIH as a suppressor of FSH.


IMMUNISATION OF CATTLE AGAINST BOTH OESTRONE AND TESTOSTERONE TO INCREASE OVULATION RATES

R.I. Cox, M.S.F. Wong and P.A. Wilson
CSIRO Division of Animal Production, P.O. Box 239, Blacktown, NSW 2148.

Cattle respond poorly and inconsistently when immunised against steroids to increase their ovulation rate (O.R.) (1,2). In previous work, immunisations were against single steroids. From data on sheep it was considered that immunisation of cattle against several steroids simultaneously might be more effective and this possibility was tested experimentally.

Eighteen mature Hereford cows were randomly divided into equal control and treated groups. The treated group was immunised against oestrone-human serum albumin (E.HSA) in 3 ml of an emulsion with Freund’s complete adjuvant (FCA). A primary immunisation was given in November 1984 and booster treatments on 22nd July, 21st August and 29th October, 1986. At the latter date and on the 10th November 1986, Estrumate® prostaglandin was administered to all 18 cows followed by oestradiol 0.6 mg intramuscularly in oil on November 11th. Endoscopy was carried out on 19th or 21st November to determine the number of corpora lutea present. Blood samples were taken by jugular venipuncture for the measurement of anti-O.T. titres. Titres averaged 1:1800 (oestrone binding) 1 week before expected ovulation.

None of the cows showed multiple ovulation.

Subsequently the treated cows were re-immunised with testosterone-human serum albumin (T.HSA) in 3 ml per ovulation on 16th December 1986 and 15th January 1987. A similar schedule to the November test was then used, boosting with T.HSA on 6th March together with estrumate treatment, further prostaglandin on the 17th March and oestradiol on the 18th March and endoscopy on 26th and 27th March.

Numbers of animals with no ovulation, single or two ovulations were 2.7/0 (controls) and 3.3/3 (E.HSA, T.HSA, immune). The groups were significantly different (P < 0.03, Fishers exact test). Titres for oestrone were similar to those in the first phase of the experiment averaging 1:1300 (oestrone binding) and 1:80 (testosterone binding) a week before expected ovulation.

These data are in accord with two preliminary tests with smaller groups of cows which had been carried out previously. These included animals which had been immunized first against testosterone or androstenedione and then against oestrone. Such immune cows had ovaries which were much more active often with several large follicles as compared to the effects of immunisation against a single steroid. Treatment with oestradiol then resulted in multiple ovulation in the animals presumably following release of endogenous LH. Alternatively treatment with NIH could be effective.

Immunisation against oestrone alone was not effective for increasing O.R. of mature Hereford cows in this experiment, whereas re-treatment to give antibodies to both oestrone and testosterone resulted in a significantly higher O.R. compared to control animals.

TIME OF OVULATION IN GOATS TREATED WITH PMSG

Kristine Battye, A.W.N. Cameron and A. Trounson.

Centre for Early Human Development, Monash University, Monash Medical Centre, 172 Lonsdale Street, Melbourne, Victoria, 3000

To study the rate of development of caprine embryos it is necessary to determine precisely the time and degree of synchrony of ovulation. Three experiments were conducted to determine the time to commencement and period over which superovulation occurs.

Feral does were superovulated in April, 1987 by treatment with intravaginal progesteragen impregnated sponges (Repromp, Upjohn, 60 mg medroxyprogesterone acetate) for 16 days and 1200 IU PMSG (Pregnoject, Merriot Agencies) administered i.m. 2 days before sponge removal. In Expt.1 half the does were injected i.m., with 50 µg GnRH (Fertagel, Intervet) 20 hours after sponge removal and the ovaries of all does were examined by laparoscopy at 6 hourly intervals commencing 24 hrs after sponge removal. In Expt.2, does were treated similarly except laparoscopy was carried out 12 hourly commencing 48 hr after removal of sponges. Observation ceased when the number of corpora lutea did not differ between two observations. In Expt.3, corpora lutea more than 1 day old at the initial laparoscopy were considered premature ovulations. To determine the origin of these corpora lutea in Expt.3 was conducted in which 10 does were treated with sponges for 16 days and 1200 IU PMSG 2 days before sponge removal (Group 1). Group 2 (N=9) did not receive PMSG. Laparoscopy were conducted prior to PMSG injection and at sponge removal.

| Table 1. Expt. 1. No. does commencing superovulation after sponge removal at:
<table>
<thead>
<tr>
<th>Hrs after sponge removal</th>
<th>N</th>
<th>24hr</th>
<th>30hr</th>
<th>36hr</th>
<th>42hr</th>
<th>48hr</th>
<th>54hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSG+GnRH</td>
<td>22</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>11</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>PMSG</td>
<td>22</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

In Expt.1, 19/21 does treated with PMSG + GnRH commenced superovulation within 48 hours of sponge removal compared to 12/22 treated with PMSG alone. Ten out of 22 does treated with PMSG alone failed to superovulate by 96 hours (Table 1). In contrast 19/21 does commenced to superovulate by 48 hours in Expt. 2 (Table 2). This suggests that repeated laparoscopy may delay superovulation in does treated with PMSG alone.

| Table 2. Expt. 2. No. Corpora Lutea observed at laparoscopy (%)
<table>
<thead>
<tr>
<th>Hrs after sponge removal</th>
<th>N</th>
<th>45hr</th>
<th>57</th>
<th>69</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSG+GnRH</td>
<td>23</td>
<td>262/290(90)</td>
<td>26/290(9)</td>
<td>2/290(0.01)</td>
</tr>
<tr>
<td>PMSG</td>
<td>21</td>
<td>19</td>
<td>116/180(64)</td>
<td>39/180(22)</td>
</tr>
</tbody>
</table>

*Does superovulate by 48 hrs.

Treatment with GnRH increased the synchrony of superovulation (Table 2) with only 9% ovulations occurring later than 48 hours compared to 35.6% when GnRH was not administered (P<0.001).

Premature ovulations were observed in 39/44 does in Expt. 1. No premature ovulations were observed in does laparoscoped prior to PMSG injection (Expt.3) whereas 2 days later, at the time of sponge removal 8/10 does in Group 1, had 1 or 2 premature ovulations compared to 0/9 does in Group 2 (P<0.001).

We concluded that administration of GnRH synchronises superovulation in does treated with PMSG. Stress related to repeated laparoscopy may delay or inhibit superovulation and that PMSG may cause ovulation in the presence of progesteragenes.

INTRA-UTERINE INSEMINATION WITH PELLET OR STRAW-FROZEN RAM SEMEN

J.R. Huntton, S.E. Flecker and W.M.C. Maxwell

Animal Breeding and Research Institute, Katanning, W.A.
Department of Agriculture, Adelaide, S.A.

There are conflicting reports on the fertility of ram semen frozen in straws (1). Satisfactory lambing has been achieved following intra-uterine insemination (I-U.I.) by laparoscopy with ram semen frozen in pellet form using a tris-based diluent. Freezing of ram semen in straws would have several advantages. This study examined the effects of dilution rate, inseminate volume and ram on the fertility of semen frozen in straws and pellets following I-U.I.

Semen was collected by artificial vagina and only ejaculates of good initial motility and a concentration of > 3x10⁹ per ml were used. In Expt.1, pooled semen from 2 rams was split for 3-fold, 12-fold and 24-fold dilution with tris-based diluent. In experiment 2, individual ejaculates from 3 rams were extended 12-fold. The extended semen was cooled to -80°C and frozen either in 0.2 ml pellet form on dry ice or in 'mini-straws' (I.N.V., France) in liquid nitrogen vapour at -80°C in a cell freezer. Ewes were synchronised with intravaginal sponges (Chronogest,Intervet) and 400iu PMSG (Folligon,Intervet). Fertilisation rates were determined by ultrasound (Expt.1) or by progesterogen assay (Expt.2).

| Table 1. (Expt.1) Effect of dilution rate and semen freezing method on pregnancy rate following intra-uterine insemination.
<table>
<thead>
<tr>
<th>Freezing method</th>
<th>Dilution Rate (semidiluent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pellet</td>
<td>3-fold</td>
</tr>
<tr>
<td>19/39</td>
<td>25/38</td>
</tr>
<tr>
<td>Straw</td>
<td>20/39</td>
</tr>
</tbody>
</table>

| Table 2. (Expt.2) Effect of inseminate volume, ram and freezing method on pregnancy rate following intra-uterine insemination.
<table>
<thead>
<tr>
<th>Freezing method</th>
<th>Inseminate volume (ml)</th>
<th>Ram A</th>
<th>Ram B</th>
<th>Ram C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pellet</td>
<td>0.1</td>
<td>25/45</td>
<td>28/45</td>
<td>32/46</td>
<td>85/140</td>
</tr>
<tr>
<td>0.2</td>
<td>21/48</td>
<td>18/39</td>
<td>26/36</td>
<td>64/121</td>
<td></td>
</tr>
<tr>
<td>Straw</td>
<td>0.1</td>
<td>17/47</td>
<td>14/44</td>
<td>52/137</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>18/48</td>
<td>23/41</td>
<td>62/130</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There are conflicting reports on the fertility of ram semen frozen in straws (1). Satisfactory lambing has been achieved following intra-uterine insemination (I-U.I.) by laparoscopy with ram semen frozen in pellet form using a tris-based diluent. Freezing of ram semen in straws would have several advantages. This study examined the effects of dilution rate, inseminate volume and ram on the fertility of semen frozen in straws and pellets following I-U.I. Treatment with GnRH increased the synchrony of superovulation (Table 2) with only 9% ovulations occurring later than 45 hours compared to 35.6% when GnRH was not administered (P<0.001).

Premature ovulations were observed in 39/44 does in Expt. 1. No premature ovulations were observed in does laparoscoped prior to PMSG injection (Expt.3) whereas 2 days later, at the time of sponge removal 8/10 does in Group 1, had 1 or 2 premature ovulations compared to 0/9 does in Group 2 (P<0.001).

We concluded that administration of GnRH synchronises superovulation in does treated with PMSG. Stress related to repeated laparoscopy may delay or inhibit superovulation and that PMSG may cause ovulation in the presence of progesteragenes.

| Table 1. Expt. 1. No. does commencing superovulation after sponge removal at:
<table>
<thead>
<tr>
<th>Hrs after sponge removal</th>
<th>N</th>
<th>24hr</th>
<th>30hr</th>
<th>36hr</th>
<th>42hr</th>
<th>48hr</th>
<th>54hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSG+GnRH</td>
<td>22</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>11</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>PMSG</td>
<td>22</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

*Does superovulate by 45 hrs.

Treatment with GnRH increased the synchrony of superovulation (Table 2) with only 9% ovulations occurring later than 45 hours compared to 35.6% when GnRH was not administered (P<0.001).

Premature ovulations were observed in 39/44 does in Expt. 1. No premature ovulations were observed in does laparoscoped prior to PMSG injection (Expt.3) whereas 2 days later, at the time of sponge removal 8/10 does in Group 1, had 1 or 2 premature ovulations compared to 0/9 does in Group 2 (P<0.001).

We concluded that administration of GnRH synchronises superovulation in does treated with PMSG. Stress related to repeated laparoscopy may delay or inhibit superovulation and that PMSG may cause ovulation in the presence of progesteragenes.

| Table 2. Expt. 2. No. Corpora Lutea observed at laparoscopy (%)
<table>
<thead>
<tr>
<th>Hrs after sponge removal</th>
<th>N</th>
<th>45hr</th>
<th>57</th>
<th>69</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSG+GnRH</td>
<td>23</td>
<td>262/290(90)</td>
<td>26/290(9)</td>
<td>2/290(0.01)</td>
</tr>
<tr>
<td>PMSG</td>
<td>21</td>
<td>19</td>
<td>116/180(64)</td>
<td>39/180(22)</td>
</tr>
</tbody>
</table>

*Does superovulate by 45 hrs.

Treatment with GnRH increased the synchrony of superovulation (Table 2) with only 9% ovulations occurring later than 45 hours compared to 35.6% when GnRH was not administered (P<0.001).

Premature ovulations were observed in 39/44 does in Expt. 1. No premature ovulations were observed in does laparoscoped prior to PMSG injection (Expt.3) whereas 2 days later, at the time of sponge removal 8/10 does in Group 1, had 1 or 2 premature ovulations compared to 0/9 does in Group 2 (P<0.001).

We concluded that administration of GnRH synchronises superovulation in does treated with PMSG. Stress related to repeated laparoscopy may delay or inhibit superovulation and that PMSG may cause ovulation in the presence of progesteragenes.
Effects of paternal caffeine consumption on his offspring: Prolonged effects on a second generation.
Irina Pollard and John Smallshaw
School of Biological Sciences, Macquarie University

It was shown that caffeine exposure of a male prior to mating affected his progeny and the progeny of a second generation. The dose chosen, 30 mg/kg/day given orally, was approximately equivalent to a caffeine intake of 10-12 cups of brewed coffee daily.

In the first (F1) generation caffeine consumption of the sires for a minimum period of 15 days prior to mating with drug naive females, caused significant fetal growth retardation of both sexes and an increased postnatal mortality of pups between weeks 1 and 2, many of which displayed characteristics of "runts". Mortality rate at birth, litter size, gestation length and sex ratios were not affected. The subsequent growth rate and adult resting plasma corticosterone concentrations was normal. Persistent caffeine effects were also found in a second (F2) generation obtained by back breeding male and female F1 offspring from control and treated groups, to control Fo rats. The F2 pups of both sexes, from the female breeding line, were born significantly heavier when compared with their control counterparts. The other birth parameters were normal as was their subsequent growth. In the male breeding line, 33% of the litters conceived were aborted in utero, and among the young F2 pups born "runts" were again evident. The subsequent growth rate of the remaining pups was normal.

At the conclusion of the breeding for the first generation the testes of the Fo sires were studied after they received caffeine for 38 consecutive days. The experimental testes showed a marked degeneration characterized by significant overall size reduction, breakdown of the germinal epithelium, accumulation of cellular debris in the lumen of the seminiferous tubules, and significant reduction in the abundance of mature spermatocytes. On ultrastructural examination there appeared to be genetic damage to the spermatogonia where nuclear cysts and pouches were seen. The appearance of the Leydig cell was not affected, normal function for which was confirmed by the unchanged libido and fertility of caffeine treated sires.

Paternal use of noxious agents may exert deleterious effects directly by genetically altering the sperm, or indirectly by exerting modifying effects on the environment surrounding the maturing germ cells. It is possible that the testicular injury induced by caffeine consumption may be indirect, for example, due to hormonal induced vasoconstriction, and/or due to direct mutagenic effects in the germ cells. A changed genetic programme in the developing germ cells of the first generation may in turn implicate a second generation.

Oviducal fluid appears to be a diluted transudate of plasma, to which specific proteins synthesized by the oviducal epithelium have been added. In sheep we have identified an oviducal fluid specific glycoprotein which is induced by oestrogen and is therefore only present in the first part of the reproductive cycle when fertilization and early embryo development occur within the oviduct (1). During these studies we also observed a faint protein band, in front of albumin, in some oviducal fluid samples subjected to alkaline polyacrylamide gel electrophoresis. The prealbumins in rat and human plasma are known to include vitamin binding proteins which are present at increased concentration during pregnancy, so it was of interest to test for vitamin binding by sheep oviducal fluid components.

We have compared oviducal fluid collected from a single ewe during dioestrous (fluid from a 10 day period was pooled) and oestrus/metoestrous (5 days) with plasma taken on day 8 (dioestrus) and day 1 (oestrus/metoestrous) of the reproductive cycle from another cyclic ewe. A sample of oviducal fluid (concentrated approximately 5-fold) or plasma containing about 50 mg protein was incubated with 100 microcuries of 14-C thiamin (Vitamin B1) in a 1.5 ml volume, at 37° for one hour, and separated by gel filtration on a Sephacryl 300 column in phosphate buffered saline. In each case, it appeared that the 14-C thiamin was binding to a single protein component with a molecular weight of approximately 125,000. This peak overlapped but slurred slightly behind the dimeric sheep albumin peak. The amount of thiamin binding per unit protein in the peak fraction (ng thiamin/gm protein) was similar for oviducal fluid (126, 74) and for plasma (159, 93) and in both cases the dioestrous sample (listed first) showed 70% more binding of thiamin than the oestrous/metoestrous sample. After allowance is made for the lower protein content of oviducal fluid, plasma shows a 10-fold higher binding of thiamin per ml.

Our data suggests that there is a thiamin binding protein in sheep oviducal fluid which is plasma-derived and transudated at a similar rate to other plasma proteins.

SODIUM-DEPENDENT AMINO-ACID TRANSPORT IN MOUSE EMBRYOS

Peter L. Kaye

Department of Physiology and Pharmacology,
University of Queensland, St. Lucia, 4067

Amino-acids enter preimplantation mouse embryos via Na-independent and dependent saturable systems as well as by non-saturable routes. The major Na-dependent uptake in blastocysts has been described as λ-like despite the absence of key features including competition from the α-specific analogue methylaminoisobutyric acid (MeAIB), (1). Recent evidence suggests that it is a more unusual system accepting a wide range of amino-acids including cations (2). Kinetics of glycine uptake although inhibited by alanine characteristically indicated co-transport of 2 Na (3). This suggested that both these amino-acids may enter by the same system, co-transporting 2 Na. The Na-dependence of alanine, leucine and methionine uptake has therefore been determined and compared with that of glycine in 2-cell embryos and blastocysts, to further substantiate this conclusion.

Embryos were collected from superovulated Quackenbush mice in modified N2 medium (3). This medium was depleted of Na for uptake assays by replacing NaCl equimolarly with choline chloride to achieve required [Na]. Uptake of 3H-amino-acids was measured over 10 min after 30 min incubation in the appropriate medium (−) amino-acid. Na-dependent uptake was calculated by subtracting the uptake in Na-free medium and the results were plotted as Hill functions.

For all amino-acids, Na-dependent uptake by blastocysts was best described as a function containing [Na]−, indicating co-transport of 2 Na, whilst this appeared to be true only for glycine in 2-cell embryos. The regression coefficients for glycine and alanine were equivalent at saturating substrate concentrations, indicating that the transport system had the same affinity for Na when transporting either of these amino-acids. Na-dependent uptake of all amino-acids had the same V max of ~200 fmol/blast/10 min. There was evidence of alanine: 2 Na co-transport in 2-cell embryos but the activity was very low (V max = fmol/g/10 min c.f. 145 for glycine) and the V max for Na was ~80 nM c.f. 20 nM when transporting glycine. Together with reported inhibition kinetics (2), these results lead to the conclusion that Na-dependent uptake of alanine and glycine and possibly leucine and methionine occurs by the one system in blastocysts, probably 2+ (2), with co-transport of 2 Na. This system is unusual for somatic cells but appears similar to that observed in sea urchin embryos (4). The Na kinetics indicate that the system may be a differentiative variant of the Gly-system apparent in 2-cell embryos (3).


This research supported by a grant from NHMRC.

INSULIN EFFECTS ON PREIMPLANTATION MOUSE EMBRYOS

Mark Harvey and Peter Kaye

Department of Physiology and Pharmacology, University of Queensland, St. Lucia, 4067

Although mouse embryos may develop from 2-cell to blastocyst in vitro in a simple medium containing only electrolytes and energy sources, there is evidence that maternal-embryo communication occurs in vivo (1); moreover, the addition of Growth Factors to the culture medium improves developmental rate (2) and affects metabolism (3,4). In particular we have found that insulin is effective in these ways, accelerating fetal growth of embryos developing after transfer (2) and have investigated if insulin affects protein synthesis during preimplantation development in vivo.

Two-cell embryos were collected from superovulated Quackenbush mice and cultured in BMOC2 medium (5) containing insulin (Humulin, Lilly) at indicated times and concentrations. At the end of incubation embryos were transferred to fresh droplets of the same medium containing 6 μM 3H-leucine (1 Ci/L) and incubated for 2h before washing, precipitation, collection and counting of material insoluble in 10% CO1, CO2 on glass fibre discs.

Incorporation of 3H-leucine by cultured mouse embryos (cpm/g/2h ± -sem, 5 experiments)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastocysts</td>
<td>2243 ± 106</td>
<td>4283 ± 164</td>
</tr>
<tr>
<td>Exp. blasts</td>
<td>3621 ± 101</td>
<td>8125 ± 480</td>
</tr>
</tbody>
</table>

Insulin (170 nM) had no effect on incorporation by 8-cell embryos, but stimulated incorporation by blastocysts which had developed in vitro from 2-cell embryos over 48 or 72 h respectively, the first 24 h in the absence of insulin. These effects were also found after 4 h exposure to insulin. Insulin concentrations as low as 1.7 pM were effective on morulae developing to blastocysts.

We conclude that insulin stimulates protein synthesis in morulae and blastocysts probably via binding with specific receptors which have been observed at these stages using immunohistochemistry (5). It may be this effect which leads to the accelerated fetal development of these embryos after transfer.


This research is supported by an NIMRC grant to PK. Humulin was a gift from Lilly, Australia.
IN VITRO DEVELOPMENT AND METABOLISM OF LACTATE BY MOUSE EMBRYOS IN THE PRESENCE OF PLATELET ACTIVATING FACTOR (PAF).


Human Reproduction Unit, Royal North Shore Hospital of Sydney.
School of Veterinary Studies, Murdoch University, WA, 6150

Pre-implantation stage human and mouse embryos produce platelet activating factor (PAF) during in vitro culture. Its activity was essential for implantation in mice (1). This study was designed to test whether PAF had a direct effect on embryo metabolism and development in vitro.

Two cell embryos were collected from superovulated Quackenbush strain mice and cultured for 72 h in vitro or utilised for short term lactate metabolism determinations. Embryos were cultured in Hepes buffered substrate free medium supplemented with increasing doses of PAF and the developmental stage was recorded at 48 and 72h. To measure the production of CO₂ from lactate, single two cell embryos were culture in 3.0 μl of Hepes buffered substrate free medium + 3 μg BSA/ml + 1.12 mM (1-14C) lactate + PAF. Culture was carried out in 1.5 ml micro-centrifuge tubes in the presence of 1.0 ml 0.15M NaOH. After 2.0 h incubation, the NaOH was quantitatively removed, counted and the amount of trapped CO₂ calculated.

Table 1: Effect of PAF on the production of 14CO₂ from (2-14C) lactate by two cell mouse embryos and their development during 72h in vitro culture.

<table>
<thead>
<tr>
<th>Conc. PAF (ug/ml)</th>
<th>CO₂ prod. from lactate (p moles/emb. h⁻¹)</th>
<th>Total Developnent stage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(emb) Blastocyst Expanding</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>48h</td>
<td>72h</td>
</tr>
<tr>
<td>0.01</td>
<td>1.08 ± 0.02 (75)</td>
<td>274 (6)</td>
</tr>
<tr>
<td>0.05</td>
<td>0.97 ± 0.04 (21)</td>
<td>38 (2)</td>
</tr>
<tr>
<td>0.1</td>
<td>1.10 ± 0.04 (19)</td>
<td>59 (2)</td>
</tr>
<tr>
<td>0.5</td>
<td>1.27 ± 0.01 (80)</td>
<td>262 (6)</td>
</tr>
<tr>
<td>5.0</td>
<td>1.06 ± 0.05 (19)</td>
<td>248 (4)</td>
</tr>
<tr>
<td>10.0</td>
<td>1.10 ± 0.10 (18)</td>
<td>206 (4)</td>
</tr>
</tbody>
</table>

* values within columns with different superscripts differ significantly (p<0.05).

There was a quadratic effect of dose of PAF on the production of 14CO₂ from lactate during the 2.0h incubation period. Maximum response was observed when embryos were incubated with 1.0 μg PAF/ml. Further, there was a modest (p<0.05) effect of low PAF concentrations (0.1μg PAF/ml) on the development of embryos through to the early blastocyst stage of development. However, higher concentrations of PAF (5.0 - 10.0 μg/ml) retarded development. This study demonstrates an effect of exogenous PAF on pre-implantation embryo metabolism indicating a possible direct role for PAF in regulating embryo viability. (1) Spinks RN and O'Neill C. (1987). Lancet, 1, 106.
TRIPRONUCLEAR HUMAN OOCYTES: ALTERED CLEAVAGE PATTERNS AND SUBSEQUENT KARYOTYPIC ANALYSIS OF EMBRYOS

Ismail Kola, Alan Trounson, Garey Dawson and Peter Rogers.

Centre for Early Human Development, Monash University, Queen Victoria Medical Centre, 172 Lonsdale Street, Melbourne, Australia, 3000.

Although 1-4% of human oocytes fertilized in vitro are triploids (contain 3 pronuclei) there is very little information about the subsequent development and chromosomal composition of preimplantation embryos which derive from these oocytes, and the data that does exist suggests a complex picture. In this study we investigate the pattern of the first cleavage division of triploidy human oocytes and the chromosomal constitution of these embryos before the second cleavage division, in order to determine both the proportion of triploidy human oocytes that develop into triploid embryos and to gain insight into the mechanisms by which these triploidy human oocytes fail (if at all) to develop into the triploid embryo.

Human oocytes with three pronuclei were obtained from the Monash/Epworth in vitro fertilization programme. These oocytes were monitored constantly for development and cleavage. Six to eight hours after the first cleavage division, embryos were incubated in colcemid (0.1μg/ml) for a further 6h and the chromosomal constitution of these embryos evaluated as described by us (1). Most (18 or 29) of the triploidy oocytes cleaved directly from 1 to 3-cells at the end of the first cleavage division. These embryos have a severely abnormal (but not triploid) chromosomal complement. Furthermore, some (6 of 29) triploidy human oocytes cleave to 2-cells plus an extrusion and these embryos are diploids, while only (7 of 29) cleave to 2-cells and these embryos are triploid after the first cleavage division.

These findings demonstrate that most triploidy human oocytes have an altered cleavage pattern at the first cleavage division, that most triploidy human oocytes (76% in this study) do not develop into triploid embryos and that a correlation exists between the pattern of the first cleavage division and the subsequent karyotype of these embryos. This study also gives insight into the mechanisms by which these triploidy human oocytes fail to develop into triploid embryos.


IN VITRO CULTURE OF SINGLE BLASTOMERES ISOLATED FROM TWO AND FOUR CELL MOUSE EMBRYOS

Leeanda Wilton, Lesley Clark, Marilyn Bakker and Alan Trounson.

Monash University Centre for Early Human Development, Queen Victoria Medical Centre, Melbourne.

We have recently developed a technique to biopsy 4-cell mouse embryos by micromanipulative removal of a single cell (blastomere) with the long term aim of performing pre-natal diagnosis of genetic diseases in the pre-implantation embryo (1). Here we report improved cell proliferation of single blastomeres in vitro using extracellular matrix components that the early embryo would be exposed to in vivo such as fibronectin (FN), laminin (LN) and a complex of laminin and nidogen (LNC).

2 or 4 cell embryos were flushed from the oviducts of superovulated F1 mice into medium 2 (M2). Zona pellucidae were removed by brief exposure to acid-Tyrode’s solution (pH 2.5) and zona-free embryos were incubated for approximately 1 hour in Ca++/Mg++-free M2 to separate individual blastomeres. Tissue culture wells were pre-coated with 2 μg of either FN, LN or LNC. 100μl of centrifuged, heat-inactivated and filtered human amniotic fluid was added to each well and covered with paraffin oil. A single blastomere was placed in each well and cultured in 5% CO2 for 5-7 days after which time cells were fixed in methanol:acetic acid (3:1), stained in 10% phosphate buffered Geimsa and the number of cell nuclei counted.

TABLE 1 NUMBER OF CELLS PER WELL (MEAN ± S.E)

<table>
<thead>
<tr>
<th>SINGLE BLASTOMERE FROM</th>
<th>EXTRACELLULAR MATRIX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SSG</td>
</tr>
<tr>
<td>2 CELL</td>
<td></td>
</tr>
<tr>
<td>EMBRYO</td>
<td></td>
</tr>
<tr>
<td>(N=38)</td>
<td>12.4±1.4</td>
</tr>
<tr>
<td>(N=26)</td>
<td></td>
</tr>
<tr>
<td>4 CELL</td>
<td></td>
</tr>
<tr>
<td>EMBRYO</td>
<td></td>
</tr>
<tr>
<td>(N=53)</td>
<td>9.5±3.7</td>
</tr>
<tr>
<td>(N=53)</td>
<td></td>
</tr>
</tbody>
</table>

#P < 0.01 compared to SSG, *P < 0.005 compared to SSG.

Table 1 shows that individual blastomeres from both 2 and 4 cell embryos can be induced to proliferate in vitro. Greater cell proliferation is achieved using the extracellular matrix components FN and LNC. It might be expected that twice as many cells could be cultured from blastomeres isolated from 2 cell embryos compared to 4 cell embryos. However, on any matrix, there was no significant difference in cell proliferation between blastomeres from 2 or 4 cell embryos suggesting that the number of cell divisions in vitro is not restricted by the developmental age of the original blastomere. Contact inhibition may play a role in preventing further cell growth and we are currently attempting to disaggregate cell plaques to encourage proliferation.

CHROMOSOMAL LOCALIZATION OF THE 70 KILODALTON HEAT SHOCK PROTEIN (HSP70) GENE IN HUMANS.

Anna Davey, and Ismail Kola.

Centre for Early Human Development, Monash University, Monash Medical Centre, 172 Lonsdale Street, Melbourne, Australia, 3000.

The one cell mouse embryo is under maternal control and does not require the transcription of the embryonic genome. At the 2-cell-stage of mouse embryo development the embryonic genome is activated. The first major transcription product of the embryonic genome has been shown to be Hsp 70 (Bensaude et al, 1983). Our current studies are aimed at evaluating the role of Hsp 70 in the preimplantation mouse embryo. One aspect of these studies involves the chromosomal localization of these genes in the mouse and the human. A 2.3 kb cDNA fragment cloned in pUC 8 was nick-translated with tritium. Probe DNA was hybridized with chromosomal DNA fixed onto glass slides. Unbound DNA was washed off after hybridization using varying stringencies. Hybridization to chromosomes was detected by autoradiography. Fifteen percent of grains were found on chromosome 6, the observed number of grains was 3 times higher than the expected number of grains. The peak of these grains was found on the short arm of chromosome 6 and more specifically in region 6 p2.1. This gene was syntenic with the c-ki-ras oncogene. This study has localized the chromosomal position of hsp 70 in humans. Further studies are underway to localize the gene position in the mouse and these studies should contribute to understanding of the function of these genes.


THE EFFECT OF X DOSAGE COMPENSATION ON PARTHENOGENETIC INVIBILITY

By Jeff R. Manna, Robin H. Lovell-Badge and David M. Danks

Centre for Early Human Development (Monash University), Queen Victoria Medical Centre, 172 Lonsdale Street, Melbourne 3000.
MRC Mammalian Development Unit, Wolfson House (University College London), 4 Stephenson Way, London NW1 2HE, U.K.
Mordoch Institute for Research into Birth Defects, Royal Children's Hospital, Flemington Road, Parkville 3052.

In certain extraembryonic tissues of normal female mouse conceptuses, X chromosome dosage compensation is achieved by preferential inactivation of the paternally derived X (1). Diploid parthenogenomes have two maternally derived X chromosomes, hence this mechanism cannot operate. To examine whether this contributes to the inviability of parthenogenomes, XO and XX parthenogenetic eggs were constructed by pronuclear transplantation (2) and their development assessed after transfer to foster mothers.

Of 108 eggs transferred (32% and 52% of which were expected to be XO and XX respectively), 72 (67%) implanted and 22 developed to postimplantation stages. In 18 of these, chromosomes could be counted. 17 were XO (possessing 39 chromosomes) and 1 was XX (40 chromosomes). This result demonstrates that two maternally derived X chromosomes contribute to parthenogenetic inviability at implantation, possibly because of a failure to achieve normal X chromosome inactivation in the trophectoderm and primitive endoderm (those lineages in which the paternally derived X chromosome is preferentially inactivated). However, XO parthenogenomes showed similar abnormalities to XX parthenogenomes at the postimplantation stage, demonstrating that parthenogenetic inviability is ultimately determined by the possession of two sets of maternally derived autosomes.

ATTEMPTS TO PRODUCE TRANSGENIC SHEEP USING A PROVEN GENE CONSTRUCT


Departments of Obstetrics and Gynaecology and Biochemistry, University of Adelaide, Adelaide, SA 5001 and Turrettfield Research Centre, Department of Agriculture, Roseisle, SA 5550.

A fusion gene construct has been developed which allowed successful incorporation of the porcine growth hormone gene into the genomes of the mouse and pig (1). In this study, this construct was microinjected into pronuclei in an attempt to produce transgenic sheep. Superovulation was induced using PMSG (1200 IU, Heriot Agencies) or FSH-P (15Smg, Sobering) and synthetic GnRH (40 ug/ewe, Intervet (Aust.) Pty Ltd) was administered 24h after progesteragen sponge removal. PMSG and FSH-P treatments produced 726 and 495 eggs respectively from 138 and 79 ewes; 83.6% and 81.2% of the eggs were fertilized. More embryos had visible pronuclei following FSH-P than PMSG treatment (63.0% v. 85.6%, P<0.001). After microinjection (approx. 600 copies), incorporation in other species.

Table 1. Development of embryos after 3-days culture in vivo.

<table>
<thead>
<tr>
<th>No. blastomeres</th>
<th>F</th>
<th>EL/D/O</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8-16</th>
<th>&gt;16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microinjected</td>
<td>1.2%</td>
<td>25.2%</td>
<td>7.0%</td>
<td>3.5%</td>
<td>8.2%</td>
<td>49.4%</td>
<td>4.7%</td>
</tr>
<tr>
<td>embryos (n=343)</td>
<td>24.7%</td>
<td>7.2%</td>
<td>2.7%</td>
<td>0.0%</td>
<td>3.6%</td>
<td>48.6%</td>
<td>13.5%</td>
</tr>
<tr>
<td>Control</td>
<td>P value</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>ns</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>embryos (n=111)</td>
<td>Fragmented, Empty zona, Degenerate one-cell.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

233 microinjected embryos (189 with 8 blastomeres or more) and 42 control embryos were transferred to final recipient ewes (2-4 embryos/ewe). 48.7% (37/76) and 72.2% (15/18) of the ewes receiving microinjected and control embryos respectively became pregnant. 20.2% (47/233) and 38.15% (16/42) of the embryos in the respective groups developed into day 50 fetuses. 44/76 and 16/42 of these foetuses resulted in lambs. In all, 52 potentially transgenic lambs were produced, including 5 lambs from intermediate recipients and 4 lambs from an in vitro culture study. Seven lambs were dead at birth compared with none in the control group (P<0.02). So far none of 30 tissue samples have proven positive to gene incorporation. This study confirms the difficulty of producing transgenic lambs even when using a construct known to be capable of incorporation in other species.


INCREASED OVULATION RATE IN MERINO EwES BY SINGLE IMMUNIZATION AGAINST SEVERAL STEROIDS WITH DRAKEOL AS IMMUNOADJUVANT

M.S.F. Wong, P.A. Wilson and R.I. Cox

CSIRO, Division of Animal Production, P.O. Box 239, Blacktown NSW, 2148

Active immunization of Merino ewes against a combination of androgen and oestrogen serum conjugates resulted in increased ovulation rate (O.R.) with less ova and embryo wastage than those treated with the standard androstenedione (A)-serum albumin in DEAE-dextran (1). Both treatments, however, required two injections 3-4 weeks apart. The aim of the present study was to determine whether the use of the more potent immunoadjuvant Drakeol with a single immunization against three steroids would increase O.R. The response was compared to that obtained with the A-serum albumin - DEAE dextran system (Fecundin ).

Merino ewes (n=146, 5-6 years old) were randomly allocated to a control group, an antigen group and a group immunized against a mixture of A, testosterone (T) and oestrone (E). In the A immune group, the immunogen was injected with DEAE-dextran as immunoadjuvant on 17th February 1987; a booster was given 21 days later. In the ATE immune group, the immunogens were injected with Drakeol as immunoadjuvant on 3rd March 1987. All the ewes were joined with entire rams for 5 weeks from 9th April 1987. Weekly endoscopy was carried out following oestrus markings.

Table 1. O.R. and oestrus in control and immunized ewes.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Adjuvant</th>
<th>No. ewes with</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>ovulations</th>
<th>ovulations</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>48</td>
<td>Drakeol</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>58</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A immune - Fecundin</td>
<td>48</td>
<td>DEAE-dextran</td>
<td>10</td>
<td>35</td>
<td>4</td>
<td>1.57*</td>
<td>98</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(normal 2 injections)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATE immune</td>
<td>49</td>
<td>Drakeol</td>
<td>10</td>
<td>36</td>
<td>3</td>
<td>1.85*</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P = 0.02, Fisher's Exact Test

Both immunization treatments significantly increased O.R. at the conception cycle (P=0.02) and all ewes showed normal oestrus behaviour (Table 1). Our previous experiments with single immunization treatments against single steroid conjugates resulted either in poor ovulatory responses or little gain at lambing. Thus it remains to be seen whether the single immunization procedure against a mixture of steroids will result in less embryo wastage and hence be an alternative technique for improving lambing performance in Merino ewes. A single immunization against a mixture of A,T and E with Drakeol as immunoadjuvant significantly increased O.R. and the response is similar to that obtained with the standard Fecundin treatments.

IMMUNIZATION OF BOOROOLA EWES WITH FECUNDIN OR AN INHIBIN-ENRICHED PREPARATION

T. O'Shea, B.M. Bindon*, L.R. Piper*, S.A.R. Al-Obaidi, and R.I. Cox**

Department of Physiology, University of New England, Armidale, NSW, CSIRO, Division of Animal Production, Armidale, NSW and **CSIRO Division of Animal Production, Prospect, NSW.

Immunization of ewes with ovarian steroids or an inhibin-enriched fraction from bovine follicular fluid, results in an increased ovulation rate (OR). As Booroola ewes have less ovarian inhibin than control ewes (1), they were immunized with these preparations to gain an insight into the control(s) of their high prolificacy.

Groups of Booroola (B) and control (C) Merino ewes were injected with Freund's adjuvant plus DEAE-dextran adjuvant (Group A), or immunized with androstenedione (Fecundin, Glaxo Aust; Group F), or an inhibin-enriched fraction (2) from bovine follicular fluid (Group I) on Days 1 and 22. Groups A and I were treated again on Day 68. Corpora lutea were counted by laparoscopy on Days 1, 41 and 93. Blood samples were taken at Day 30.

The mean OR are presented in Table 1. Immunization with Fecundin (P<0.05) or "inhibin" (P<0.01) increased OR in C ewes but had no effect on the B ewes. Androstenedione antibody titres were similar in the Fecundin immunized groups (mean ± SE of reciprocal Booroola, 4164 ± 1227, N = 15; control strain 4844 ± 1139, N = 21) and were very low in the A (16 ± 3, N = 15) and I (18 ± 3, N = 19) sheep.

In a second study B and C ewes gave the same results on injection with adjuvant or immunization with inhibin.

Table 1. Ovulation Rate following immunization with adjuvant (A), Fecundin (F) or inhibin (I).

| Day | Treatment | A I F A I F A I F |
|-----|-----------|---------|---------|---------|
| 1   | Control   | 19 19 21 19 21 17 18 * 19 |
| 41  | Merinos   | 1.47 1.47 1.29 1.47 1.64 1.95* 1.78 3.06** 1.26 |
| 93  | Booroola | 16 16 16 16 15 15 15 |
| 16  | Merinos   | 3.75 3.28 3.05 5.63 4.38 4.75 3.23 3.88 4.60 |
| SE  | 0.55 0.50 0.48 0.55 0.52 0.46 0.55 0.24 0.62 |

Within-ewe increment in OR significantly different to A group. P<0.01
** Within-ewe increment in OR significantly different to A group. P<0.001

The lack of response of Booroolas to immunization with inhibin supports the concept that they are already effectively deregulated with regard to this hormone due to their low ovarian concentration. It is possible that steroid immunization also acts by way of lowering or opposing endogenous inhibin.


SUPEROVULATION FOLLOWING VACCINATION AGAINST OESTRADIOL-17B


Dept. of Wool Science, U.N.S.W., NSW 2033
*Division of Animal Production, CSIRO, Blacktown, NSW, 2184.

Boland (1) observed an increase in ovulation rate (OR) above PMSG treated controls in small groups of ewes with low titre levels of anti-oestradiol-17B (anti-E2). This experiment describes a field study, using larger groups of ewes, aimed at examining the OR and yield of embryos in PMSG treated ewes in which titre levels of anti-E2 were induced within the optimum range defined by Boland.

One hundred and eighty aged Merino ewes were treated with oestradiol-17B conjugated with dextran to produce reciprocal titre levels of anti-E2 of <1000 or >1000. Control ewes were treated with dextran adjuvant only. Each group was then treated with either 600 iu or 1200 iu PMSG at the end of a 12 day progesterone sponge treatment (Repromap, Upjohn). Blood titres of anti-E2 were determined from blood samples taken on the day of sponge removal and incidence of oestrus, OR and yield of embryos were recorded at slaughter 6 days after sponge removal.

Table 1. Oestrus and Ovarian Response of PMSG treated ewes to anti-E2 Titres.

<table>
<thead>
<tr>
<th>Main Effect</th>
<th>Number Ewes (%)</th>
<th>Oestrus</th>
<th>Ovulating</th>
<th>OR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSG 600 iu</td>
<td>87</td>
<td>58 (67%)</td>
<td>62 (72%)</td>
<td>6.0±1.5</td>
</tr>
<tr>
<td>1200 iu</td>
<td>89</td>
<td>76 (86%)</td>
<td>80 (90%)</td>
<td>13.4±1.3</td>
</tr>
<tr>
<td>Anti E2 Nil</td>
<td>58</td>
<td>56 (97%)</td>
<td>57 (99%)</td>
<td>9.6±1.0</td>
</tr>
<tr>
<td>Titre &lt;1000</td>
<td>96</td>
<td>68 (71%)</td>
<td>76 (80%)</td>
<td>9.2±0.9</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>22</td>
<td>10 (46%)</td>
<td>9 (41%)</td>
<td>10.0±2.6</td>
</tr>
</tbody>
</table>

* per ewe ovulating.

As anti-E2 titres increased there was a reduction in both the proportion of ewes exhibiting oestrus and the proportion of ewes ovulating. Further, there was no benefit in OR in or yield of embryos over untreated ewes from high or low levels of anti-E2.

We conclude that the vaccination method employed was reliable in producing low levels of anti-E2 but that this provides no benefit in the production of embryos in superovulation programmes.

FERTILIZATION AND EMBRYO LOSS IN BOOROOLA CROSS EWES: EFFECT OF THE F GENE


Department of Obstetrics and Gynaecology, University of Adelaide, S.A. and Department of Agriculture, Adelaide, S.A.

High prenatal wastage has been reported for Booroola Merino ewes. Various reasons for the embryonic loss in ++ relative to F+ ewes have been put forward, but the results are still a chance event. We conclude that the majority of embryonic loss in ++ relative to F+ ewes occurred during the first few weeks of pregnancy. Explanations for this wastage require further study.

TABLE 1. Effect of the F gene on OR, FR and ovum plus embryo loss (OELOSS) at days 21, 45 and 90 post insemination, respectively.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>GENOTYPE</th>
<th>F+</th>
<th>P</th>
<th>++</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>OR</td>
<td>3.22 (206/64)</td>
<td>1.50 (48/32)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>OR</td>
<td>3.10 (193/63)</td>
<td>1.63 (57/35)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>OR</td>
<td>0.56 (108/192)</td>
<td>0.65 (37/57)</td>
<td>&gt;.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>++</td>
<td>0.57 (110/192)</td>
<td>0.65 (37/57)</td>
<td>&gt;.05</td>
<td></td>
</tr>
</tbody>
</table>

Fertilization failure was independent of genotype and was not a significant source of reproductive wastage. Most embryonic loss occurred between fertilization and early implantation (day 21) in both genotypes. Reasons for the high level of wastage in ++ relative to F+ ewes are not apparent other than the results are a chance event. We conclude that the majority of embryonic loss in ++ relative to F+ ewes occurred during the first few weeks of pregnancy. Explanations for this wastage require further study.

PLASMA FSH CONCENTRATIONS IN RAM LAMBS CARRYING THE BOOROOLA FECUNDITY (E) GENE.

I.W. Purvis and J.R. Ford.

Animal Science Group, School of Agriculture, University of Western Australia, Nedlands, 6009.

Male carriers of the Booroola Merino E gene are currently identified by systemic testing, which is an expensive and time consuming procedure. The E gene could be utilized more efficiently if rams carrying the gene were identified as lambs. At 30 days of age, Merino ewes and Booroola rams that carry the E gene have higher plasma FSH concentrations than ewes that do not carry the gene (1). Unfortunately, at similar ages, no difference was observed between plasma FSH in crossbred male E gene carriers and non-carriers (2). However, these ram lambs were observed at only 3, 5 and 10 weeks of age, so the present experiment was designed to characterise more thoroughly the plasma FSH concentrations in male Merino E gene carriers and non-carriers at ages between 1 and 10 weeks.

Blood was sampled (two samples 1 h apart) and liveweights were measured at 1, 2, 3, 4, 5, 8 and 10 weeks of age in male progeny of Merino ewes and a Booroola ram, shown by progeny test to be homozygous for the E gene, or 3 Merino rams (pooled semen) not carrying the E gene. Plasma FSH was measured by RIA (NIAMDD-RR-1 as standard).

These results suggest that inhibition of FSH by gonadal feedback develops earlier in ram lambs carrying the E gene than in lambs not carrying the gene. However, the lambs carrying the gene were the progeny of only one Booroola ram and some of the differences in FSH may be due to sire differences in other genes which influence the FSH concentration. On the other hand, the difference in FSH at 3-5 weeks of age was so large that the E gene probably accounts for a major part of it. With these qualifications in mind, the concentration of FSH in peripheral plasma may be useful as a test for the presence of the E gene in ram lambs.


Exposure to progesterone before oestrus has been shown to be important in regulating fertility in sheep (1). It may be that the duration and concentration of plasma P$_4$ preceding oestrus in the bovine has a similar role. The aim of the studies reported here was to vary both the duration and concentration of plasma P$_4$ and observe the effects on fertility following oestrus. In Experiment 1 ovariectomized cows (n=18) were treated with P$_4$ (PRID; Ceva, Australia) in a 2 x 2 factorial design to produce high and low plasma P$_4$ concentrations for 7 or 14 days (H7, H14, L7 or L14). Plasma samples were collected daily during treatment and assayed for P$_4$ content by radio-immunoassay (Amerlex - M; Ameraham, Australia). In Experiment 2, 171 cows with suckling calves 1-2 months old received the same PRID treatments as cows in Experiment 1 and either 375 or 750 IU pregnant mare serum gonadotrophin (Pregnoil; Livestock Laboratories, Melbourne) intramuscularly at PRID removal and 500 µg cloprostenol (Estrumate; ICJ, Australia) intramuscularly at PRID insertion. The combination of PRID and cloprostenol was used to minimize P$_4$ secretion by endogenous luteal tissue (2). In Experiment 1, mean plasma P$_4$ during the first week of treatment in H14 and L14 were similar, 6.6 and 6.0 ng/ml, but during the second week were different, 5.4 x 3.8 ng/ml (PG0.01). H7 had a higher weekly mean than L7 (6.3 x 4.2 ng/ml, PG0.01). In Experiment 2 an overall calving rate of 32% was observed following insemination 54-58 hours after PRID removal. There was a significant interaction (PG0.025) between the effects of duration and dose of PRID treatment on calving rate (Table 1). Calving rate was only significantly depressed when plasma P$_4$ failed to reach concentrations typical of luteal phase levels. These results indicate that high calving rates following oestrus require plasma P$_4$ to be above minimum luteal phase concentrations for a period which may be considerably shorter than the length of the complete luteal phase.

**Table 1.** Plasma concentrations of progesterone in cows which received 1 of 8 treatments in Exp. 1 and calving rates from cows which were inseminated after receiving identical treatments in Exp.2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Highest daily</th>
<th>Highest weekly</th>
<th>Calving Rate</th>
<th>Lowest daily</th>
<th>Lowest weekly</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µg/ml)</td>
<td>(µg/ml)</td>
<td>(%)</td>
<td>(µg/ml)</td>
<td>(µg/ml)</td>
</tr>
<tr>
<td>H14</td>
<td>9.4*</td>
<td>6.0</td>
<td>33</td>
<td>4.3</td>
<td>5.4</td>
</tr>
<tr>
<td>L14</td>
<td>9.6</td>
<td>6.0</td>
<td>41e</td>
<td>3.0</td>
<td>3.8</td>
</tr>
<tr>
<td>H7</td>
<td>9.5</td>
<td>6.3</td>
<td>40</td>
<td>5.0</td>
<td>6.2</td>
</tr>
<tr>
<td>L7</td>
<td>5.9</td>
<td>4.2</td>
<td>12e</td>
<td>3.4</td>
<td>4.4</td>
</tr>
</tbody>
</table>

* Different subscripts within a column denote significant differences, P < 0.05.


EFFECT OF LUPIN SUPPLEMENTATION AND GnRH ON THE OVARIAN OVULATORY AND STEROIDogenic RESPONSE OF SUPEROVULATED Ewes

G. Evans, H.N. Jabbour, W.M.C. Maxwell & J.P. Ryan

1 Department of Animal Husbandry, University of Sydney, NSW 2006
2 Animal Breeding and Research Institute, Katanning, WA 6317

Feeding lupin grains can increase the ovulation rate in sheep. GnRH may also be used to synchronize ovulation in superovulated ewes. This study was designed to investigate the combined effects of GnRH and lupin grain supplementation on the ovarian ovulatory and steroidogenic response of superovulated Merino ewes.

Merino ewes were treated in March 1986, at Katanning, WA, with intravaginal prostaglandin pessaries (Repromap, Upjohn) for 12 days, 400 IU PMSG (Folligon, Intervet) 48 h before pessary withdrawal (PW) and 12 mg of FSH-P as 6 12-hourly doses starting at the time of PMSG injection (3, 3, 2, 1 & 1 mg). The animals were divided randomly into 4 groups (n=5). Groups 1 and 2 were fed supplementary lupin grains ad lib from the time of sponge insertion up to day 7 after PW. Groups 1 and 3 each received an i.v. injection of GnRH (100 µg Fertagyl, Intervet) 24 h after PW. Oestradiol 17β (E) and LH levels (assayed against NIH-WRR-2B as standard) were measured in jugular venous plasma samples collected at 4-hourly intervals for 56 h from PW. Onset of oestrus was determined using raddle teaser rams. The number of ovulation points (CL) and large unruptured follicles (LF) were recorded at laparoscopy on day 7 after PW.

Total follicular development (TFD = CL + LF), CL, peak preovulatory E levels adjusted per follicle and time to LH peak are presented in Table 1.

Table 1. Ovarian & endocrine responses of superovulated Merino ewes to supplementary lupin grains (L) and GnRH injection (G) (±s.e.m.).

<table>
<thead>
<tr>
<th>Group</th>
<th>CL + LF</th>
<th>CL</th>
<th>E per Follicle (pg/ml)</th>
<th>Time to LH Peak (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gp1</td>
<td>15.4±1.8</td>
<td>13.6±1.6</td>
<td>4.3±0.4</td>
<td>28.0±1.8</td>
</tr>
<tr>
<td>Gp2</td>
<td>17.4±1.4</td>
<td>14.7±1.6</td>
<td>5.7±0.3</td>
<td>27.2±0.8</td>
</tr>
<tr>
<td>Gp3</td>
<td>17.0±1.4</td>
<td>16.3±1.6</td>
<td>5.7±0.3</td>
<td>27.2±0.8</td>
</tr>
<tr>
<td>Gp4</td>
<td>17.4±1.4</td>
<td>16.3±1.6</td>
<td>5.7±0.3</td>
<td>27.2±0.8</td>
</tr>
</tbody>
</table>

Values with different superscripts within each row differ (P<0.05).

GnRH increased peak LH levels (5.9±0.6 vs 3.7±0.8 ng/ml, P<0.01), but lupin grain feeding had no effect. Lupin grain treatment advanced the time to onset of oestrus (31.3±0.8 vs 30.5±0.6 h following PW, P<0.01), and GnRH and lupin grain treatment each advanced the LH surge. GnRH treatment, without lupin grains (Gp 3), increased ovulation rate. Animals treated with lupin grains without GnRH (Gp 2) had high preovulatory E levels.

This study demonstrated the beneficial effects of using GnRH in a superovulatory treatment regime, but failed to find any beneficial effect of lupin grain feeding. The relatively high E levels resulting from lupin grain feeding without GnRH treatment may have adverse effects on fertility.
PLASMA PROGESTERONE CONCENTRATIONS AND OESTROUS SYNCHRONY IN CIDR-TREATED GOATS

D.R. Barnes, A.P. Oakley, K.L. Macmillan and T.J. Braggins

Ruakura Animal Research Station, Hamilton, New Zealand

Two types of CIDR (S & G; AHI Plastic Moulding Co.) can be used to synchronise oestrus in goats. Differences between CIDR types in their ability to maintain plasma progesterone concentrations (PPC) have not been reported previously. Neither has the use of CIDRs been evaluated as a means of stimulating an early onset to the breeding season. Two trials were completed, the first to compare PPC's in ovariectomised (ovx) goats treated with a CIDR S or G; and the second with entire Saanen goats treated with CIDR G before the commencement to the autumn breeding season.

A CIDR G was inserted into the vagina of each of 10 ovx does for 18 days and blood samples taken at selected intervals for assaying for PPC's. This procedure was repeated with CIDR S, except in 3 non-parous does which had small vaginas. In the breeding trial, 27 parous Saanen does were run with vasectomised (vx) bucks from 5 January and another 27 kept isolated from buck-influence. A CIDR G was inserted into each doe for 18 days from 27 January and 3 entire bucks run with each group from CIDR removal (14 February) until 20 March when vx bucks were returned. Blood samples were taken for PPC's on 4 dates (6, 9, 11 & 13 March) when returns-to-service were expected. Pregnancy diagnosis was confirmed by electronic scanning from 90 to 92 days after the synchronised mating.

The CIDR G maintained higher PPC's than the CIDR S (p<0.01) for the duration of treatment (Table 1).

<table>
<thead>
<tr>
<th>CIDR</th>
<th>Pre- Interval from CIDR insertion (days): 6 h post removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 25 1 4 7 12 17 18</td>
</tr>
<tr>
<td>S</td>
<td>0.09 7.0 6.7 6.6 5.0 3.2 2.3 2.4 0.5</td>
</tr>
<tr>
<td>(n=7)</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>0.19 7.9 8.6 7.1 6.0 5.1 3.1 3.7 0.6</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
</tr>
</tbody>
</table>

The 5 goats (9.3%) which lost CIDR's were all in the group without vx bucks. Thirty three (61%) of the 54 CIDR-treated Saanen does were served from 30 to 54 h after CIDR removal and another 13 (24%) in the following 24 h. Most of the remaining 15% were served on about the same date as those does which returned to service. None of the does run with vx bucks were served in the pre-mating period. Forty three (80%) of the 54 does conceived to a first service around 48 or 72 h after CIDR removal. The presence of vx bucks did not alter the response patterns even though the synchronised matings occurred 3 weeks before the onset of the breeding season.

TIME OF OVULATION FOLLOWING SYNCHRONY OF OESTRUS IN THE NONSUPEROVULATED MERINO


Turretfied Research Centre, Department of Agriculture, Rosedale, SA 5350 and Department of Obstetrics and Gynaecology, University of Adelaide, GPO Box 495, Adelaide, S.A. 5001

Merino ewes treated with progestagen pessary/PMSG (400 IU) commence ovulation between 51-72h after treatment with a median of 63h(1). This study examined between and within flock variability in time of ovulation following synchrony of oestrus (Study 1). The effect of type of intravaginal pessary (Study 2) and brand of PMSG (Study 3) on timing were also studied. Time of ovulation was assessed by laparoscopy at 6-h intervals. Depending on the study, ewes were treated for 12 days with either medroxypregesterone acetate (MPA), fluorogestone acetate (FGA) or progesterone in controlled internal drug release (CIDR) pessaries. Ewes were injected with 400 IU of either Pregnoeel (Hartick Agencies) or Foligon (Intervet (Aust). Pty Ltd). Observations began at either 45h (CIDR treatment) or 51-57h (MPA and FGA treatments) after pessary removal.

Table 1. Time of ovulation relative to pessary removal in six S.A. Merino flocks following MPA/400 IU Pregnoeel treatment.

<table>
<thead>
<tr>
<th>Observation no</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flock no</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Time (h) from 51-72</td>
<td>69-81</td>
<td>57-75</td>
<td>57-81</td>
<td>57-81</td>
<td>69-81</td>
<td>69-81</td>
</tr>
<tr>
<td>Time (h) of 1</td>
<td>63</td>
<td>75</td>
<td>63</td>
<td>69</td>
<td>69</td>
<td>75</td>
</tr>
<tr>
<td>median</td>
<td>2</td>
<td>63</td>
<td>69</td>
<td>63</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N= 10-34 per observation.

In Study 1, the time of median ovulation differed significantly (F0.01) between the flocks (Table 1). Three flocks were observed on two occasions and in Flock 2 the time of median ovulation differed significantly (F0.05) between observations. In Study 2, CIDR-treated ewes commenced ovulating significantly (F0.001) earlier than MPA or FGA-treated ewes (45v. 57h v. 57h respectively) but the time required for all ewes to commence ovulation was similar (24h v. 24h v. 10h respectively). In Study 3, there were no differences between the PMSG treatments. It is concluded that the within and between flock variation in the time of ovulation is substantial. The brand of PMSG used is unlikely to be an additional source of variation but CIDR treatment advances the time of ovulation.

TIMING OF GnRH TREATMENT AND EMBRYO QUALITY IN THE MERINO

S.E. Walker, D.H. Smith* and R.F. Seamark

Department of Obstetrics and Gynaecology, University of Adelaide, GPO Box 495, Adelaide SA 5001 and *Turretfield Research Centre, Department of Agriculture, Rosea, SA 5550

Highly synchronised ovaitions are desirable in superovulation regimes designed to collect pronuclear embryos. GnRH is often administered for this purpose 24h after sponge removal thus preventing the endogenous preovulatory LH surge(1). This study examined the effect of timing of GnRH treatment on embryo quality in superovulated and non-superovulated ewes. Ewes were stimulated to superovulate using either 1200 IU PMSG (Intervet (Aust.) Pty Ltd) or 6x2.5mg FSH-P (Schering) following a 12-day progestagen sponge treatment (Study 1). Non-superovulated ewes were treated with a 12-day progestagen sponge/400 IU PMSG regime (Study 2). Synthetic GnRH (40ug/ewe, Intervet (Aust.) Pty Ltd) was administered either 24h or 36h after sponge removal. Ewes were naturally mated and artificially inseminated with fresh semen. Day 5 embryos were collected, fixed and stained and nuclei counted. Study 2 was repeated and foetal number determined (day 50).

Table 1 Proportion of normal and retarded embryos and unfertilized oocytes following GnRH treatment in superovulated ewes.

<table>
<thead>
<tr>
<th>Gonadotrophin</th>
<th>Timing*</th>
<th>Normal GnRH Embryos</th>
<th>Retarded** GnRH Embryos</th>
<th>Unfertilized Oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSG 1200 IU</td>
<td>Control 24h</td>
<td>51/60 (85.0%)</td>
<td>3/60 (5.0%)</td>
<td>6/60 (10.0%)</td>
</tr>
<tr>
<td></td>
<td>36h</td>
<td>58/64 (90.6%)</td>
<td>1/64 (1.6%)</td>
<td>5/64 (7.9%)</td>
</tr>
<tr>
<td>FSH-P 15mg</td>
<td>Control 24h</td>
<td>75/88 (87.2%)</td>
<td>8/88 (9.3%)</td>
<td>3/88 (3.5%)</td>
</tr>
<tr>
<td></td>
<td>36h</td>
<td>105/124 (84.7%)</td>
<td>12/124 (9.7%)</td>
<td>7/124 (5.6%)</td>
</tr>
</tbody>
</table>

*Relative to time of sponge removal **< 16 nuclei day 5.

In Study 1, timing of GnRH treatment did not affect embryo quality as assessed by incidence of retarded embryos or the percentage of unfertilized oocytes in ewes treated with 1200 IU PMSG. However, treatment at 24h significantly (PGO.05) increased the percentage of unfertilized oocytes in FSH-treated ewes (Table 1). In Study 2, GnRH treatment at 24h reduced the percentage of normal embryos (85.0% - 88.1% to 53.5%, P=0.08) and increased the percentage of retarded embryos (4.5% - 5.8% to 16.7%, P=0.06) and unfertilized oocytes (7.5% - 9.2% to 30.0%, P<0.05) compared with other treatments (N=67, 30 and 120 for control, 24h and 36h treatments respectively). There were fewer pregnant ewes when GnRH was administered at 24h compared with control and 36h treatment groups (15/35 v 26/42 and 54/62 respectively, P=0.13).

It is concluded that the optimal timing of GnRH treatment varies with the type and dosage of the gonadotrophin used.


MULTIPLE OESTRUS-WITHOUT-OVULATION IN PERIPUBERTAL MEDIUM-PePpIn MERINO LAMBS

G.T. Stevenson, G.N. Hinch and T.N. Edey

Department of Animal Science, University of New England, Armidale, NSW

The influence of genetic differences between Merino strains on age and weight at first oestrus (PF), which is often used as an indicator of puberty (PUR), has been investigated in view of the possible use for selection for increased fertility (1). However, the onset of puberty requires (essentially) simultaneous oestrus and ovulation, which doesn't always occur as seen in the incidence of ovulation without oestrus ('silent heat', GnRH) and information on the occurrence of oestrus without ovulation (EWO) in Merino ewe lambs prior to puberty (2).

Observations were made on September born ewe lambs of two Merino strains including Medium-Peppin (MP) and a local fine wool (FW), together with two cross-breeds including first and second crosses (1stX and 2ndX). The lambs were reared under varying nutritional regimes (Table 1) and were observed for distinct behavioural oestrus (via vasectomised rams) and associated ovulation (by laparoscopy) from December to August.

Flock Breed Nutrition Growth n % Strain level rate(g/d) PUB (frequency prepubertal EWO)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Level</th>
<th>Rate (g/d)</th>
<th>Pub</th>
<th>Frequency prepubertal EWO</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>MP low</td>
<td>53</td>
<td>95</td>
<td>.39 .28 .19 .13</td>
</tr>
<tr>
<td>B</td>
<td>MP medium</td>
<td>63</td>
<td>112</td>
<td>.22 .19 .20 .22</td>
</tr>
<tr>
<td>C</td>
<td>MP high</td>
<td>81</td>
<td>80</td>
<td>.19 .13</td>
</tr>
<tr>
<td>D</td>
<td>FW high</td>
<td>71</td>
<td>44</td>
<td>.18 .04 0 0</td>
</tr>
<tr>
<td>E</td>
<td>1stX</td>
<td>87</td>
<td>133</td>
<td>.46 .17 .04 0 0</td>
</tr>
<tr>
<td>F</td>
<td>2ndX</td>
<td>100</td>
<td>136</td>
<td>.65 .17 .04 0 0</td>
</tr>
</tbody>
</table>

*The MP Merino strain lambs experienced more prepubertal EWO than either the FW strain or the cross breeds when reared under similar nutritional conditions (compare flocks C x D,E) and had similar mean 'cycle' lengths (i.e. interval between EWO), ranging from 6 to 195 days, to that of the remaining flocks combined (36 and 33 days respectively). Analysis of the proportions of the MP flocks B and C showing oestrus indicated a bi-modal pattern with peak activity 6 and 15 weeks after ram introduction. Within the EWO strain, improved nutrition had little effect on the incidence of multiple EWO (compare flocks A x B x C), while within flock B two well-grown lambs recorded 8 incidents of EWO before attaining puberty.

These results for the MP Merino represent a significant increase in the recorded maximum of two EWO with 'cycles' of 16 days previously recorded for Merinos (2). Clearly these lambs require a long period for oestrus 'practice runs' prior to puberty, and there would appear to be a large strain effect on EWO similar to that observed for PF by (1).

COMPARATIVE EFFICACY OF FSH AND PMSG FOR SHEEP ZYGOTE PRODUCTION


CSIRO, Division of Animal Production, Blacktown, NSW 2148

PMSG (Folligon, Intervet Aust) has been used routinely in the production of zygotes for pronuclear injection of recombinant DNA to produce transgenic sheep (1). We have now compared the efficacy of FSH-P (Burns-Biotech Labs Inc.) with that of PMSG to produce zygotes for injection.

Eight replicate experiments each using 16 Merino donor ewes divided into 2 treatment groups (PMSG and FSH) were carried out over 4 weeks. Treatment with 1200 i.u. PMSG 34 h before sponge removal has been described previously (1). The FSH groups received 18 mg FSH-P in 6 doses of 4,4,4,2,2,2 mg administered at 12-hourly intervals from 46 h before to 14 h after sponge removal. All sheep received 40 µg GnRH 24 h after sponge removal and were inseminated laparoscopically at 40 h. Zygotes were collected and pooled from 4 ewes per treatment group alternately at 63-64 h after sponge removal. Zygotes with visible pronuclei which survived injection were transferred to recipient ewes. Eggs apparently without sperm or pronuclei were re-examined with orcein stain. Data were combined and compared by Chi² and t-test analyses.

TABLE 1. Ovulation rates, egg recovery rates and fertilization rates for PMSG and FSH-treated donor ewes (64 per treatment group).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ovulations total OR</th>
<th>Egg recoveries %</th>
<th>Fertilization rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSG</td>
<td>689</td>
<td>535</td>
<td>51.2</td>
</tr>
<tr>
<td>FSH</td>
<td>814</td>
<td>544</td>
<td>66.8*</td>
</tr>
<tr>
<td>Total</td>
<td>1503</td>
<td>697</td>
<td>59.7</td>
</tr>
</tbody>
</table>

TABLE 2. Pronuclear visibility, injection survival rates and injected embryos transferred from PMSG and FSH-treated donor ewes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pronuclear visibility (%)</th>
<th>Embryos injected total</th>
<th>Embryos transferred total</th>
<th>Fertilized no.</th>
<th>% survival</th>
<th>Fertilized no./donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSG</td>
<td>72.8</td>
<td>90.2</td>
<td>257</td>
<td>66.9</td>
<td>172</td>
<td>2.69</td>
</tr>
<tr>
<td>FSH</td>
<td>79.0*</td>
<td>90.9</td>
<td>439</td>
<td>62.1</td>
<td>267</td>
<td>4.17*</td>
</tr>
<tr>
<td>Total</td>
<td>76.6</td>
<td>90.6</td>
<td>687</td>
<td>63.9</td>
<td>439</td>
<td>3.43</td>
</tr>
</tbody>
</table>

All significant differences favoured the FSH-P treated groups: ovulation rate (P=0.008); recovery rate (P=0.001); fertilization rate (P=0.041); eggs/experiment (P=0.003); pronuclear visibility (P=0.038). There was no difference in survival to injection (P=0.23). Thus the number of injected zygotes provided for transfer by each FSH-treated donor was significantly higher (P=0.028). However to produce about 40 embryos for transfer during 1 day, the cost per group would be $725 for FSH (10 donors) and $565 for PMSG (15 donors), sheep purchase, maintenance and drugs considered. The cost effectiveness favours PMSG when used with this schedule.


PROGESTERONE CONCENTRATION, CORPORA LUTEA AND EMBRYO SURVIVAL AT DAY 28 OF PREGNANCY IN EwES TREATED WITH PMS

J.F. Wilkins, N.W. Bruce* and J.R. Kreitich*.

Departments of Animal Science and *Anatomy and Human Biology, University of Western Australia, Nedlands, Western Australia, 6009.

Progesterone produced by the corpus luteum (CL) is vital to the success of conception and early embryo development (1,2). Here, we examine associations between progesterone concentration, number and mass of CL, and the number and weights of embryos recovered at day 28.

Mature Merino ewes were synchronised with prostagagen sponges (Repromap, Upjohn) and given intramuscular injections of whole pregnant mare serum (PMS) at sponge withdrawal, producing a large range in number of ovulations. They were mated with Merino rams. The ewes were sampled for plasma progesterone concentration on day 27 and killed on day 28. Reproductive tracts were recovered at the abattoir and the embryos examined and weighed. Ovaries were fixed in formalin for later dissection of the CL. The results are presented in Table 1.

Table 1. Group means for weights (mg) of CL, plasma progesterone concentrations (ng/ml) and numbers of total and live embryos in ewes with different numbers of CL and with at least one viable embryo.

<table>
<thead>
<tr>
<th>No.of CL/ewe</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>&gt;8</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.of CL/ewe</td>
<td>11</td>
<td>33</td>
<td>28</td>
<td>16</td>
<td>14</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Total CL mass</td>
<td>452</td>
<td>787</td>
<td>1010</td>
<td>1061</td>
<td>1444</td>
<td>1716</td>
<td>2037</td>
</tr>
<tr>
<td>Progesterone*</td>
<td>4.70</td>
<td>4.32</td>
<td>4.62</td>
<td>3.94</td>
<td>5.96</td>
<td>6.78</td>
<td>7.54</td>
</tr>
<tr>
<td>Total embryos*</td>
<td>1</td>
<td>1.55</td>
<td>1.69</td>
<td>1.75</td>
<td>2.50</td>
<td>2.45</td>
<td>2.83</td>
</tr>
<tr>
<td>Live embryos*</td>
<td>1</td>
<td>1.48</td>
<td>1.68</td>
<td>1.75</td>
<td>2.29</td>
<td>2.45</td>
<td>2.58</td>
</tr>
</tbody>
</table>

* Significant differences between groups (One-way ANOVA; PKG.01).

Total CL mass increased approximately linearly with CL number although this was offset in part by a significant decline in individual CL mass from 452 ± 33 mg (mean ± SMD) to 234 ± 30 mg for groups with 1 and >8 CL respectively. Progesterone concentrations and total CL mass were highly correlated for group means (r=0.87) but a weaker correlation for individuals (r = 0.52; n = 141) exposed unaccounted variation. There were no significant differences in the mean weights of surviving embryos over the range of litter sizes 1-4 and thus no evidence of competition at this stage. The proportion of non-viable embryos (indicating deaths from about 20-28 days) was not associated with number of CL or progesterone concentration.

The recovery of dead embryos was not obviously related to progesterone insufficiency. Proportions of embryos conceived/CL were not enhanced by increased progesterone as assessed here.


Supported by A.M.L.R.D.C. and A.R.G.S.
Luteinizing hormone releasing hormone (LHRH) has proved useful in the treatment of reproductive dysfunctions resulting from suppressed gonadotrophin secretion. A major factor contributing to postpartum (PP) anoestrous in cattle is thought to be a state of chronic gonadotrophin insufficiency. The present study therefore investigated both the gonadotrophin and ovarian responses in PP cows to continuous LHRH therapy. Alzet® osmotic pumps (model 2ML4) were used as a first step in the development of novel delivery systems for LHRH. Osmotic pumps calculated to deliver 100ng LHRH/kg BW/h were placed subcutaneously in a group of suckling Zebu cross cows to continuous LHRH therapy. Alzet® osmotic pumps (model 2ML4) were used as a first step in the development of novel delivery systems for LHRH. Osmotic pumps calculated to deliver 100ng LHRH/kg BW/h were placed subcutaneously in a group of suckling Zebu cross cows

Experiment 1 was a factorial trial carried out during Autumn (N=157) and Spring (N=164). There were 9 gonadotrophin treatments used in each season consisting of 3 doses of PMSG (0, 800 or 1600 iu) with each of 3 doses of FSH-P (0, 12 or 18 mg). The method for synchronization of oestrus and the gonadotrophin injection regime utilised has been previously described (1). On day 6 (day 0 = day of oestrus) ewes were subjected to laparotomy, when the status (normal or regressed) of CL was recorded. In experiment 2, ewes were superovulated during Autumn (N=89) and Spring (N=88) with 400 iu PMSG + 12 mg FSH-P. Half of the ewes treated in Autumn were fed a whole lupin grain supplement for 18 days prior to ovarian recovery. Ewes were subjected to laparoscopy on day 6 of the oestrous cycle when the status of CL was assessed.

Table 1: Incidence of superovulated ewes with PR-CL as affected by the type of egg and the season.

<table>
<thead>
<tr>
<th>Season</th>
<th>PMSG: 0 v 800</th>
<th>FSH-P: 0 v 12</th>
<th>Lupin: Yes v No</th>
<th>CL Status</th>
<th>Prob</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>53/143 (37)</td>
<td>32/156 (21)</td>
<td>1/143 (0)</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>17/98 (17)</td>
<td>30/104 (29)</td>
<td>1/98 (0)</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>13/101 (13)</td>
<td>22/89 (22)</td>
<td>1/101 (0)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>21/89 (24)</td>
<td>5/88 (6)</td>
<td>1/89 (0)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

The occurrence of PR-CL in superovulated Merino ewes, run under field conditions in south-west WA, was greater in Autumn than in Spring. This seasonal effect may have been due to changes in the nutritional status of the ewes, as the feeding of lupin grain reduced the incidence of PR-CL in ewes treated in Autumn. Irrespective of season, the incidence of PR-CL was shown to increase with superovulatory dose of gonadotrophin (PMSG, FSH-P or a combination of both).

INFLUENCE OF PROGESTERONE AND OESTRADIOL 17\beta ON BLASTOCYSTS IN SEASONAL DIAPAUSE IN THE TAMMAR WALLABY

T.P. Fletcher, M.B. Renfree and A.E. Jettont

Department of Anatomy, Monash University, Melbourne, 3168 and
Department of Neurobiology & Physiology, Northwestern University, Evanston, Illinois 60201.

During seasonal diapause (May-December) lactating female tammar wallabies carry a diapausing blastocyst which remains quiescent even after the sucking pouch young is removed. However, diapausing embryos can be reactivated by progesterone (P) injections, but 10 mg/day is needed for 10 days, and there is a 50% embryonic loss, mostly at early stages of development (1). This suggests that P may not act alone to induce normal reactivation. Recently, a transient rise in circulating oestradiol 17\beta (E) at diapause day 5 of pregnancy has been described, (2) coinciding with the early rise in P (2,3). Thus it seemed possible that E may be acting in concert with P to stimulate reactivation after diapause. This study was designed to test whether E alone, or acting synergistically with P could reactivate diapausing blastocysts during seasonal quiescence.

Six groups of six wallabies were injected with P (10 mg/day/18d) or E (1 \mu g or 10 \mu g/day/10d) alone or in various combinations (Fig.1) or vehicle (peanut oil). Plasma P was elevated to concentrations seen in late pregnancy for the duration of the treatment. Low dose (1 \mu g/day) E groups showed no measurable plasma increase but in the high dose (10 \mu g/day) plasma E was elevated to oestrous levels. At autopsy the embryos and reproductive tracts were assessed (Fig. 1). While P reactivated about 50% of the embryos as previously described (1), blastocysts subjected to E alone either remained in diapause (low dose) or disappeared from the uterus (high dose). One blastocyst in each E group collapsed after some slight expansion. No synergistic effect was noted in groups receiving both E & P. We conclude that oestradiol is not capable of stimulating normal growth of tammar blastocysts, and its role during early pregnancy remains unclear.

The annual changes in daylength that regulate seasonal reproduction are mediated by the pineal gland hormone, melatonin. However, the persistence of seasonality after pinealectomy in various mammals, including the tammar (1), demonstrates that the pineal is not totipotent and other endogenous or exogenous factors must be involved. As extra-pineal sources of melatonin (2,3,4) may provide photoperiodic information and therefore maintain seasonality, we compared the effects of immunization against melatonin on seasonal reproduction in the tammar, with those reported after pinealectomy (1).

In August, during the period of seasonal quiescence, ten non-lactating tammars presumed to have a quiescent corpus luteum received a primary i.m. injection (0.5 ml in 2 sites) of a 5-methoxytryptamine hemisuccinate:Human Serum Albumin conjugate in Freund’s Complete Adjuvant (FCA). Four control tammars received the FCA alone. As a direct comparison with the effects of pinealectomy (1) each animal received a vaccine booster or control injection on October 17. Twice weekly their pouches were examined for newborn young, the urogenital sinus for a copulatory plug indicative of oestrus, and blood samples were taken to measure antibody titres and progesterone concentrations.

The antibody titres in the treated group peaked in November at 1:3749 ± 1200 (mean ± s.e.m.) and declined to 1:685 ± 255 by January 6. In each of the immunized and control tammars birth and/or oestrus occurred at the normal time between January-March and the interval from boost to birth/oestrus was not significantly different between the two groups (P>0.05).

These results are similar to those reported after pinealectomy at this time of year (1) and both studies therefore suggest that melatonin from any source is not necessary for the maintenance of seasonal quiescence, nor for reactivation of the corpus luteum at the start of the breeding season.

Protein secretion by the epididymis and incorporation into sperm has been studied as epididymal proteins have been implicated in the process of sperm maturation in the mammalian epididymis (1). An earlier report described the changing electrophoretic pattern of proteins along the epididymis of the tammar (2). However, there are problems in interpreting the electrophoretic patterns as: (a) proteins may originate from 3 sources (sperm, testicular fluid and blood); (b) RF values of proteins are affected by the concentrations of other substances in samples; (c) proteins may be modified after secretion. Consequently, we have examined the secretion of protein into a Ringer-bicarbonate solution perfused through the lumen of the duct, and the significance of sperm as a source of luminal protein.

Tammars were anaesthetized with Inactin (Byk Gulden Pharm.,Konstang, W. Germany) and the epididymides prepared for micropuncture (2). A length of 20 cm of duct in the caput, corpus and cauda epididymidis were perfused. These regions corresponded to the 3 main epididymal segments (3). The length of duct was cannulated at each end with polyethylene tubing (ID = 280 µm) drawn to a 100-200 µm tip. The proximal end was connected to a perfusion pump and the distal end emptied into a haemaccelot tube. The duct was then flushed free of sperm and perfused at a rate of 0.6 - 1 µl/min. Protein analyses was described previously (2).

Table 1 shows that protein secretion into the cauda was greater and more variable than into the caput and corpus epididymidis.

<table>
<thead>
<tr>
<th>Component</th>
<th>Caput epididymis</th>
<th>Corpus epididymis</th>
<th>Cauda epididymis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein concentration</td>
<td>µg/ml</td>
<td>ng/cm/min</td>
<td>ng/cm/min</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.19 ± 0.014</td>
<td>5.47 ± 0.91</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.073 ± 0.020</td>
<td>3.69 ± 0.68</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.207 ± 0.095</td>
<td>8.75 ± 2.68</td>
<td></td>
</tr>
</tbody>
</table>

However, polyacrylamide electrophoresis of the perfusates indicated that only blood proteins were secreted into the cauda epididymidis whereas 3 proteins which were not present in blood or rete testis fluid were secreted into the caput and corpus epididymidis (MW = 29.4K, 17.7K and 12.8K). In addition, one protein was secreted only into the caput (MW = 75.7K) and another into the corpus (MW = 12.1K) epididymidis. Comparisons of gels of perfusates and epididymal plasma confirmed that some proteins (particularly the 29.4K protein) were being modified in the lumen of the ductus epididymidis. Three proteins (MW = 47.2K, 29.4K & 28.0K) present in sperm from the rete testis were absent in caudal sperm, however, these proteins did not contribute significantly to the electrophoretic pattern of epididymal plasma. Sperm gained one new protein (MW = 30.4) during epididymal transit.

Epididymal development in Antechinus stuartii (MARSUPIALIA)

D.A. Taggart and P.D. Temple-Smith
Department of Anatomy, Monash University, Clayton, Victoria, 3168.

Although the ultrastructure and function of the ductus epididymis in marsupials is now documented for several species there are no published studies of the fine structure of the developing epididymis in any marsupial species. This study examined the regional development and cellular differentiation of the epididymis in A. stuartii, a small, forest dwelling dasyurid marsupial.

Four male A. stuartii were trapped each month from April-August for this study. Each was perfused with buffered glutaraldehyde fixative and testes, epididymides and prostate were removed and weighed. Epididymides were subdivided into twelve regions, and processed for routine light and electron microscopy. Tubule diameter and epithelial heights were determined from 50 transverse sections of the duct in each region per animal using a Leitz ASM.

Like other mammals, the epididymis of A. stuartii can be divided macroscopically into caput, corpus and caudal (CL) regions. Lumen size increased during development and lumen shape remained circular in all regions except the CL segments where, from June, it became narrow and slit-like. Tubule diameter increased throughout development with the greatest increase occurring in CL segments. Epithelial height (EH) increased in all segments throughout development and peaked in the CL segments where considerable variability in EH was observed. An apparent wave of epididymal development occurred along the duct from April to June and maximum cellular differentiation occurred in May/June. In April the epithelium appears undifferentiated. Unspecialized epithelial cells lining the duct contained large nuclei which occupy most of the cell and few cytoplasmic organelles, and were devoid of cell surface specializations. A large increase in the abundance of cell organelles and surface specializations occurred between April and June. Mature, principal (PC), basal (BC) and mitochondria-rich (MRC) and clear cells (CC) were identified throughout the duct in June. PC and BC were most abundant while CC and MRC occur less frequently. In adult animals, cell ultrastructure along the duct varied in PC only. Stereocilia covered the luminal surface of PC between June and August except in CL regions where they are replaced by a brush border of microvilli, which was not derived from stereocilia. Spermatogenesis commenced in March and concluded in June. At this time viable sperm were observed throughout the epididymis, except in caudal regions where they were in various stages of degeneration suggesting that conditions were unsuitable for maintaining sperm viability. Intact sperm were found throughout the duct in July and August.

Epididymal development coincided with the passage of testicular fluids and presumably androgens, along the epididymis and with increases in prostatic weight. Rising levels of plasma androgens during the period from April to July (1) appear to confirm an androgenic influence on epididymal development in A. stuartii. With the exception of the CL specialization, the general sequence of epididymal development and cellular differentiation in A. stuartii was similar to that described for the few eutherian mammals which have been examined.

REGULATION OF MOTILITY OF SPERM FROM THE JAPANESE QUAIL

J. Clulow1, C. Pholpramool2 & R.C. Jones1

1 Department of Biological Sciences, University of Newcastle, N.S.W., 2308.
2 Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand.

Our earlier work showed that sperm develop the capacity for motility in the proximal extratubular genital ducts and that "mature" sperm may be collected from the ductus deferens of the Japanese quail (1). This report describes studies on the regulation of motility of sperm collected by microincision from the ductus deferens of quail anaesthetised with Inactin (Byk Gulden, Konstanz, W. Germany). The samples (20 nl) were diluted and stored under oil. Their motility was assessed microscopically and scored on a scale from 0 to 10.

It was found that sperm were immotile in vivo, but developed vigorous motility when collected into a microincision pipette. The motility was maintained when samples were diluted in Krebs-Ringer phosphate at 30°C (mean score, MS = 8.8± 0.5) or the ductus deferens (MS = 8.1± 0.7) or the ductus deferens (MS = 3.6± 1.3) at 42°C. Incubation of sperm at 42°C in Krebs-Ringer phosphate lowered motility (MS = 0.9± 0.3). However, the effect was reversible as motility could be restored by recollaring to 30°C (MS = 8.6± 0.3). Dilution of sperm in MES buffer at pH 5.5 inhibited motility completely, but full motility was restored by recollaring in HEPES buffer at pH 7.4. Aerobic incubation also resulted in loss of motility (MS = 0.8± 0.5) which was restored under anaerobic conditions (MS = 8.8± 0.03).

It is concluded that sperm motility in the quail can be reversibly inhibited by low extracellular pH, body temperature and anaerobic conditions. However, we have yet to resolve how these factors regulate motility in vivo as the pH of the luminal fluid in the ductus deferens is 7.33± 0.02 and conditions are aerobic if they are similar to the mammalian epididymis (3).


METABOLISM OF ANDROGENS IN THE BRAIN OF THE LIZARD TILIGUA RUGOSA, IN VITRO

P.A. Elahi, A.R. Bourns, T.G. Watson

Biological Sciences, Deakin University, Geelong, Victoria 3217.

The metabolism of androgens in the vertebrate central nervous system (CNS) is considered necessary for certain behavioural and neuroendocrine responses. Enzyme systems such as aromatase, 5α-reductase, and 17α-oxidoreductase (17α-OR) have been found in the brain of representatives of most vertebrate classes (1). The aromatase system is considered to be phylogenetically ancient and is associated in most vertebrates with metabolism of androgen action on the brain (1). The male lizard Tiliguara rugosa has high activity of 17α-oxidoreductase in the testes as well as unusually high concentrations of epitestosterone in the plasma (2). The presence of this enzyme has been reported in the brain of other reptiles and an avian species (1). The purpose of this study is to determine the androgen metabolising enzyme systems present in the brain of T. rugosa. Animals were captured during the mating period, decapitated and the brain removed and stored in liquid nitrogen. In vitro incubations of homogenates of whole or sectioned brains with [3H]-androstenedione and [14C]-testosterone were carried out (in Krebs-Henseleit buffer pH 7.4, with cofactors NADPH and NADH) at 32°C for 1-2 hr and the products subsequently identified using thin-layer chromatography, high performance liquid chromatography, microchemical methods and recrystallisation to constant specific activity. Approximate enzyme activities were expressed as pmol of product per hour per mg of tissue. The activity of 17α-OR in the male was measured at 300 pmol/mg/hr spread uniformly in fore-, mid- and hindbrain areas. Similar activity was also found in the female brain. Aromatase and 17α-OR activity were low and close to the detection limit. No evidence of 5α-reductase was found.

Our results indicate that 17α-OR activity in the CNS of T. rugosa is considerably higher than for other vertebrates and may make a significant contribution to levels of epitestosterone in the peripheral plasma of this animal. Its uniform distribution may be analogous to the presence of 5α-reductase in other vertebrates. Aromatase activity is high in T. rugosa but may be localised in specific areas in the brain as in other vertebrate classes.

effects of captivity on plasma androgens in the lizard tiliguarna nigra
T.G. Watson, A.R. Bourne and D. Windmill
Biological Sciences, Deakin University, Geelong, Vic. 3217.

In many seasonally breeding vertebrates it has been shown that captivity has marked effects on the breeding cycle. However there have been few investigations of the changes in circulating levels on gonadal hormones in captive animals. Our previous studies of the male lizard Tiliqua nigra have shown that it has a well defined breeding cycle with a peak in plasma androgens and testis weight during spring (1). The present study examines the effect of captivity on the seasonal pattern of male hormones.

Adult male lizards were collected monthly from areas near the Grampians. Captive animals (n = 5) were defined as animals which had been in captivity for a minimum period of three months. Field animals (n = 54) were those which were either sampled in the field or within one day of capture. Blood samples were taken following pentobarbital anaesthesia and animals were then killed by overdose. Testes were removed and weighed. Plasma androgens (testosterone and epitestosterone) were measured using specific radioimmunoassays (1).

Covitity appeared to have no effect on testicular weight and there was no difference in values between captive and field animals throughout the year. In this animal the testes are regressed during summer and development resumes in Autumn reaching a peak in Spring. Spermiogenesis is maximal during this latter period. Normally plasma androgen levels parallel the changes in testicular weight. In field animals the mean plasma concentrations of testosterone and epitestosterone were approximately 150 and 400 nmol/l respectively. In captive animals the plasma androgen levels were significantly (p < 0.05) lower than in field animals during Spring. There was no sign of a seasonal cycle in plasma androgens in captive animals. Mean plasma concentrations for testosterone and epitestosterone were approximately 40 and 300 nmol/l respectively in captive animals which are equivalent to values found during the non-breeding season.

These results indicate that the stress of captivity on Tiliqua nigra, as in some other reptiles (2), has a differential effect on androgenesis and spermatogenesis.


THE ROLE OF THE OPTIC GLAND HORMONE IN REPRODUCTION AND POST-REPRODUCTIVE SENESCENCE IN OCTOPUS

R. W. TAIT
Laboratoire Arogo, Université de Paris, Bangues-sur-mer, France and Physiology Section, Animal Research Institute, Werribee, 3030

Adults of Octopus vulgaris, the common Mediterranean octopus, grow to a large size (up to 10 kgs) within a brief life span (1-2 yrs) females lay a single clutch of eggs and die soon after these hatch. In this species, maturation is controlled by a hormone secreted by the paired optic glands situated on the optic lobe peduncle of the brain (1). Following reproduction, animals of either sex cease feeding, their general body condition deteriorates and they die within a short period. This study was intended to demonstrate whether the endocrine glands controlling reproduction are also responsible for the degeneration of the somatic tissues associated with reproduction.

Four experimental groups were used: immature, fed animals; immature starved animals; post-reproductive (senile) animals, and senile animals with the optic glands removed after reproduction. Of the somatic tissues examined, the musculature of the arms and mantle, comprising 80-90% of the total body weight, was found to be the most severely affected by reproduction. Table 1 gives some parameters of nitrogen metabolism for the four groups: muscle protein synthesis (cts incorp./hr/mg wet wt) and catabolic rates (cts released/hr/cts present as protein), total ammonia loss (in mmol/hr/kg =~ 20°C) and urinary amino acid (m leucine equiv.) and soluble protein (mg/l BSA equiv.) levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein synth. (cts incorp./hr/mg wet wt)</th>
<th>Protein catabol. (cts released/hr)</th>
<th>Protein excret. (cts released/hr)</th>
<th>Ammonia excret. (mmol/hr)</th>
<th>Urinary protein (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature</td>
<td>305.1±(13) 0.178±(3)</td>
<td>3.117±(157) 2.99±(3)</td>
<td>515.2±(33)</td>
<td>17.0±(2)</td>
<td>113.6±(17)</td>
</tr>
<tr>
<td>Starved</td>
<td>117.0±(13) 0.337±(8)</td>
<td>0.276±(35) 1.15±(5)</td>
<td>561.3±(17)</td>
<td>16.4±(1)</td>
<td>113.6±(17)</td>
</tr>
<tr>
<td>Senile</td>
<td>123.4±(6) 0.506±(4)</td>
<td>0.416±(93) 11.6±(14)</td>
<td>113.6±(17)</td>
<td>4.6±(3)</td>
<td>1756.0±(8)</td>
</tr>
</tbody>
</table>

(a,b,c indicate significant difference groups at p < 0.05: * p = 0.05)

It is evident that the degeneration of the musculature seen following reproduction is due to a large increase in the net catabolism of the muscle (approx. 5-fold). In the absence of feeding, the fatal end result is inevitable. Removal of the optic glands reverses this effect and promotes conservation of protein nitrogen, even though the senile 000 animals did not resume feeding. Contrary to previous findings however (2), post-reproductive survival was not extended.

These results indicate that the optic glands are responsible for both a gonadal and anabolic effect and a somatic catabolic effect. It is suggested that their interdependence of reproduction and somatic degeneration, mediated by the optic glands, may provide an evolutionary constraint responsible for the lack of variation in reproductive tactics in this group.

(2) Vo¤insky, J. Science, N.Y., 198: 348-551 (1977)
Factors affecting activation and duration of motility in Sillago ciliata spermatids

J.A. Goodall, A.W. Blackshaw, M.F. Capra*

Department of Physiology and Pharmacology, University of Queensland, St Lucia, 4067.
*Department of Public Health and Nutrition, Queensland Institute of Technology, Brisbane, 4001.

The summer whiting (Sillago ciliata) has significant aquaculture potential. The factors affecting sperm motility and therefore fertility in the fertilising media have been explored.

Sperms were collected by abdominal pressure and mixed with the experimental activating media and the motility of the sperm was visualised microscopically. The effect of temperature, osmolarity, cations and organic substrates on the duration of motility was studied.

600

Table 1.

| Temp* | mS/mol | Glu | NaCl | KCl | Ca | Mg | NaHCO3 | Pyruvate | Citrate | Lactate | Glucose | Seawater |
|-------|--------|-----|------|-----|----|----|--------|----------|---------|---------|---------|---------|---------|
| C:Wm | 541 | 150 | 0 | 0 | 0 | 8:1 | 308 | 0.0 | 459 | 127 |
| C:Wm | 309 | 300 | 0 | 0 | 0 | 1:4 | 317 | 0.1 | 358 | 138 |
| W:Wm | 449 | 600 | 16 | 702 | 581 | 2:1 | 361 | 0.5 | 906 | 112 |
| W:Wm | 239 | 900 | 287 | 577 | 1:1 | 322 | 1.0 | 506 | 148 |
| 1200 | 316 | 238 | 287 | 1:2 | 372 | 1.1 | 446 | 111 |
| 1:4 | 483 | 1.5 | 915 | 99 |
| 1:8 | 567 | 5.0 | 693 | 5.1 | 1394 |
| +4 | 575 | 1244 |

*artificial seawater as basis

C:Wm = cold (on ice) stored sperm; cold activating media
W:Wm = warm (room temp) stored sperm; warm activating media

Sperm are best stored on ice as opposed to room temperature and the duration of motility is greatest when the activating solution (artificial seawater) is kept on ice.

Osmolarities greater than 300 mOs/m will activate motility in ionic and anionic solutions. Ionic solutions were clearly superior but there was no significant difference between NaCl and KCl. The sperms in the 300 mOs/m and 150 mOs/m solutions were capable of activation on the addition of a hyperosmotic solution. Ratios of NaCl:KCl greater than 1 greatly improved the duration of motility.

There were significant overall responses to increasing Ca and Mg levels. At zero or low levels of Mg there was a linear response to Ca and at high levels of Mg a maximum Ca response occurred at 1 [Ca] and this response was unchanged at 5 [Ca]. The effect of Mg reflects the greater proportion of Mg naturally occurring in seawater.

Organic substrates, especially metabolisable substrates increased duration of motility.

Hyperosmotic solutions activate sperm, 600 mOs/m being superior to 1200 mOs/m (seawater). Presence of Ca and Mg and energy providing substrates improves duration of motility.
EFFECT OF SERTOLI CELL EXTRACELLULAR MATRIX, FETAL CALF SERUM AND SERTOLI CELLS ON MYOID CELL GROWTH AND EXTRACELLULAR MATRIX

Samir S. Raychoudhury, M.G. Irving and A.W. Blackshaw*

School of Science, Griffith University, Nathan, Qld. and *Department of Physiology and Pharmacology, University of Queensland, St. Lucia, Qld.

This study investigates hormonal regulation of myoid cell function.

Sertoli cells (S) and myoid cells (M) were prepared from 20-22 day old rat testes and cultured (1). There were eight treatment combinations of M, S, Sertoli cell matrix (X) and fetal calf serum (F), viz; M, S, SM, X, XM, XF, MF and XMF. All culture types were labelled with Na35SO4 (10Ci/ml) for 96 hrs at 37°C and treated with (BU)2cAMP, (0.5 mM) or FIRT (FSH, 25ng/ml; insulin, 5ug/ml; retinol, 0.35muM; testosterone, 0.7muM). Before harvesting, the cultures were incubated with 3H-leucine (1mu/plate) for 60 mins at 37°C. Parameters measured included 3H-leucine and 35SO4 incorporation into cellular and secreted proteins (2), 35SO4 incorporation into cellular, secreted and extracellular matrix (ECM) glycosaminoglycan (GAG). Myoid cell proliferative response was quantified by measuring total DNA and cellular protein in all treatment groups.

In the absence of hormones, Sertoli and myoid cells (SM) in co-culture were synergistic, increasing both DNA and cellular protein. However, X had only a minor effect on DNA levels and reduced cellular protein content. The incorporation of 3H-leucine and 35SO4 into protein, 35SO4 into GAG was inhibited by S while X stimulated 35SO4 incorporation into cellular and ECM GAG. F increased both the DNA and protein content of cultures and strongly stimulated the secretion of 3H- and 35SO4 labelled protein; it also increased cellular levels of GAG but greatly reduced the amount of GAG in ECM. In M and XM, FIRT increased cell proliferation, total cell protein, specific protein synthesis and secretion. cellular and ECM GAG. The response of MF and XMF to FIRT was relatively small except for secreted and ECM GAG, but in co-culture the small ECM GAG response was greatly enhanced by FIRT. However, (BU)2cAMP markedly increased protein synthesis in MF and XMF; medium and ECM GAG was strongly stimulated in all cultures.

Secretion of GAG by myoid cells is an important contribution to the ECM of the seminiferous tubules. It appears to be modulated by Sertoli cell matrix and is sensitive to hormonal stimulation.


TESTOSTERONE CONCENTRATIONS IN TESTICULAR INTERSTITIAL FLUID AND VENOUS BLOOD OF ADULT RATS FOLLOWING LOCAL HEATING OF THE TESTES.

S. Maddocks, J.L. Zupp, S.F. Sowerbutts and B.P. Setchell

Department of Animal Sciences, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, South Australia 5064.

When the testes of rats are heated at 43°C for 30 minutes, spermatogenesis is severely but reversibly disrupted (1). The interstitial tissue becomes hypertrophied, as in the cryptorchid testis in which cytological changes are suggestive of increased steroid secretion (2). However, peripheral blood levels of testosterone (T) do not show any significant changes after these treatments suggesting that other metabolites are being secreted (2) or that testicular blood flow is limiting secretion (3). In this study, we have investigated the concentrations of T in testicular interstitial fluid (IF) collected with a push-pull cannula (4), and in testicular (TV) and peripheral (PV) venous blood of adult male rats whose testes were heated at 43°C for 30 minutes. Samples were collected under pentobarbitone anaesthesia at various times after heating.

PV concentrations of testosterone were not consistently affected. TV levels were significantly increased from 14 to 35 days, but were significantly lower than control values at 49 and 63 days. IF concentrations rose until 35 days, when they were not significantly different from TV levels. By 63 days, IF levels were again lower than TV levels but remained significantly higher than control IF levels.

Days after heating | Testosterone concentrations (ng/ml) |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IF</td>
</tr>
<tr>
<td>0</td>
<td>5.5 ± 1.3</td>
</tr>
<tr>
<td>7</td>
<td>8.0 ± 3.9</td>
</tr>
<tr>
<td>14</td>
<td>16.9 ± 3.2</td>
</tr>
<tr>
<td>21</td>
<td>14.9 ± 2.5</td>
</tr>
<tr>
<td>28</td>
<td>19.3 ± 4.5</td>
</tr>
<tr>
<td>35</td>
<td>42.1 ± 8.7</td>
</tr>
<tr>
<td>49</td>
<td>20.9 ± 5.6</td>
</tr>
<tr>
<td>63</td>
<td>13.1 ± 3.8</td>
</tr>
</tbody>
</table>

*: significantly different from control (P < 0.05). Values across rows with different superscripts are significantly different.

Similar changes in TV T concentrations after localised heating have been correlated with changes in testis weight due to disruption of spermatogenesis, and changes in testicular blood flow (3). When our results are compared with changes in spermatogenesis and testicular histology at the same times after heating, we believe the results provide further evidence of important interactions between the seminiferous tubules, the Leydig cells, and the vascular system in the testis for the control of T production and secretion.

THE ROLE OF TESTOSTERONE IN THE MAINTENANCE OF SPERMATOGENESIS IN HYPOPHYSECTOMIZED RATS

Sun Yun-Tian, Dan C. Irby, David M. Robertson and David M. de Kretser

Studies on the hormonal control of spermatogenesis have suggested that FSH is necessary for the initiation, but not maintenance, or restoration of spermatogenesis after prolonged hormonal withdrawal. It has been postulated but not established that testosterone alone can maintain spermatogenesis quantitatively in rats without FSH.

The aim of this study was to explore whether testosterone alone could maintain spermatogenesis quantitatively and whether FSH had any influence on this process. Adult male rats were hypophysectomized and then implanted immediately with different lengths (0-20cm) of testosterone silastic capsules for a period of 7 or 13 weeks. Testis weights, daily sperm production (DSP), serum testosterone (T), testicular interstitial fluid testosterone (IFT), serum FSH and serum LH were measured.

Table: Daily Sperm Production (mean ± s.d., × 10⁶)

<table>
<thead>
<tr>
<th>T Implants (cm)</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact rats</td>
<td>118±9</td>
<td>44±12</td>
<td>36±24</td>
<td>89±19</td>
<td>96±10</td>
<td>96±15</td>
</tr>
<tr>
<td>Hypox 7 wks</td>
<td>1.1±0.1</td>
<td>9±9</td>
<td>4±3</td>
<td>7±3</td>
<td>12±1</td>
<td>2±1</td>
</tr>
<tr>
<td>13 wks</td>
<td>0</td>
<td>2±1</td>
<td>-</td>
<td>-</td>
<td>5±14</td>
<td>-</td>
</tr>
</tbody>
</table>

The results showed that spermatogenesis as assessed by DSP was maintained at 80-90% of control levels in intact animals and at 70-80% in hypophysectomized animals by testosterone implants >6cm alone. However, spermatogenesis was suppressed to 31% and 3% of control levels by 6cm implants. Furthermore, DSP in 13-weeks groups showed greater suppression and less recovery with same doses of T implants compared to 7-weeks groups. These suggested that pituitary support presumably by FSH was responsible for this difference. FSH also had an important action in maintaining spermatogenesis.

The recovery of spermatogenesis observed in animals with lengths of implants between 6cm and 8cm was associated with no significant changes in IFT and serum T (6.4±2.2 and 7.9±1.0 ng/ml), although the levels were within the control range (3.9±4.2 ng/ml). Further studies are required to explore the manner by which testosterone is able to stimulate spermatogenesis, particularly in view of the failure of intratesticular and plasma T levels to rise significantly.

Electron Microscopy of Spermatoogenesis in Triploid Fowls

M. Lin, M.H. Thorne, I.C.A. Martin, B.L. Sheldon and R.C. Jones

1 Department of Biological Sciences, University of Newcastle, N.S.W., 2308.
2 Division of Animal Production, C.S.I.R.O., North Ryde, N.S.W., 2113.
3 Department of Veterinary Physiology, University of Sydney, N.S.W., 2006.

Our earlier report (1) described the histology of the gonads of triploid fowls of 3A.ZZ (supermale) and 3A.ZSW (intersex) genotypes and compared them with normal 2A.ZZ males. Spermatogenesis occurred in both triploid genotypes, however, neither meiosis nor spermiogenesis were normal and we were uncertain whether any normal spermatozoa are produced. Consequently, we have examined spermatogenesis in three 3A.ZZ and four 3A.ZSW fowls using the transmission and scanning electron microscopes.

We found that the mitotic cells were normal except that their chromatin and nucleoli are more electron dense than in diploid fowls. The primary spermatocytes also appear normal in both genotypes up until the zygotene stage of meiosis. Examination of this stage revealed that homologous triple pairing of chromosomes occurs, but it involves the formation of a pentapartite synaptonemal complex (SC) rather than the tripartite SC which forms during meiosis of diploid cells. Examination of the pentapartite structure indicated that recombination nodules (RN) occur at random on the two central elements from which it appears that reciprocal exchange of pieces of chromatin can occur irregularly among any pair of the homologues. During diplotene, the RNs are surrounded by condensed chromatin and become chromatid nodules which are probably precursors of chiasmata. Secondary spermatocytes and spermatids apparently form normally except that some spermatids appear to be diploid. The latter were identified by the possession of larger nuclei than other spermatids and duplication of some organelles such as centriolar complexes and acrosomal vesicles.

Most diploid spermatids develop to form large, horseshoe- or comma-shaped heads, two nucleoli and two tails. The irregular heads are a consequence of derangement of nuclear condensation and shaping during spermiogenesis. Also, the circular and longitudinal manchette, two sets of microtubules which surround the nucleus of spermatids in diploid fowls, are either absent or irregularly arranged in triploid fowl spermatids. Malformations were also observed in haploid spermatids.

It is concluded that normal spermatozoa are rare in the lumen of the seminiferous tubules of ZZ fowl, and they may be absent in ZSW birds.

THE ACTION OF (S)-3-CHLOROLACTALDEHYDE ON THE METABOLIC ACTIVITY OF BOAR SPERMATOZOA: INHIBITION OF TRIOSEPHOSPHATE ISOMERASE

A.R. Jones and S.J. Cooney
Department of Biochemistry, The University of Sydney, Sydney, NSW 2006

Studies with the male antifertility agent (S)-o-chlorohydrin (I) have revealed that the compound owes its biological activity to a metabolite, (S)-3-chlorolactaldehyde (II). This metabolite, which is produced by mature spermatozoa in vitro, specifically inhibits glycerolaldehyde 3-phosphate dehydrogenase thereby decreasing the capability of the spermatozoa to synthesise NADP (II). The use of this specific inhibitor to study spermatozoal metabolism has, however, been thwarted for two reasons. Firstly, a chemical synthesis of (S)-3-chlorolactaldehyde has not yet been achieved so that use of the racemic (R,S)-mixture involves permeability problems and complications arising from the presence of the unwanted (R)-isomer. Secondly, as the generation of (S)-3-chlorolactaldehyde from (S)-o-chlorohydrin is catalysed by a NADP+ dependent dehydrogenase which is involved in the oxidation of glycerol, the presence of glycerol inhibits formation (I). These problems have been overcome with the use of 3-chloro-1-hydroxyacetone (III) which is converted by spermatozoal triosephosphate isomerase into (S)-3-chlorolactaldehyde (II). Not only has this enabled the metabolism of glycerol to be studied in its presence but it has led to an important observation about the mechanism of the antiglycolytic action of (S)-3-chlorolactaldehyde.

In the presence of 3-chloro-1-hydroxyacetone (0.5mM) and with D-fructose (1mM) as the substrate, incubation of boar spermatozoa for 1h at 34°C led to a decrease in endogenous lactate and to the accumulation of fructose-1,6-bisphosphate and dihydroxyacetone phosphate which is indicative of the inhibition of glycerolaldehyde 3-phosphate dehydrogenase. However, with glycerol (2mM) as the substrate, the presence of 3-chloro-1-hydroxyacetone led to a decrease in endogenous lactate but not to the accumulation of these two key glycolytic intermediates. This could be explained on the basis that triosephosphate isomerase was also being inhibited thereby preventing the formation of the glycerolaldehyde 3-phosphate essential for the synthesis (and the accumulation) of fructose-1,6-bisphosphate. Incubation of boar spermatozoa for 1h at 34°C with fructose (1mM) and 3-chloro-1-hydroxyacetone (0.5mM), followed by assay of enzyme activities confirmed that this was the case: glycerolaldehyde 3-phosphate dehydrogenase activity was 26.6±2.9% (of control value of 112.0±11.8nmol substrates oxidised/min/mg protein, n=4) and triosephosphate isomerase activity was 31.9±4.0% (of control value of 1.40±0.06nmol substrate converted/min/mg protein, n=5). This is the first demonstration that the antiglycolytic action in mature spermatozoa of (S)-3-chlorolactaldehyde and, consequently, of (S)-o-chlorohydrin, involves the inhibition of two enzymes.


89

The Action of (S)-3-Chlorolactaldehyde on the Metabolic Activity of Boar Spermatozoa: Inhibition of Triosephosphate Isomerase

A.R. Jones and S.J. Cooney
Department of Biochemistry, The University of Sydney, Sydney, NSW 2006

Studies with the male antifertility agent (S)-o-chlorohydrin (I) have revealed that the compound owes its biological activity to a metabolite, (S)-3-chlorolactaldehyde (II). This metabolite, which is produced by mature spermatozoa in vitro, specifically inhibits glycerolaldehyde 3-phosphate dehydrogenase thereby decreasing the capability of the spermatozoa to synthesise NADP (II). The use of this specific inhibitor to study spermatozoal metabolism has, however, been thwarted for two reasons. Firstly, a chemical synthesis of (S)-3-chlorolactaldehyde has not yet been achieved so that use of the racemic (R,S)-mixture involves permeability problems and complications arising from the presence of the unwanted (R)-isomer. Secondly, as the generation of (S)-3-chlorolactaldehyde from (S)-o-chlorohydrin is catalysed by a NADP+ dependent dehydrogenase which is involved in the oxidation of glycerol, the presence of glycerol inhibits formation (I). These problems have been overcome with the use of 3-chloro-1-hydroxyacetone (III) which is converted by spermatozoal triosephosphate isomerase into (S)-3-chlorolactaldehyde (II). Not only has this enabled the metabolism of glycerol to be studied in its presence but it has led to an important observation about the mechanism of the antiglycolytic action of (S)-3-chlorolactaldehyde.

In the presence of 3-chloro-1-hydroxyacetone (0.5mM) and with D-fructose (1mM) as the substrate, incubation of boar spermatozoa for 1h at 34°C led to a decrease in endogenous lactate and to the accumulation of fructose-1,6-bisphosphate and dihydroxyacetone phosphate which is indicative of the inhibition of glycerolaldehyde 3-phosphate dehydrogenase. However, with glycerol (2mM) as the substrate, the presence of 3-chloro-1-hydroxyacetone led to a decrease in endogenous lactate but not to the accumulation of these two key glycolytic intermediates. This could be explained on the basis that triosephosphate isomerase was also being inhibited thereby preventing the formation of the glycerolaldehyde 3-phosphate essential for the synthesis (and the accumulation) of fructose-1,6-bisphosphate. Incubation of boar spermatozoa for 1h at 34°C with fructose (1mM) and 3-chloro-1-hydroxyacetone (0.5mM), followed by assay of enzyme activities confirmed that this was the case: glycerolaldehyde 3-phosphate dehydrogenase activity was 26.6±2.9% (of control value of 112.0±11.8nmol substrates oxidised/min/mg protein, n=4) and triosephosphate isomerase activity was 31.9±4.0% (of control value of 1.40±0.06nmol substrate converted/min/mg protein, n=5). This is the first demonstration that the antiglycolytic action in mature spermatozoa of (S)-3-chlorolactaldehyde and, consequently, of (S)-o-chlorohydrin, involves the inhibition of two enzymes.


89

Occurrence of a Homologue to the Initial Segment of the Mammalian Epididymis in the Avian Sperm Ducts?

R.C. Jones, G. Kidd, N. Spinks and J. Clulow
Department of Biological Sciences, University of Newcastle, N.S.W. 2308

There is good evidence from studies on animals from each subclass of Mammalia that the mammalian ductus epididymis has a characteristic initial segment. The segment is characterised by a distinctive epithelium which contains high 5α-reductase activity and is dependent on the luminal fluids coming from the testis. Further, it seems to play an important role in the maturation of sperm after they leave the testis. This report describes studies on the Japanese quail, Coturnix coturnix, which indicate that its extratesticular sperm ducts do not contain a region homologous to the initial segment of the mammalian epididymis.

Our structural studies on the quail confirm reports on the domestic fowl (1) which found no structural differentiation of the epithelium lining the wide connecting ducts, ductus epididymis and ductus deferens.

The dependence of the duct epithelium on the luminal fluids from the testes was examined in an experiment involving unilateral and bilateral orchidectomy under Fluothane anaesthesia. Table 1 shows that unilateral orchidectomy had little effect on the duct epithelium whereas bilateral orchidectomy caused regression of the epithelium lining the ductus epididymis and ductus deferens.

Table 1. Effects of Unilateral (left testis) and Bilateral Orchidectomy on Epididymis Height (EH) and Duct Diameter (DD) of the Sperm Ducts of the Quail. (Means ± SE from 5 animals, µm)

<table>
<thead>
<tr>
<th>Ductulus</th>
<th>Ductus</th>
<th>Ductus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effentera</td>
<td>Epididymis</td>
<td>Deferens</td>
</tr>
<tr>
<td>EH</td>
<td>DD</td>
<td>EH</td>
</tr>
<tr>
<td>Sham</td>
<td>9.9±1.2</td>
<td>53±11</td>
</tr>
<tr>
<td>operation</td>
<td>11.6±1.0</td>
<td>54±9</td>
</tr>
<tr>
<td>Unilateral</td>
<td>11.8±0.8</td>
<td>51±5</td>
</tr>
<tr>
<td>orchidectomy</td>
<td>10.5±0.4</td>
<td>57±4</td>
</tr>
<tr>
<td>Bilateral</td>
<td>9.6±1.8</td>
<td>46±8</td>
</tr>
<tr>
<td>orchidectomy</td>
<td>9.6±1.8</td>
<td>47±11</td>
</tr>
</tbody>
</table>

Polyacrylamide gel electrophoresis of the luminal contents of the sperm ducts showed that the ductus epididymis and ductus deferens secrete one protein (MW = 16,500) which is not present in rete testes fluid or blood. The secretion is dependent on systemic testosterone but not on intraductal fluids from the testsis. It is suggested that the absence of a homologue of the initial segment in the quail may explain why there is little post-testicular sperm maturation in the bird.

The initial segment of the mammalian epididymis has a characteristic ultrastructure and is dependent on its luminal fluids originating from the testis. As Nicander et al. (1) showed that structural signs of regression of the initial segment epithelium occurs within 6 hours of different duct ligation, we have developed a microperfusion technique to study the effects in vivo of modifying the composition of the luminal fluids of the initial segment.

Mature Wistar rats were anaesthetized with Inactin (Byk Gulden Pharm., M. Germany) and prepared for surgery as described previously (2). A length of initial segment (18.0 cm) was cannulated at each end with polyethylene tubing drawn to a tip diameter of 100-120 μm. The proximal end was connected to a perfusion pump and the distal end emptied into a haematocrit tube. The duct was then flushed free of sperm with a synthetic medium with the same inorganic composition as ram rete testis fluid and containing 0.25% inulin. Subsequently the duct was perfused for at least 6 hours at 0.5 μl/min with either the synthetic medium or rete testis fluid from the ram. The perfusate was analysed quantitatively for protein and inulin and samples were examined using polyacrylamide gel electrophoresis.

Estimates of inulin ratio showed little net transport of fluid across the duct mucosa. However, protein was secreted into the ram rete testis fluid at a greater rate than into the synthetic medium (Table 1).

### Table 1. Secretion of protein into 2 solutions perfused through the initial segment of the epididymis of the rat.

<table>
<thead>
<tr>
<th>Source</th>
<th>Approximate Volume (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>Approximate Volume (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

Ram RTF 40384 ± 5157 74343 ± 8810 11034 ± 18459
Rat RTF 62486 ± 15166 85330 ± 20786 101059 ± 21081
Tammar RTF 48121 ± 7676 78922 ± 8550 104040 ± 16854
Quail RTF 14139 ± 1934 40280 ± 12084 -
Rat CEF 48389 ± 7213 68222 ± 9785 -
Tammar CEF 115862 ± 31245 130916 ± 28564 -

It is proposed that the microperfusion technique provides a method for assaying for the component in rete testis fluid which supports the function of the initial segment of the epididymis.


The role of seminiferous growth factor in regulating the initial segment of the mammalian epididymis?


1 Department of Biological Sciences, University of Newcastle, NSW 2308
2 Department of Animal Physiology, Waite Agricultural Research Institute, University of Adelaide, SA 5064
3 Department of Veterinary Physiology, University of Sydney, NSW 2006

The seminiferous growth factor has been identified as a peptide in extracts of testicular homogenates from the mouse, guinea pig, rat and bull(1), and in rete testis fluid (RTF) from the ram(2). It is suggested that it may regulate spermatogenesis and/or the initial segment of the epididymis, however, there is no evidence to confirm these proposals. We have obtained circumstantial evidence to test the latter proposal. The potency of the growth factor in RTF from the ram, rat and tammar (Macropus eugenii) was determined to assess whether it is present in a variety of animals with an initial segment which is dependent on luminal fluids from the testis. The potency of RTF from the Japanese quail (Coturnix coturnix) was also determined, as its extra testicular sperm ducts are not dependent on their luminal fluids. Further, the potency of caudal epididymal fluid (CEF) from the rat and tammar was determined, as if the growth factor regulates the initial segment of the epididymis, its potency in CEF would be much less than in RTF.

The procedures for collecting RTF and CEF have been described previously (2,3). Growth factor activity was determined by measuring the uptake of tritiated thymidine by cultured BALB/c 3T3 cells (1). The procedures for collecting RTF and CEF have been described previously (2,3). Growth factor activity was determined by measuring the uptake of tritiated thymidine by cultured BALB/c 3T3 cells (1).

It is concluded that the seminiferous growth factor probably plays no role in regulating the initial segment of the mammalian epididymis.

HISTOCHEMICAL STUDY OF THE EPIDIDYMIS OF THE GUINEA-PIG (Cavia porcellus) AND THE EFFECTS OF ENZYMES IN VITRO ON THE ROULEAUX FORMATION OF EPIDIDYMAL SPERMATOZOA

S.J. Gatie, T.D. Glover* and A.W. Blackshaw

Department of Physiology and Pharmacology and *Department of Animal Sciences and Production, University of Queensland, St. Lucia, 4067.

Epididymal tissue was removed under ether anaesthesia, frozen and cut in a cryostat, but lectin-binding was examined in methanol-fixed tissue. Rouleaux formation was examined in vitro by incubating mature spermatozoa from the terminal segment of the epididymis with different hydrolytic enzymes, namely, pronase, hyaluronidase,  ś -galactosidase, sulphatase and uterine fluid. Sperm smears were stained with Giemsa or reacted with lectins.

Epithelial cells of the epididymis showed variable enzyme activity depending upon the level of the duct. In the proximal part of the middle segment, acid and alkaline phosphatase activity and lactate dehydrogenase activity were pronounced, whilst at other levels of the duct the enzymes showed only moderate activity. However, in the terminal segment, there was conspicuous lactate dehydrogenase activity. Sucinate dehydrogenase gave only a weak reaction at all levels of the epididymis. Lipids were prominent in the upper part of the middle segment (Oil Red O and Sudan Black B) which confirms the finding of Laurant (3). Fluorescein-labelled concanavalin A (Con A) and wheat germ agglutinin (WGA) bound strongly to the acrosomes of the spermatozoa, but significant binding of WGA only, was to be found on the epithelium of the proximal part of the middle segment.

After only 15 min of incubation, pronase caused loss of the acrosome in most spermatozoa and thus resulted in almost total separation of previously stacked spermatozoa. This effect was presumably due to the digestion of acrosomal protein. By contrast, hyaluronidase and ś -galactosidase caused only a 40% separation of the spermatozoa, even after 2 hr of incubation. Sulphatase and uterine fluid, partially affected rouleaux formation by separating about 90% of the spermatozoa after 2 hr of incubation.

These results suggest that glycoproteins, and perhaps sulphated glycoproteins, play a role in the stacking of epididymal spermatozoa in the guinea-pig epididymis. These glycoproteins appear to be secreted mainly from the proximal middle segment of the epididymis.


IN VITRO FERTILIZING CAPACITY OF EPIDIDYMAL SPERM FROM SULPHAZPYRIDINE-TREATED HAMSTERS

C.L. Shatin, N.T. Hong Kong

Our data showed that sulphapyridine (SP) caused an impairment of fertility in male hamsters despite no changes in testicular functions. The aim of the present study was to investigate whether its anti-fertility effect was manifested at the epididymal level in a reduced fertilizing capacity of sperm when tested in vitro with homologous zona intact hamster eggs. Adult male hamsters were given 400 mg/kg/day SP in corn oil or the vehicle (control) by gavage. Fertility was tested at 8 wk after treatment by mating with two females and determining the litter size at Day 14 of pregnancy. At the 10th wk, in the same group of hamsters, spermatozoa were expressed from excised epididymides and capacitated for 75 min in a modified Tyrode's solution (TALP) (1). Insemination was performed using 1-2 x 10^6 motile sperm/ml in 200 μl fertilization medium (2). Hamster eggs were obtained by superovulating mature female hamsters with 25 IU PMSG followed by 25 IU hCG 58 h later. Egg-cumulus complexes were recovered from the oviduct 12-14 h post-hCG and placed with the spermatozoa. Fertilization was indicated by the pronuclei formation.

Results showed that after 8 wk of SP treatment 9 out of 11 males failed to impregnate their female partners despite normal testis weight and intratesticular sperm count (Table 1). However, the apparent sterility was not associated with a reduced in vitro fertilization rate determined at 10 wk after treatment, which was comparable to that of the control. Repeat of this study using the parent drug of SP - sulphasalazine, gave similar results. In conclusion, either SP affects the fertilization capacity of hamster sperm at a site distal to the epididymis or its effect can be easily negated during the preparation of epididymal spermatozoa for in vitro fertilization.

Table 1: Effect of sulphapyridine on (a) the fertility of hamsters and (b) the in vitro fertilising capacity of epididymal sperm

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SP-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) after 8 wk treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Litter size (no. of foetuses)</td>
<td>12.2 ± 0.8</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>Total no. of foetuses</td>
<td>245/245 (96.0%)</td>
<td>26/27 (95.7%)</td>
</tr>
<tr>
<td>(b) after 10 wk treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of ova examined</td>
<td>454</td>
<td>472</td>
</tr>
<tr>
<td>No. of ova fertilised</td>
<td>333</td>
<td>328</td>
</tr>
<tr>
<td>% of fertilisation</td>
<td>72.9 ± 4.0</td>
<td>57.8 ± 4.5</td>
</tr>
<tr>
<td>Paired testis wt. (gm)</td>
<td>4.22 ± 0.10</td>
<td>4.13 ± 0.14</td>
</tr>
<tr>
<td>Intratesticular sperm count (10^6)</td>
<td>274 ± 7</td>
<td>236 ± 11</td>
</tr>
<tr>
<td>No. of animals</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

Where applicable, data represent mean ± SEM. Statistical analyses were performed using Mann-Whitney test of Student's t-test by comparing against the control group. *P<0.001.

NUCLEAR CHROMATIN UNCONDENSATION; A TEST FOR SPERM IMMATURITY, IS IT OF CLINICAL VALUE?

De Yi Liu, Yvonne Du Plessis, H.W. Gordon Baker

University of Melbourne Department of Obstetrics & Gynaecology & Reproductive Biology Unit The Royal Women's Hospital Melbourne

During epididymal transit sperm complete maturation by the formation of disulphide bonds in the chromatin which result in an increase in the physical stability of the nuclei. The proportion of ejaculated human spermatozoa which undergo decondensation of nuclei when exposed to detergents (eg. sodium dodecyl sulphate - SDS), or with or without reducing agents (eg. dithiothreitol - DTT), correlates with the number of immature sperm(1). Thus determination of nuclear chromatin decondensation (NCD) in vitro could provide a useful clinical test of sperm maturity. To investigate this, the present study was carried out on 74 consecutive IVF treatments. Sperm in semen and insemination medium after selection by swim-up, were washed with 0.9% NaCl (10min) and then exposed to 1% SDS+EDTA DTT. Sperm in semen were also washed with 0.3M EDTA to remove zinc. After 30min (DTT+SOS) or 60min (SOS alone) incubation at room temperature the reaction was stopped by addition of 2.5% glutaraldehyde and the percentage of sperm (N=200) with swollen heads was determined under a phase contrast microscope. Sperm concentration (C), vitality, motility, motility index, normal morphology (NM) in semen and C in insemination medium were also measured for each patient.

PERCENTAGE OF SPERM WITH NCD WITH LOW (<SOS, n = 20) AND NORMAL ( >SOS, n = 54) FERTILIZATION RATE IN VITRO (MEAN±SEM)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Sperm Count (×10⁹/ml)</th>
<th>Motility (0-4)</th>
<th>Fructose Citrate (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunflower seed meal</td>
<td>1.7± 2.9 ± 3.6± 71.8 ± 6.7± 6.6±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotton seed meal</td>
<td>0.4± 0.4± 0.1± 3.2± 1.3± 1.5±</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It is clear that feeding cotton seed at this level had no substantial effects on sperm production or semen quality and the fructose and citric acid concentrations in the seminal plasma were not decreased. Since these compounds are produced by the seminal vesicles and are androgen dependent, testosterone production was presumably not impaired by the cotton seed meal.

There is evidence that ruminants can detoxify gossypol, putatively by binding to protein in the rumen (2) and this may account for the lack of an effect of the cotton seed meal. We conclude that CSM is safe to use as a protein supplement for rams.

EFFECT OF ETHANOL ON MOTILITY CHARACTERISTICS AND METABOLISM OF RAM SPERM

R. Vishwanath, M.A. Swan & I.G. White

Departments of Veterinary Physiology, and Histology and Embryology, University of Sydney, N.S.W. 2006

The fertilizing ability of mouse sperm in vitro is impaired by ethanol, putatively by inhibiting capacitation (1). Ethanol could act by directly inhibiting motility or indirectly by inhibiting metabolism. This study assesses the effect of ethanol on the motility characteristics of partially demembranated ram sperm and on the metabolism of the intact sperm.

Ram sperm were partially demembranated by extracting with 0.1% Triton X-100, and then reactivated with 1mM ATP(2). In the presence of 4% and 2% ethanol the mean ± S.E. % reactivation of demembranated sperm by 1mM ATP was decreased from 77.9 ± 1.2 (control) to 56.8 ± 1.2 and 55.4 ± 4.0 respectively (n=5); 1% and 0.5% had no effect on reactivation (76.4 ± 4.4 and 77.6 ± 4.4 respectively). Cinemographic analysis of demembranated sperm revealed no change in the mean amplitude of the wave motion at the neck and midpiece junctions in the presence of 1% ethanol. There was, however, a significant increase in the beat frequency (Table 1).

Table 1: Effect of ethanol on the motility characteristics (Mean ± S.E., n=5) of partially demembranated sperm.

<table>
<thead>
<tr>
<th>Ethanol</th>
<th>Region</th>
<th>Mean Amplitude (nm)</th>
<th>Beat Frequency (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Neck</td>
<td>6.7 ± 0.7</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Midpiece</td>
<td>9.8 ± 1.2</td>
<td>6.2 ± 0.8</td>
</tr>
<tr>
<td>1%</td>
<td>Neck</td>
<td>7.2 ± 0.6</td>
<td>13.2 ± 0.7*</td>
</tr>
<tr>
<td></td>
<td>Midpiece</td>
<td>12.2 ± 1.1</td>
<td>13.2 ± 0.5*</td>
</tr>
</tbody>
</table>

* significantly increased (P < 0.01).

This was confirmed by stroboscopic measurements of beat frequency of the sperm models. However, while ethanol (1%) enhanced the beat frequency (11.6 Hz ± 0.2) compared to the control (9.7 Hz ± 0.1), methanol (1%) decreased it (7.7 Hz ± 0.2). N-Bulanol and isopropanol completely inhibited reactivation of the sperm models by 1mM ATP.

The motility of intact sperm diluted in Ringer phosphate with 0.1% glucose was unchanged after incubating with 0.01%, 0.1% or 1% ethanol for 4 hrs. The oxygen uptake of intact sperm (μl per 10^8 cells) incubated with 1% ethanol for 60 min (15.8 ± 0.8) was not significantly different from the control (15.2 ± 2.6). We conclude that the surprising increase in the beat frequency is due to a fairly specific effect of ethanol on the motility apparatus of ram sperm rather than on their oxidative metabolism.


A COMPARATIVE STUDY OF AUTOMATED (CELLSOFT) AND MANUAL SEMEN ANALYSIS

M. Ismail M. Tambi, Elizabeth J. Howard, Wayne F. Thompson, David N. de Kretser & R.W. Gordon Baker

Reproductive Medicine Clinic, Medical Research Centre, Prince Henry's Hospital, Department of Anatomy, Monash University and University of Melbourne Department of Obstetrics & Gynaecology, Royal Women's Hospital, Melbourne, Vic., 3000.

Automation of semen analysis should remove subjectivity and improve precision. To validate the CellSoft method, reproducibility has been assessed and results have been compared with those for standard manual methods (WHO Lab. Manual 1980) on the same samples from normal and infertile men. The same video tapes of 12 samples were reanalysed four times by CellSoft using the 20 frame set up parameters as suggested by the manufacturers or a 10 frame modification to reduce analysis time. The errors of measurement expressed as pooled coefficients of variation (CV) were between 3 and 9% for sperm concentration (C), percentage motility (M), velocity (V), linearity (L) and amplitude of lateral head displacement (LHA) except that LHA was 13% with the 10 frame modification. Correlation coefficients (r) were 0.94 for C and M and between 0.84 and 0.87 for V, L and LHA. Duplicate analyses of the same 10 semen samples by CellSoft gave CV for log C 5%, M 17%, V 5.7%, L 1.2% and LHA 7.6% (r=0.58 - 0.78). Duplicate manual counts with the Makler chamber used for CellSoft analysis gave a 4.5% CV for C (r=0.98) and duplicate visual assessments of M from the video screen gave a CV of 14% (r=0.86). Comparison of results of samples analysed by CellSoft and the standard manual methods (n >90) gave CV 18% for log C (r=0.93) and 52% for M (r=0.62). Comparing Makler chamber and video screen visual counts on the same samples with Cellsoft gave CV 6.4% for log C (r=0.97) and 28% for M (r=0.85). CellSoft results on two or three different semen samples from the same six normal donors gave pooled CV's - C 31%, M 18%, V 17%, L 5.7% and LHA 23%.

In conclusion, the CellSoft method provides extra information of characteristics of sperm motility (V, L, LHA) not obtainable by manual methods with reasonable precision (≤3%). Precision for C and M is less but probably adequate in the clinical setting in view of the large day to day variation in semen analysis results in normal men.
before the control group (Table 1). Animals placed in
Soc. separated into three groups: 16L:8D, decreasing
Biol. Repflod., ± and blood samples were obtained every 20 days. Testes
results are presented as a percentage of the maximum TV shown by
animals were kept in an outdoor cage in natural
typical TV although maximum levels in artificial
peaked (5 pg/ml) but
weight increase
± rose in all groups
10 93 ± 7*
117 pg/ml*), rose to a
the 16L:
207pg/ml; p<0.01), declined in early
increased
5
photoperiod showed the
result characteristic poliocephalus 
&", as
± slower but
poliocephalus 8L:16D on day 172) and 8L:16D. Testis dimensions,
10* ± 5 93 ± 3
114 pg/ml) but
weights of decreasing photoperiod appeared necessary to produce typical
reproductive changes.

Table 1. Percentage of maximal TV, ± s.d., n = 3 unless specified
(n). Statistics: ANOVA vs. Control *p<0.05, **p<0.01; vs. 16L:8D
#p<0.05, ##p<0.01.

<table>
<thead>
<tr>
<th>DAY</th>
<th>CONTROL 16L:8D</th>
<th>DECREASING 8L:16D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>49 ± 6</td>
<td>57 ± 11 (9)</td>
</tr>
<tr>
<td>100</td>
<td>48 ± 10</td>
<td>66 ± 7 (9)**</td>
</tr>
<tr>
<td>138</td>
<td>57 ± 7</td>
<td>71 ± 3*</td>
</tr>
<tr>
<td>122</td>
<td>70 ± 10</td>
<td>93 ± 7*</td>
</tr>
<tr>
<td>239</td>
<td>96 ± 5</td>
<td>93 ± 3</td>
</tr>
<tr>
<td>300</td>
<td>67 ± 5</td>
<td>81 ± 6*</td>
</tr>
</tbody>
</table>

All three artificial photoperiod groups resulted in maximal TV
occurring before the control group (Table 1). Animals placed in
either short or decreasing photoperiod showed a peak in TV in either
December or early January whereas TV in controls peaked in March (day
239). By day 300 (mid-May) TV in the controls had decreased whereas
TV in the 8L:16D groups had begun to rise after a period of
regression. TV increased more slowly but was maintained for a longer
period in the 16L:8D group compared to the other regimens. Only the
animals in decreasing photoperiod showed the characteristic increase in
body weight around the time of maximal TV which was evident in the
control animals. Plasma testosterone concentration rose in all groups
concomitant with increasing TV although maximum levels in artificial
photoperiod groups were lower than those seen in the controls.

In this species, although prolonged long photoperiod results in
increased testis growth, presumably due to photorefractoriness, a
period of decreasing photoperiod appears necessary to produce typical
reproductive changes.


P. poliocephalus is a short-day breeder; a previous investigation
(1) showed that seasonal-like reproductive changes were induced in
males placed in long photoperiod followed by decreasing photoperiod.
The present study uses several photoperiod regimens in order to
determine the nature of the response to photoperiod.

Three control animals were kept in an outdoor cage in natural
photoperiod groups were lower than those seen in the controls.

To further elucidate patterns of hormone secretion in
breeding/early pregnant females we followed peripheral hormone levels
in 16 captive breeding females. Since androgens can be useful markers
of ovarian function (2), we measured androstenedione as well as E and
P. Blood was collected monthly from December-March, weekly in the
breeding season and early pregnancy: significant increases
which occur later in pregnancy probably reflect placental synthesis
(1). In Pteropus spp. preimplantation development of the endometrium
is restricted to that part close to the corpus luteum (CL). Thus
pregnancy may be established with minimal ovarian secretion of E and
P, embryo implantation requiring high levels only locally and
peripheral values remaining unchanged.

The most consistent pattern was seen with androstenedione (A).
Levels remained unchanged until March (693 ± 117 pg/ml*), rose to a
peak in late March (1,545 ± 207 pg/ml p<0.01), declined in early
April (696 ± 137 pg/ml) and rose to 3,677 ± 416 pg/ml (p<0.001) in
late April. Levels of E generally remained low (~5 pg/ml) but
several animals showed very transient peaks (>10 pg/ml) in April. The
March peak in A may mark the development of large antral follicles,
which occurs at this time (1), with the subsequent decline and
transient E spikes indicating the selection and brief existence of
single preovulatory follicles. The later rise in A may mark the
development of a functional CL.

Peripheral levels of P varied widely between individuals, and
showed no consistent pattern within individuals or in relation to the
March peak in A. Statistically, however, they increased significantly
through March and early April, to decline and rise again in late
April. The source and biological significance of this progesterone
remain an enigma, as increased levels occur well before any appearance
of preovulatory follicles/CL, or progestational development of the
endometrium.

1078-1083.

*Results are means ± SEMs.
COUNTER-CURRENTexchange, whereby small quantities of ovarian hormones may pass from the ovarian vein to the ovarian artery and travel via a common vessel appears to enclose the ovarian artery for about two-thirds of its length. This arrangement appears to provide more than sufficient area for counter-current exchange, whereby small quantities of ovarian hormones may pass from the ovarian vein to the ovarian artery and travel, via the anastomosis with the uterine artery, to the cranial tip of the uterine horn ipsilateral to the single corpus luteum.

Five animals were euthanased and their reproductive tracts fixed in situ in Boulin's fluid. Serial sections showed that in all cases, the entire arterial coil is surrounded by a large venous sinus, which derives largely from the ovarian drainage. Cranial to the coil, this sinus anastomoses with a major venous sinus from the uterus and the common vessel appears to enclose the ovarian artery for about two-thirds of its length.

The ovarian arteries branch from the aorta immediately below the renal arteries and usually from a common stem. Just cranial to the ovary each artery forms a tight coil. Arterioles run from the coil to supply the ovary. The ovarian artery then runs caudally along the posterio-lateral side of the uterine horn to anastomose with the uterine artery and is the main blood supply to the cranial end of the uterine horn.

Nine non-pregnant and 2 pregnant P. australis, 2 post-partum P. poliocephalus and 1 juvenile P. alecto were used to elucidate mechanisms which give rise to the local endometrial reaction. Eight animals were euthanased and the descending aorta and vena cava cannulated and flushed with isotonic saline warmed to 37° C. Orange microfil and white latex were perfused into the vena cava and aorta respectively. Each preparation was refrigerated overnight to set. The reproductive tract was then dissected out, fixed in Boulin's fluid, dehydrated in ethanol and cleared in benzyl benzoate, to visualise the anatomy of the blood supply.

The ovarian arteries branch from the aorta immediately below the renal arteries and usually from a common stem. Just cranial to the ovary each artery forms a tight coil. Arterioles run from the coil to supply the ovary. The ovarian artery then runs caudally along the posterio-lateral side of the uterine horn to anastomose with the uterine artery and is the main blood supply to the cranial end of the uterine horn.

Five animals were euthanased and their reproductive tracts fixed in situ in Boulin's fluid. Serial sections showed that in all cases, the entire arterial coil is surrounded by a large venous sinus, which derives largely from the ovarian drainage. Cranial to the coil, this sinus anastomoses with a major venous sinus from the uterus and the common vessel appears to enclose the ovarian artery for about two-thirds of its length.

This arrangement appears to provide more than sufficient area for counter-current exchange, whereby small quantities of ovarian hormones may pass from the ovarian vein to the ovarian artery and travel, via the anastomosis with the uterine artery, to the cranial tip of the uterine horn ipsilateral to the single corpus luteum.

In many species of bats, females exhibit a permanent reproductive asymmetry in that only one ovary functions and pregnancy always involves the one uterine horn. Pteropus spp. are monovulatory but both ovaries function, and ovulation is thought to alternate from side to side with successive seasons. However, a preferential development successive from the uterine horn to anastomose with the largely anastomosis with the uterine artery, to the cranial tip the ovarian drainage. Cranial to the coil, this occurs in situ.

The ovarian arteries branch from the aorta immediately below the renal arteries and usually from a common stem. Just cranial to the ovary each artery forms a tight coil. Arterioles run from the coil to supply the ovary. The ovarian artery then runs caudally along the posterio-lateral side of the uterine horn to anastomose with the uterine artery and is the main blood supply to the cranial end of the uterine horn.

Nine non-pregnant and 2 pregnant P. australis, 2 post-partum P. poliocephalus and 1 juvenile P. alecto were used to elucidate mechanisms which give rise to the local endometrial reaction. Eight animals were euthanased and the descending aorta and vena cava cannulated and flushed with isotonic saline warmed to 37° C. Orange microfil and white latex were perfused into the vena cava and aorta respectively. Each preparation was refrigerated overnight to set. The reproductive tract was then dissected out, fixed in Boulin's fluid, dehydrated in ethanol and cleared in benzyl benzoate, to visualise the anatomy of the blood supply.

The ovarian arteries branch from the aorta immediately below the renal arteries and usually from a common stem. Just cranial to the ovary each artery forms a tight coil. Arterioles run from the coil to supply the ovary. The ovarian artery then runs caudally along the posterio-lateral side of the uterine horn to anastomose with the uterine artery and is the main blood supply to the cranial end of the uterine horn.

Nine non-pregnant and 2 pregnant P. australis, 2 post-partum P. poliocephalus and 1 juvenile P. alecto were used to elucidate mechanisms which give rise to the local endometrial reaction. Eight animals were euthanased and the descending aorta and vena cava cannulated and flushed with isotonic saline warmed to 37° C. Orange microfil and white latex were perfused into the vena cava and aorta respectively. Each preparation was refrigerated overnight to set. The reproductive tract was then dissected out, fixed in Boulin's fluid, dehydrated in ethanol and cleared in benzyl benzoate, to visualise the anatomy of the blood supply.

The ovarian arteries branch from the aorta immediately below the renal arteries and usually from a common stem. Just cranial to the ovary each artery forms a tight coil. Arterioles run from the coil to supply the ovary. The ovarian artery then runs caudally along the posterio-lateral side of the uterine horn to anastomose with the uterine artery and is the main blood supply to the cranial end of the uterine horn.
SEXUAL DIFFERENTIATION OF THE TAMMAR WALLABY ON THE DAY OF BIRTH.

W.S. G. Shaw, H.B. Renfree and R.V. Short.
Department of Anatomy & Physiology, Monash University, Melbourne, 3168.

In mammals the formation of a testis is controlled by a gene or genes on the Y chromosome, and an ovary develops in its absence. All subsequent sexual differentiation is thought to be controlled by gonadal hormones. However, preliminary observations in the tammar show that scrotal anlagen are already present on the day of birth in males, before there is any obvious gonadal differentiation. To confirm and extend these observations, pouch young were killed within 24 h of birth by decapitation and tissues were taken for karyotyping. The pouch young were fixed in neutral buffered formalin, embedded in wax and serial transverse sections cut at 5 μm. The cross-sectional areas of gonads, and Mullerian and Wolffian ducts (including lumina) were measured on every tenth section using an A.S.M. digitizing tablet (Leitz) and the volumes of these structures were calculated using Simpson’s formula (± s.e., Table 1).

Table 1. Sexual differentiation of newborn tammar wallabies

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karyotype</td>
<td>14,XX</td>
<td>14,XY</td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Gonad volume (mm³)</td>
<td>0.068 ±0.014</td>
<td>0.071 ±0.021</td>
<td>P=0.1, NS</td>
</tr>
<tr>
<td>Mullerian duct volume (mm³)</td>
<td>0.0012±0.0005</td>
<td>0.0012±0.0003</td>
<td>P=0.1, NS</td>
</tr>
<tr>
<td>Wolffian duct volume (mm³)</td>
<td>0.008±0.0020</td>
<td>0.010±0.0036</td>
<td>P&gt;0.1, NS</td>
</tr>
<tr>
<td>Scrotal anlagen</td>
<td>always absent</td>
<td>always present</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Mammary gland anlagen</td>
<td>always present</td>
<td>always absent</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

No histological differences were noted between male and female gonads on the day of birth. As the first sign of gonadal sexual differentiation in eutherian mammals is the relative increase in the size of the testis (3) the similarity in the volumes of the male and female gonads in this study showed that they were undifferentiated on the day of birth. The lack of any sex difference in the volume of the Mullerian and Wolffian ducts is a good indication of a lack of gonadal hormone action. The appearance of the scrotal anlagen only in the male and the mammary gland anlagen only in the female is therefore unlikely to be mediated by gonadal hormones and may represent an extra-gonadal effect of the sex chromosomes.

We conclude from this data that some aspects of somatic sexual dimorphism precede gonadal sexual differentiation in the tammar wallaby.


SURREOVULATI0N OF A MONOVULAR MARSUPIAL THE BRUSH-TAILED POSSUM

J.C. Rodger & K.E. Mate

Dept. of Biological Sciences, University of Newcastle, NSW, 2308.

There have been several attempts to induce ovulation or superovulation of marsupials using exogenous gonadotrophins but neither a reproducible method nor fertility has resulted (1,2). The present work is a fresh attempt to establish such techniques for studies of development and practical artificial breeding.

We report here our initial experience using pregnant mare serum gonadotrophin (PMSG: Folligon, Intervet; s.c.) and synthetic gonadotrophin releasing hormone (GnRH: Fertagyl, Intervet; i.m.) to induce ovulation in the possum Trichosurus vulpecula. Current results suggest three factors are important: 1) reproductive state of the female; 2) dose of follicle stimulating gonadotrophin; and 3) a specific ovulatory stimulus is required.

Immature (n=7); ancestral (n=3), pregnant (n=7) and lactating (n=7) female possums all responded to PMSG and up to 10-20 medium (2.5-3.0mm diam.) to large (3.0-4.0mm diam.) Graafian follicles developed in each ovary within 3-5 days. In contrast the ovaries of preovulatory cycling females (n=7) were refractory to this treatment. A range of single doses (1-1,000U) and regimens of multiple moderate doses (50 or 100U) of PMSG were tested. Only the lower single doses produced ovarian responses and reproductive tract development of normal oestrous character. Current practice has settled on a single dose of 100U which stimulated a moderate number of follicles to grow to the preovulatory state (5-10/ovary) and led to very little premature luteinization or degenerative changes in the oocytes.

Although PMSG stimulated a crop of follicles to grow to preovulatory size the oocytes showed only early signs of nuclear maturation and ovulation never occurred implying that a luteinizing hormone (LH) surge did not result from the treatment. Attempts were made to induce an endogenous surge by administering GnRH. Initially single doses of GnRH were tested but this produced no greater ovarian response than PMSG alone (n=4). In the monovular tammar wallaby, the only marsupial for which there are data, one such GnRH treatment induces a LH surge of 1-2h duration (3), whereas the endogenous LH peak lasts 8-12h (4). Therefore, multiple doses of GnRH (3 doses of 50μg, 90min apart) were tested in an attempt to produce a LH surge of several hours duration. This treatment regimen after a single 100U PMSG injection 3 days earlier (n=8) yielded normal mature preovulatory or ovulated oocytes (5-10/ovary) 1-2 days after GnRH treatment. Work is continuing to refine these techniques and to apply them to other monovular and polyovular species.

HAEMOPOIETIC DEVELOPMENT IN THE FETAL LAMB FROM 85 TO 135 DAYS

T.C. Smeaton

Department of Immunology, John Curtin School of Medical Research, A.N.U., Canberra A.C.T.

Haemopoiesis first appears in the embryonic yolk sac, then progressively in the liver and bone marrow (BM) of the developing foetus (1). In the fetal lamb, we have studied haemopoietic activity in the BM compartment by assessing mitotic activity of the BM of the long bones of the limbs by incorporation of (methyl-H) thymidine (TDR). Cell numbers in the BM population and the distribution of cell types have also been measured. Fourteen fetal lambs of known gestation were taken from ewes killed by intravenous injection of pentobarbitone sodium. Six bones from each foetus (humeri, tibiae and femora) were split open and the BM removed with a stream of Eagle's minimum essential medium with Earle's salts (PIGS Gibco, N.Y.). Four replicate samples of each cell suspension (5x10^6 viable cells) were incubated at 37°C with 1 μC TDR for two hours and incorporation into DNA measured after harvest. A differential count of BM smears was done for two boxes of each foetus. Soft tissues, the thymus, spleen and liver, were also tested for TDR incorporation in a similar manner. The number of BM cells obtained from the bones from 85 to 135 days is shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>No. bone pairs</th>
<th>No. BM cells x 10^6</th>
<th>Left/Right *</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>85-90</td>
<td>12</td>
<td>8.8</td>
<td>99.4 ± 7.9</td>
<td></td>
</tr>
<tr>
<td>100-105</td>
<td>9</td>
<td>103.0</td>
<td>95.6 ± 12.1</td>
<td></td>
</tr>
<tr>
<td>115-120</td>
<td>12</td>
<td>189.0</td>
<td>104.3 ± 8.6</td>
<td></td>
</tr>
<tr>
<td>130-135</td>
<td>9</td>
<td>469.0</td>
<td>93.6 ± 10.3</td>
<td></td>
</tr>
</tbody>
</table>

* The number of cells obtained from the left bone is expressed as a percentage of the right to estimate variation in extraction of BM cells.

The rate of incorporation of TDR into the DNA of the BM cells was consistent throughout the period studied (range 0.0414-0.4860 cpm/μl cells) with no clear trend related to foetal age. Thus, with the expansion in the total cell number, the mitotic capacity of the BM compartment increased tenfold. At the same time there was a decline in the capacity of the liver, reflecting a shift in haemopoiesis from the liver to the BM. Smears from BM cells contained a diverse population of cells, representing most of the cell types found in the adult. With increasing gestational age the percentage of myeloblasts decreased while mature polymorphonuclear neutrophils and band cells increased.

Details of this study have been published elsewhere (2).

The rate of ovarian blood flow required by the corpus luteum to sustain normal progesterone secretion is controversial (1,2). Most studies have related blood flow and secretion rates in CL at different functional states or experimental conditions which could affect CL function indirectly.

Here, we modified a venous outflow technique (3) to measure blood flow and progesterone secretion rates in 16 Day pregnant rat with an intact arterial supply. The arterial blood was then directed through a peristaltic pump so flow rates could be varied from about 0.5X to 2X the intact flow rate. Eight rats were anesthetized with pentobarbitone sodium and after surgery and positioning of cannulae, were placed in a saline bath (37°C) which promotes physiological stability of the preparation. Arterial and ovarian venous blood samples were taken at 5 min intervals to determine total progesterone secretion (progesterone and 20 alpha dihydroprogesterone as measured by RIA) and oxygen consumption (measured with a Hemoximeter. Radiometer).

Results are shown in Table 1: measurements for the intact circulation (blood flow. 0.41 ± 0.02 ml/min; oxygen consumption, 5.9 ± 1.1 ml/min/kg and progesterone secretion, 1.29 ± 0.15 nmol/min/kg) were averaged over 20 min and those for each perfusion rate for 30 min.

Table 1: Mean (±SEM) progesterone secretion (nmol/min/kg) and oxygen consumption (ml/min/kg) during mechanical perfusion (ml/min).

<table>
<thead>
<tr>
<th>Perfusion</th>
<th>Oxygen</th>
<th>Progestagens</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>3.8±0.6</td>
<td>0.39±0.05</td>
</tr>
<tr>
<td>0.72</td>
<td>4.1±0.5</td>
<td>0.47±0.10</td>
</tr>
<tr>
<td>0.4</td>
<td>2.7±0.2</td>
<td>0.29±0.03</td>
</tr>
<tr>
<td>0.24</td>
<td>3.8±1.7</td>
<td>0.18±0.03</td>
</tr>
<tr>
<td>0.4</td>
<td>2.1±0.1</td>
<td>0.22±0.05</td>
</tr>
</tbody>
</table>

Figures in parenthesis are percentage of intact values: all were significantly lower than the intact value (paired t tests).

Since the animal's own blood was used for the perfusion it was expected that flow rate would be the major variable affecting ovarian function. Progestagen secretion, however, was substantially reduced even when blood was perfused at twice the normal rate, precluding any simple relationship between blood flow and secretion. The ovary continued to consume relatively high levels of oxygen despite the decreased secretion rate. Histological examination showed no major areas of infarction. These results raise the possibility that pulsatility which was reduced with pumping. rather than flow rate, selectively influenced ovarian metabolism.


Although the association between alcohol consumption during pregnancy and an increased incidence of developmental abnormalities in the offspring has now been well established in humans as well as in many other mammalian species. the mechanisms underlying this phenomenon. expressed in its most extreme form as the Fetal Alcohol Syndrome (FAS). are still poorly understood. The results of a recent report (1) suggested that, in the QS mouse during early post-implantation pregnancy, part of the adverse action that alcohol exerts on embryonic development stems from indirect effects that involve uterine functions relating to the nurture of the conceptus via glycolysis. The objective of the present study was to examine this response to alcohol in greater detail giving emphasis to the time course of ethanol-induced alterations in glucose metabolism and effects of the drug on the activity of the tricarboxylic acid (TCA) cycle.

QS mice on day 9 of pregnancy exposed to an acute teratogenic dose of ethanol (3.5g/Kg body wt. by intraperitoneal injection) achieved a peak blood ethanol level of 3.25 ± 0.13mg/ml 15 min. after administration and cleared the ethanol 6h later. Uterine levels of ethanol followed a similar pattern to that observed in the blood. Maternal liver glycogen levels significantly decreased 1h after exposure to ethanol and further declined after 6h. In the same animals, blood glucose levels changed dramatically, with a state of hypoglycaemia being registered at 1h followed by severe hypoglycaemia at 4h and 6h. Plasma lactate levels were depressed during the entire period of exposure to ethanol. A striking feature of the response to alcohol was a transient depression in the levels of glucose and hexose phosphates in the uterus 30 min. after treatment. The rate of accumulation of several glycolytic intermediates in the uterus then increased after a further period of exposure (1-4h) and returned to control values 6h after alcohol administration. The uterine activity of the TCA cycle was also disturbed 2h after the administration of ethanol and the levels of several intermediates, including citrate, α-ketoglutarate and malate, were significantly enhanced without any accompanying alteration in redox state as evidenced by the ratio of NAD to NADH.

The results indicate that the pattern of glucose metabolism in the uterus of the mouse during post-implantation pregnancy is modified within 30 min. of an acute exposure to ethanol. It is suggested that a stress response, resulting in the mobilization of liver glycogen stores, plays an important role in the ethanol-induced alterations and may be involved in the pathogenesis of the FAS stemming, in particular, from binge drinking.

GLUCOSE METABOLISM IN RELATION TO THE EMBRYO-TOXICITY OF ALCOHOL. II. DIRECT EFFECTS ON THE CONCEPTUS.

B. Slim* and R.N. Murdoch

Department of Biological Sciences, University of Newcastle, Newnastle, N.S.W., 2308, and *Department of Clinical Chemistry, Newcastle Mater Hospital, Marathah, N.S.W., 2306.

The early post-implantation stage of pregnancy in the mouse is a time when the embryo is particularly susceptible to teratological insult (1). During this period, the conceptus is heavily reliant on glucose as its major energy source (2), and hence alterations in either the supply or utilisation of glucose are major sites open for modification by potential teratogens. The aim of the present study was to investigate the effects of ethanol, and its metabolite acetate on glucose metabolism in the isolated early somite mouse conceptus, and hence assess the possibility that this may be a mechanism contributing to the adverse developmental effects associated with the prenatal ingestion of alcohol.

Concepti were dissected from decidua eq day 9 of pregnancy and cultured in the presence of 4 uCi/mI [U-14C] glucose. Ethanol or acetate was added to the culture medium to give final concentrations of 3g/l and 2 mM, respectively. Under these conditions the early somite mouse conceptus exhibited high rates of lactate production from glucose with only minimal amounts being oxidized to CO2. Both ethanol and acetate significantly decreased lactate production from glucose. However, acetate significantly increased CO2 production, while ethanol tended to depress CO2 production, although not significantly.

To gain an indication of whether similar effects also occur in vivo, fetal membrane fluid was collected from day 14 pregnant mice 24 after an acute teratogenic dose of alcohol (3.5 g/kg) and assayed for acetate, ethanol, glucose and lactate. It was found that both ethanol and acetate appeared in the fetal membrane fluid after the maternal administration of alcohol and caused a significantly increased accumulation of glucose in the fluid. The results suggest that ethanol and acetate can modify the rate at which the developing conceptus utilizes glucose. In view of the metabolic reliance of the conceptus on glucose during the early post-implantation stage of pregnancy, these alterations may constitute important mechanisms through which alcohol causes developmental abnormalities.


THE EFFECT OF CHRONIC HYPOBARIC HYPOXIA ON THE ONTOGENY OF INDIVIDUAL AMINO-ACIDS IN PLASMA OF FETAL SHEEP


Chronic maternal hypoxaemia results in both a reduced rate and altered pattern of fetal growth [1]. To determine if this is associated with disturbed amino-acid metabolism within the gravid uterus the concentrations of 15 individual amino-acids were measured in arterial plasma in catheterised normal and chronically hypoxaemic fetal sheep from 118 to 140 days of gestation.

Catheters were implanted in the fetal and maternal carotid arteries of 14 sheep under general anaesthesia at 110 days of gestation. From 120 to 140 days 7 sheep were subjected to simulated high altitude in a hypobaric chamber which reduced (p < 0.05) fetal arterial P O2 (mean ± sem) from 22.1 ±1.2 to 12.9 ±2.0 mm Hg within 15 min.

After 21 days of hypobaric hypoxaemia, fetal weight was reduced (p < 0.05) to 84% of controls (4.15 ± 0.51 vs 3.46 ± 0.72 kg). Fetal arterial plasma concentrations of glycine, tyrosine, phenylalanine and ornithine increased with gestational age in the control fetuses and in a lesser extent or not at all in hypoxaemic fetuses. Plasma levels of the branched chain amino-acids valine, leucine and isoleucine did not show developmental changes in the control fetuses and decreased over this period in hypoxaemic fetuses. Fetal plasma concentrations of serine declined with increasing age, and fell more rapidly during hypoxaemia. Levels of asparagine, glutamine, methionine, glutamate, lysine and histidine in fetal plasma did not change significantly with gestational age in either group, but tended to be lower during chronic fetal hypoxaemia. In contrast with all other amino-acids studied, alanine concentrations in fetal plasma were higher and increased more rapidly with gestational age in chronic fetal hypoxaemia.

These changes suggest that except for alanine chronic hypoxaemia reduces placental transfer of amino-acids to the fetus to a greater extent than their utilization for growth and oxidative metabolism.

FETAL AND PLACENTAL GROWTH IN RATS EXPOSED TO CARBON MONOXIDE GAS.

A.M. Lynch and N.W. Bruce.

Department of Anatomy and Human Biology, University of Western Australia, Nedlands, W.A. 6009.

Maternal hypoxaemia such as that associated with anaemia and cigarette smoking retards fetal growth and can lead to placental hypertrophy (1). However, it is not known whether placental hypertrophy protects the fetus or when during pregnancy it can be induced.

In the present work, rats were exposed to carbon monoxide (CO) either throughout or at selected stages in pregnancy. These stages included early embryonic development and placentation, and late pregnancy when fetal growth is rapid and a decline in placental functional capacity is suspected (2).

Hypoxaemia was induced by exposing pregnant rats (Day 1 = mating) to CO (100ppm) in air. This raised maternal carboxyhaemoglobin saturations to 14% as calculated from blood samples and half-life estimations obtained from conscious, chronically catheterized rats. These levels are equivalent to the maximum levels reported in women who smoke (3). The six groups of pregnant rats (Table 1) were kept in identical chambers, in either air or air containing CO, and were examined on Day 22.

Table 1. Fetal and placental weights (mg) at Day 22 (group means ± SEM).

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treated Days</th>
<th>No. in group</th>
<th>Fetal wt.</th>
<th>Placental wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>15</td>
<td>4765 ± 46</td>
<td>429 ± 8</td>
</tr>
<tr>
<td>2</td>
<td>1-22</td>
<td>12</td>
<td>4401 ± 57**</td>
<td>483 ± 12**</td>
</tr>
<tr>
<td>3</td>
<td>1-16</td>
<td>12</td>
<td>4707 ± 95</td>
<td>449 ± 13</td>
</tr>
<tr>
<td>4</td>
<td>4-12</td>
<td>12</td>
<td>4751 ± 61</td>
<td>447 ± 8</td>
</tr>
<tr>
<td>5</td>
<td>10-22</td>
<td>14</td>
<td>4514 ± 68**</td>
<td>486 ± 16**</td>
</tr>
<tr>
<td>6</td>
<td>18-22</td>
<td>9</td>
<td>4620 ± 86</td>
<td>498 ± 11**</td>
</tr>
</tbody>
</table>

Treated group different to control value; **p <0.01 (L.S.D statistic).

The treatment regime had no obvious effects upon maternal well-being, or fetal survival and gross development. Placental hypertrophy was seen in treated Groups 2, 5 and 6. Fetal weight was significantly reduced by approximately 10% in Groups 2 and 5 but not in Group 6.

These findings show that placental growth adapts to CO exposure and that this response appears limited to the second half of pregnancy. Placental hypertrophy demonstrated in Group 6 in association with the relative maintenance of fetal weight suggests that the growth response does benefit the fetus, presumably by improving oxygen transport.


THE EFFECT OF HYPERTHERMIA ON FETAL SHEEP THYROID STRUCTURE

Peter Andrianakis, R.G.A. Stephenson, D.W. Walker and G.D. Thorburn

Department of Physiology, Monash University, Clayton, Vic. 3168, Australia

The exposure of pregnant sheep to high ambient temperatures results in fetal growth retardation and a high incidence of neonatal mortality (1). Previous work in adult sheep (2) has shown thyroid secretion rate to decrease with increased ambient temperatures.

This study was to examine the response of the fetal sheep thyroid gland to an increase in ambient temperatures. Twenty pregnant merino sheep at Julia Creek, Queensland were used. At 133 days gestation 10 sheep (Group 1) were exposed to high ambient temperatures in a climate chamber cycling at 44 ± 1°C for 10 hours and 24 ± 2°C for 14 hours. Another 10 sheep (Group 2) were kept in a sheltered shed exposed to ambient daily temperatures between 24 to 36°C.

At 133 days gestation fetal thyroid glands were removed and fixed in 10% formal saline for histology. Blgoid was also collected by cardiac puncture and plasma frozen at -20°C for thyroid hormone analysis by radioimmunoassay.

Results show no significant difference between fetal thyroid weight (gm) as a fraction of fetal body weight (kg). However, histology showed a significant increase in mean size of follicles and colloid content of Group 1 (2532 ± 181) not seen in Group 2 (1650 ± 186, p<0.01). The epithelial cells of the follicles were flattened in the thyroid glands of Group 1 fetuses whereas they occur columnar in Group 2 fetuses.

Plasma free thyroxine concentrations were not significantly different between the two Groups, (Gp1; 39.86 ± 3.69 pg/ml and Gp2; 41.57 ± 3.97 pg/ml). Food intake of both groups was monitored during the study assuring the same level of nutrition in both groups.

The difference seen in the fetal thyroids of Group 1 appear to be related to temperature rather than nutrition. This effect may be mediated via the hypothalamic stimulation of thyrotropin (TSH). Other data shows that fetal prolactin secretion is increased during hyperthermia (3). Hyperthermia may also cause changes in deiodinase activity and peripheral uptake of thyroxine.

COMPARISON OF PROTEIN SECRETION PATTERNS BY OVINE ENDOMETRIAL EPITHELIAL CELLS GROWN ON MICROCARRIERS AND ON PLASTIC DISHES

R.A. Cherry and J.K. Findlay
Medical Research Centre, Prince Henry's Hospital, Melbourne, 3004, Vic.

The substrates upon which cells are cultured in vitro have differing effects on cell proliferation, growth and differentiation and the cellular responses to hormonal stimuli (1). Specifically, collagen, the major constituent of extra cellular matrix, influences a variety of parameters when used as a substrate. In this study, the effects of steroid hormones on the pattern of newly synthesised protein secreted by ovine endometrial epithelial cells were examined in culture upon 2 different substrates. Ovariectomised Corriedale ewes (N=5) were treated with implants of oestradiol 17β (E2) 10 days prior to hysterectomy. Endometrial epithelial cells were isolated by enzyme dissociation and density gradient centrifugation on Ficoll 400 (Pharmacia) and inoculated onto either plastic tissue culture dishes (2) or 15 mg of Cytodex 3 collagen-coated dextran microcarrier beads (Pharmacia) at densities of 1 x 10⁶ and 4 x 10⁶ cells per dish, respectively. The initial culture medium, M199 + 5% charcoal- and heat-treated FCS, was replaced 2 or 6 days after attachment with methionine-free MEM. To this was added 100 uCi of (³⁵) methionine and either E₂ (10⁻⁸ M final concentration), progesterone, (P, 10⁻¹⁰ M), E₂ + P (each 10⁻⁸ M) or medium. After 24 h (ie, d3 or d7), the medium from each of the dishes was separately spun and frozen. Protein in the media was precipitated by acetone and subjected to 2-dimensional polyacrylamide gel electrophoretic analysis and autoradiography.

A previously described (2) 46 K glycoprotein while prominent in gels from cells cultured on plastic, was almost absent in preparations derived from cells grown on microcarriers.

There were no significant differences between the patterns produced or the amount of label incorporated into secreted protein by the cells under different hormonal conditions in either culture system. Accordingly, pooled means from the various hormonal treatment groups (cpm x 10⁶ per 1 x 10⁶ cells) were used to show that incorporated label from d7 culture on beads was significantly lower (P < 0.01) than either d3 and d7 cultures on plastic or d3 on beads: d3 plastic 2.02 ± 0.47, d3 beads 1.52 ± 0.36, d7 plastic 3.96 ± 0.42, d7 beads 0.56 ± 0.46. This decrease in incorporation of label was not accompanied by proportionate cell detachment or death.

This work confirms previous findings that effects of steroid hormones demonstrated in vivo cannot be reproduced in vitro. We conclude, however, that the substrate upon which ovine endometrial epithelial cells are cultured influences the quantity of new methionine incorporated and the pattern of release of newly synthesised protein.


This work was supported by the William Buckland Foundation.

DISTRIBUTION AND FUNCTION OF PREGNANCY PROTEINS SECRETED BY THE SHEEP UTERUS

B.G. Miller and X. Zhang
Department of Animal Husbandry, University of Sydney, Camden, 2570

The intercotyledonary endometrium of the pregnant ewe secretes a group of glycoproteins (pregnancy proteins, PP) whose molecular weights are about 20 Kd. PP are present in uterine fluid (uterine milk, UTM) obtained from the ligated, non-pregnant horns of unilaterally pregnant ewes. An antiserum (AS) against PP was prepared by challenging mature rabbits with UTM from a ewe at Day 91 of pregnancy and absorbing the AS against plasma from a non-pregnant, ovariectomised (OVX) ewe. When this AS was allowed to react with UTM which had been subjected to electrophoresis in agar gels, at least 8 antigens were detected. Most migrated towards the anode. Using the same AS, at least 3 antigens were detected in allantoic fluid from a ewe at Day 124 of pregnancy, but no antigens were detected in plasma or kidney and liver homogenates from the same ewe, or in uterine flushings from non-pregnant ewes.

UTM suppresses lymphocyte proliferation (LP) induced by phytohaemagglutinin. Dialysed UTM from a ewe at Day 91 of pregnancy was tested in the same LP assay. LP, expressed as a % of control (cultures containing no test material), was reduced by dialysed UTM but not by serum from an OVX ewe (Table 1).

| TABLE 1. Effect of dialysed UTM on PHA-induced lymphocyte proliferation |
|-----------------|--------|--------|--------|
| mg protein/ml  | 0.3    | 0.9    | 2.7    |
| serum-OVX      | 102.5 ± 3.6* | 96.7 ± 3.4 | 92.9 ± 2.0 |
| dialysed UTM   | 92.4 ± 3.6 | 76.6 ± 5.0 | 50.5 ± 6.4* |

* mean ± SE for 3 experiments; a serum + UTM or PP; b UTM + PP; P<0.05.

PP were partially purified by fractionating UTM on a column of Sephadex G-200 and tested for their suppressive effect on LP in mixed lymphocyte cultures (MLC). Control cultures containing lymphocytes from two ewes were incubated for 5 days and pulsed with [³H]thymidine 20 h before the end of the incubation period. Both partially purified PP and unfractionated UTM inhibited LP in MLC, and PP was more potent than UTM (Table 2). These results indicate that (a) at least some PP are translocated to allantoic fluid; (b) PP appear to be specific to the uterus and pregnancy and (c) PP may act as immunosuppressive factors to prevent maternal immune rejection of the conceptus.

EFFECT OF HYPOPHYSECTOMY ON THE MAINTENANCE OF PREGNANCY IN THE GOAT

J.C. Malecki*, I.R. Young, G. Jenkin and G.D. Thorburn

Department of Physiology, Monash University, Clayton, Victoria and *Department of Agriculture and Rural Affairs, Regional Veterinary Laboratory, Bairnsdale, Victoria.

The corpus luteum is the major source of progesterone throughout pregnancy in the goat. It is thus essential for the maintenance of pregnancy. Hypophysectomy of pregnant goats results in abortion (1) which can be prevented, in some instances, by administration of luteinizing hormone (2,3). We have, however, shown that any major surgery can cause abortion in goats (4). Thus, since adequate sham-operated control animals were not included in (2) and (3), the effect of hypophysectomy and sham-hypophysectomy on pregnancy maintenance in the goat was investigated.

Four multiparous, Angora x feral goats were hypophysectomized at 84 to 98 days gestation by a paramedian transnasal transphenoidal approach. Sham-hypophysectomy was performed on 3 goats at the same stage of gestation. All goats were treated with progesterone, 100 mg i.m. twice daily for 5 days after surgery. The remaining two goats were treated with progesterone, 25 mg twice daily for 2 days. Blood, for radioimmunoassay of plasma progesterone, LH and prolactin concentrations, was obtained via an indwelling jugular vein catheter.

Abortion occurred in 3 of the 4 hypophysectomized goats and in 2 of the 3 sham-operated controls between 12.5 and 17.5 days after surgery. To assess functional completeness of hypophysectomy a TRF/LHRH stimulation test was performed on all goats between 3 and 4 weeks after surgery. Following a bolus i.v. injection of 30 μg TRF and 200 μg LHRH, none of the hypophysectomized goats showed any significant increase in plasma LH (< 0.1 ng/ml in all samples). The sham-operated goats exhibited a peak LH response of 8.4 to 9.7 ng/ml. Prolactin concentrations in two hypophysectomized goats remained below 1.0 ng/ml after TRF treatment. The remaining two showed peak prolactin responses of 85 and 120 ng/ml. At post-mortem they were found to have isolated remnants of pars distalis tissue in the anterior of the pituitary fossa. Sham-operated goats exhibited peak prolactin responses ranging from 285 to 440 ng/ml.

These findings suggest that maternal pituitary LH may not be essential for the maintenance of pregnancy in the goat. The results confirm that the stress and trauma of major surgery can result in abortion in this species and emphasise the need for adequate controls in such experiments.

This study was funded by the NH&MRC Australia.

QUANTITATIVE ASPECTS OF LUTEAL STRUCTURE DURING PREGNANCY IN THE EWE

J. D. O'Shea and K. McCoy

School of Veterinary Science, University of Melbourne, Victoria 3052.

The corpus luteum (CL) is no longer essential as a source of progestrone after about Day 50, but its histological structure is well-maintained until much later. We have examined quantitative changes in the size and composition of the CL from early pregnancy until near term. CL from 153 pregnant ewes of various breeds, from an abattoir, were collected and weighed, and stage of pregnancy estimated from foetal crown-rump length. CL from 5 additional Merino ewes on each of Days 30, 60, 100 and 142 were fixed by immersion for ultrastructural morphometry (1). Semi-thin sections were cut from 5 blocks per CL, and counts of mitotic figures and dead cells were performed by light microscopy (2500 cells/CL). Data were analysed by analysis of variance and the Student-Newman-Keuls method.

Weight per CL did not change between Days 20-120 (overall mean 0.56±SD 0.11g) in 110 ewes with single or twin CL. Luteal weight fell to 0.42±0.13g (n=32) and 0.31±0.11g (n=6) between Days 120-140 and >140 respectively (P<0.01). Mitotic index (%) on Days 30, 60, 100 and 142 was 0.04, 0.03, 0.02 and 0.02 respectively, and not different, and no mitotic figures were seen in large luteal cells. Percentages of dead cells on the same days were 0.00, 0.01, 0.00 and 0.23. The incidence of cell death on Day 142 was greater than on any other day (P<0.01), and at this stage some CL were infiltrated with many leucocytes.

Morphometric data on large luteal (LL) and small luteal (SL) cells (Table 1) showed no significant differences between days.

Table 1. Quantitative changes in LL and SL cells during pregnancy

<table>
<thead>
<tr>
<th>Feature type</th>
<th>Days of pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume density (%)</td>
<td>30</td>
</tr>
<tr>
<td>LL</td>
<td>41.1±5.4</td>
</tr>
<tr>
<td>SL</td>
<td>16.2±3.6</td>
</tr>
<tr>
<td>Cells/mm³×10⁻³</td>
<td>3</td>
</tr>
<tr>
<td>SL</td>
<td>73.4±15.0</td>
</tr>
<tr>
<td>Cell volume (μm³×10⁻³)</td>
<td>LL</td>
</tr>
<tr>
<td>SL</td>
<td>2.6±0.6</td>
</tr>
</tbody>
</table>

Numbers of LL and SL cells per unit volume up to Day 100 were similar to those reported during mid-cycle (1). Since luteal weight up to this stage was also similar to that at mid-cycle, absolute numbers of LL and SL cells during pregnancy could be accounted for by persistence of the populations formed during genesis of the CL, without additional sources. Since luteal weight fell during the final month, it is possible that absolute numbers of both LL and SL cells may have declined before term.


LEVELS OF mRNA ENCODING INHIBIN SUBUNITS IN BOVINE FOLLICLES AND CORPORA LUTEA

R.J. Rodgers, J.K. Findlay, R.G. Forage* and H.G. Burger

Medical Research Centre, Prince Henry's Hospital, Melbourne, Vic. 3004, and Biotechnology Australia Pty Ltd, Roseville, N.S.W. 2069.

Inhibin is a gonadal glycoprotein hormone which regulates the secretion of FSH by the pituitary. It is composed of two crosslinked subunits, A and B, which are separate gene products (1). To examine the relative amounts of mRNA encoding these subunits, as a function of follicular and luteal development, bovine ovaries were collected at an abattoir. A range of healthy follicles of differing size (2 pools of 20 and 10 follicles < 3 mm, 2 pools of 6 and 12 follicles 4-6 mm, 2 pools of 3 and 4 follicles 7-10 mm and individual follicles 11 mm and 13 mm) and corpora lutea of different stages of development (2 recently ovulated follicles or immature corpora lutea 2 days old, 3 mature corpora lutea and 1 regressing corpus luteum) were dissected. RNA was extracted and poly(A)⁺ RNA prepared using oligo(dT)-cellulose (2). mRNA (5 μg) was subjected to electrophoresis through agarose/formaldehyde gels and electrophoblated onto Zeta-probe. Prehybridizations and hybridizations were carried out at 65°C in a solution containing 10 x Denhardt's solution, 6 x SSC, 1 mM EDTA, 0.5% SDS and 100 μg denatured salmon sperm DNA per ml. cDNA's, complementary to bovine A mRNA (720 bp SpiH, -Small fragment from pBTA24) and bovine B mRNA (420 bp PstI + XbaI fragment from pBTA29) (1), were labelled with α-³²P-DCTP to a specific activity of 3.4 x 10⁵ cpm/μg DNA and used at 10⁴ cpm/ml. Blots were washed under stringent conditions at a final concentration of 0.1 x SSC at 50°C.

Inhibin A mRNA was not detected in ovarian stroma or in mature or regressing corpora lutea but was present in all antral follicles examined and in recently ovulated follicles. As a proportion of total mRNA the levels of Inhibin A mRNA were highest in large (> 7 mm diameter) follicles. Inhibin B mRNA was detected only in large follicles (> 7 mm diameter) and the levels were lower than those of inhibin A mRNA. Henderson and Franchimont (3) have shown that only bovine follicles and not ovarian stroma or corpora lutea produce inhibin in culture. The present results suggest that this is because inhibin mRNA is found only in bovine follicles. The finding of undetectable levels of inhibin A mRNA in mature bovine corpora lutea is in contrast to detectable levels in cyclic rat corpora lutea (4) and human cyclic and pregnancy corpora lutea (5).


Supported by Queen Elizabeth II Award and the NH&MRC of Australia.
EFFECT OF endometrial microvasculature: IN VIVO OBSERVATION OF UTERINE AUTOGRAPHS TRANSPLANTED TO THE ANTERIOR CHAMBER OF THE EYE

Peter A.W. Rogers, Anne Macpherson, Linda Beaton.
Department of Obstetrics & Gynaecology, Monash University Queen Victoria Medical Centre, Melbourne, Australia.

Estrogen has a well documented effect on the uterine vasculature, causing marked increases in both blood flow and permeability. However, most of these observations have been made using indirect methods, such as radio-labelled microspheres or other markers, or by measuring whole uterine blood flow with electromagnetic flow probes. As a result of this indirect methodology, little is known about the localized response of the endometrial microvasculature to estrogen. In particular, there may be a number of mechanisms by which the estrogen induced increase in uterine blood flow and volume may occur. These include increased flow through microvessels that were patent at the time, an increase in the number of microvessels that are patent, an increase in flow through arteriovenous anastomoses, or a volume increase mediated through post capillary capacitance vessels. The aim of the present study was to investigate the direct response of endometrial microvasculature to estrogen.

Autoimplants consisted of 1-2mm thick slices of uterus from which the longitudinal muscle layer had been removed. Grafts were left for a minimum of 2 weeks to successfully revascularize following transplantation to the anterior chamber of the eye. Rats were ovariotomized 2-4 days prior to estrogen injection. Observation of the endometrial microvasculature in anesthetized rats was made using incident light fluorescent microscopy, imaging directly through the cornea. Three IV fluorescent tracers were used, Evans blue (which binds strongly to serum albumin, giving an effective molecular weight of 67,000), FITC-BSA (MW 67,000) and FITC-Dextran (MW 156,000).

There is a dramatic sequence of endometrial microvascular events in response to a large dose of estrogen (estradiol 17b, IV, 20 mg/rat; Sigma). Approximately 20 minutes after estrogen injection there is a cessation of blood flow in most of the endometrial capillaries. By 120 minutes blood flow has re-established in some of these vessels and this is accompanied by a massive leakage of 67,000 MWT fluorescent tracer into the tissue space. This increase in vascular permeability continues until 156,000 MWT fluorescent tracer starts to leak out of the vessels by about 150 minutes. Most of the capillary stasis appears to be due to the closure of post-capillary sphincters, rather than due to a reduction on the arteriolar side. Smaller doses of estradiol 17b (1.25 mg) result in an increased permeability to 67,000 MWT tracer by 2 hours, without producing the same preliminary capillary stasis. There is no significant increase in microvascular diameter for vessels between 10-100 um 2 hours after 1.25 mg injection of estrogen. It has not yet been possible to measure red blood cell velocities and hence calculate capillary blood flow rates during these experiments. In conclusion, this model enables a detailed description to be made of the response of endometrial capillaries to estrogen, and can be extended to include a number of other vasoactive substances, including those produced by the implanting embryo.
THE FUNCTIONAL PROPERTIES OF ISOLATED ADULT RAT LEYDIG CELLS.

M.P. HEDGER AND E.M. EDDY

Gazette Biology Section, Laboratory of Reproductive and Developmental Toxicology, NIEHS, NIH, Research Triangle Park, N.C., U.S.A.

Reported differences between the steroidogenic capacities of isolated Leydig cells (LC), which sediment with different apparent buoyant densities in Percoll or Metrizamide gradients, have been attributed to either multiple LC populations(1), or limitations in the methods used to identify intact LC(2). This question was re-investigated using collagenase-dispersed intertubular cells from adult rat testes, prepared under conditions designed to minimize LC trauma and fractionated on linear Percoll gradients(3). The presence of the LC enzymes, 3 β-hydroxysteroid dehydrogenase (3 β-HSD) and non-specific esterases, were determined by routine cytochemical techniques following lysis of the plasma membrane (PM) to allow entry of the cytochemical reagents. The presence of an LC-antigen defined by monoclonal antibody LC-IC6(3), was determined by indirect immunofluorescence assay. LC function was assessed by incubation for 3 h with or without a maximally-stimulating dose of human chorionic gonadotrophin (hCG), and testosterone (T) release was measured by radioimmunoassay(3). By electron microscopy, LC were found to be predominantly in gradient fractions II (specific gravity 1.096–1.070 g/ml), III (1.070–1.064 g/ml) and IV (1.064–1.054 g/ml). There was a close cellular correlation between the presence of 3 β-HSD and the other LC markers in these fractions. LC with damaged PM were identified by 3 β-HSD reactivity in freshly-prepared unlysed cell preparations. Whole cells were differentiated from cytoplasmic fragments by counter-staining with the nuclear dye, propidium iodide. Greater than 80% of LC in each of the 3 fractions were intact (ie. possessed an intact PM and nucleus). In vitro hCG-stimulated T production/intact LC increased 2.5-fold with an increase in cell concentration from 10,000 to 50,000 LC/assay well, while addition of other testicular cell types had no effect on T production, indicating close cellular correlation between the presence of 3 β-HSD and the other LC markers in these fractions. LC with damaged PM were identified by 3 β-HSD reactivity in freshly-prepared unlysed cell preparations. Whole cells were differentiated from cytoplasmic fragments by counter-staining with the nuclear dye, propidium iodide. Greater than 80% of LC in each of the 3 fractions were intact (ie. possessed an intact PM and nucleus). In vitro hCG-stimulated T production/intact LC increased 2.5-fold with an increase in cell concentration from 10,000 to 50,000 LC/assay well, while addition of co-operativity between LC in vitro. At lower concentrations (< 10,000 LC/well), intact LC from fraction IV produced significantly less T (p < 0.01) than LC from fraction II, in response to hCG-stimulation. However, prolonging the exposure of isolated intertubular cells to the dispersal conditions resulted in declines in the apparent buoyant densities and steroidogenic capacities of all LC, which were not related to detectable changes in LC integrity. These results indicate that adult rat LC are homogeneous in steroidogenic capacity, but their function in vitro is modified by isolation procedures and culture conditions.


STIMULATION OF IMMATURE RAT LEYDIG CELL TESTOSTERONE SYNTHESIS BY A PROTEIN PURIFIED FROM PRIMARY SERTOLI CELL CULTURE MEDIUM.

V. Papadopoulos, S. Carreau, C.Y. Cheng, and C.W. Bardin

Laboratoire de Biochimie, U.A. CNRS 609, C.H.U., Caen, France
* The Population Council, New York, N.Y. 10021, U.S.A.
+ Present Address: Department of Endocrinology, Prince of Wales Hospital, Sydney, 2031 Australia.

We have previously reported that Sertoli cells (s) stimulates Leydig cell testosterone (T) production in vitro at all ages. In order to identify which Sertoli cell factor(s) were responsible for this effect, experiments were done using purified Leydig Cells and CMB proteins purified from primary Sertoli cells-enriched culture medium by asion exchange High Performance Liquid Chromatography. 5–10 x 10^7/0.5ml Leydig cells were incubated for 5 hours at 32°C under (95%/5%) O2/C02, with increasing concentration of CMB proteins (0–1000ng/ml) in either the presence or absence of a saturating amount of oLH (25ng/ml). T and cAMP levels were measured by RIA. Of the several CMB proteins tested, CMB-21 (a FSH and T responsive protein) was noted to produce a dose related increase of T production: from 2 to 500pg/ml of CMB-21 no changes were observed in T (51pg/10^6 cells) but 1 to 1000ng/ml of this protein produced a linear increase of T ranging from 86 to 869pg. In the presence of oLH (25ng/ml) which induced a 10-fold augmentation of T (500pg), increasing doses of CMB-21 resulted in a further stimulation of T output (775 to 2272pg/10^6 cells). The cyclic AMP analogue dbcAMP (1mM) also stimulated T secretion by Leydig cells similarly to oLH; CMB-21 also increases the dbcAMP dependant steroidogenesis. cAMP levels, which were increased up to 4-fold by oLH, remained unchanged by the presence of CMB-21 either alone or with oLH. In conclusion, in contrast to seminiferous tubule medium which stimulates Leydig cell T levels 2-fold, CMB-21 increase the basal T levels 10-fold and potentiates the action of oLH. We propose that CMB-21 may be one of the factors of seminiferous tubular origin that regulates T synthesis in immature rat Leydig cells.
PHOSPHOLIPIDS IN ISOLATED GUINEA PIG SPERM OUTER ACROSOMAL MEMBRAND AND PLASMA MEMBRANE BEFORE AND AFTER CAPACITATION IN VITRO.

University of Melbourne, Reproductive Biology Unit, Pathology Department, Royal Women's Hospital and Infertility Medical Centre, Epworth Hospital, Melbourne, Victoria, 3000.

Mammalian spermatozoa must undergo biochemical changes, referred to as capacitation, before they undergo fusion of the outer acrosomal membrane (OAM) with the overlying plasma membrane (PM) known as the acrosome reaction. To date, the mechanisms of capacitation are not fully understood, but appear to involve biochemical and physical changes in sperm membranes in the head. We report here for the first time, major changes in the phospholipid profiles of guinea pig plasma membranes, during the process of capacitation. Adult male guinea pigs (Dunkin Hartley) were anaesthetized with ether and killed by cervical dislocation. The isolated cauda epididymal spermatozoa (1) were either suspended in a medium based on the composition of the cytosol, MediuM A (2) with added 10mM HgCl₂ (pH 6.2) at 4°C or capacitated in a K⁺, Ca²⁺ free minimal culture medium (MCN-PL, pH 8.3) for 2hrs 37°C (1) and subsequently suspended in medium A with added 10mM HgCl₂ and 0.2% Ficoll 70 at 4°C. The percentage motile spermatozoa and "acrosome reactions were assessed after adding Ca²⁺(5mM) to an aliquot of capacitated sperm. Both sperm suspensions in MediuM A were subsequently homogenized (2) and centrifuged (2000xg, 4°C). The resultant supernatants contained PM which was isolated by applying the supernatants onto a sucrose gradient (0.4M, 1.33M, 1.70M; 100,000xg, 3hrs, 4°C). The PM which layered out at the 0.4M/1.33M interface was collected and subjected to another 0.4M sucrose wash (100,000xg, 3hrs, 4°C). Acrosomal caps, a source of OAM, were isolated from the homogenate pellet as previously outlined (2). The acrosomal caps were suspended in a solution containing 1M NaHCO₃ and 1mM p-aminobenzamidine for 16-20hrs at 4°C to lyse the caps and the resultant OAM was then collected by a two step centrifugation procedure. Firstly, a low speed centrifugation (8,000xg, 30min, 4°C) to remove particulate matter and the supernatant then overlayed onto a 1.33M sucrose solution and the OAM collected at the bottom of the tube after a high speed centrifugation (100,000xg, 3hrs, 4°C) followed by a 0.4M sucrose wash. Examination of the sperm homogenate by electron microscopy revealed PM removed from the acrosomal cap but remaining predominately intact posterior to the equatorial segment of the head, midpiece and tail. Analysis of membrane phospholipid profiles, using a Waters HPLC system, revealed a complete loss of phosphatidycholine (PC) from PM after incubation in MCN-PL for 2 hrs. A time course study revealed that PM LC loss occurred between 60 and 90 minutes of incubation. Incubation of spermatozoa with an acrosome reaction inhibitor, 10mM 2-deoxyglucose (1), restored PM phospholipid levels back to the non-capacitated state. These data provide direct evidence that phospholipids are involved in guinea pig sperm capacitation.


COTTON SEED CONTAINS GOSSEPOL WHICH DISRUPTS SPERMATOGENESIS WHEN FED TO MONOGASTRIC ANIMALS (E.G. MAN, DOG, RAT, RABBIT) RENDERING THEM INFERTILE (1). SINCE COTTON SEED MEAL (CSM) HAS BEEN FEED TO SHEEP, PARTICULARLY DURING DROUGHT PERIODS, IT SEEMED IMPORTANT TO CHECK THE SEMEN QUALITY OF RAMS ON SUCH A DIET.

Four Merino rams were fed a diet with sunflower seed (250g/kg) as the sole source of protein, and four similar rams a diet containing an equivalent protein as CSM (=167 ppm gossypol). The diets were equal in all other respects and maintained the body weight of the rams. Semen was collected by electroejaculation once a week over 20 weeks. The volume of each ejaculate was recorded, the sperm counted, motility scored (0-4) and the percent motility estimated. The fructose and citric acid concentrations of the seminal plasma were also monitored (Table 1).

There was no significant trend in the semen characteristics of either group over the 20 weeks, which would have been sufficiently long to detect any disruption of spermato genesis.

Table 1. Semen characteristics of rams fed diets containing sunflower seed meal (control) and cotton seed (gossypol). Mean values ± S.E. (N).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Volume (ml)</th>
<th>Sperm Count (x10⁶/ml)</th>
<th>Motility Score (0-4)</th>
<th>Fructose (mg/ml)</th>
<th>Citrate (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunflower seed</td>
<td>1.7±</td>
<td>2.9±</td>
<td>3.6±</td>
<td>71±</td>
<td>6.7±</td>
</tr>
<tr>
<td>seed meal</td>
<td>0.4±(79)</td>
<td>0.4±(79)</td>
<td>0.1±(79)</td>
<td>3.2±(79)</td>
<td>1.3±(26)</td>
</tr>
<tr>
<td>Cotton seed</td>
<td>1.9±</td>
<td>2.1±</td>
<td>3.5±</td>
<td>60±</td>
<td>8.5±</td>
</tr>
<tr>
<td>meal</td>
<td>0.3±(75)</td>
<td>0.7±(75)</td>
<td>0.2±(75)</td>
<td>13.4±(75)</td>
<td>0.7±(26)</td>
</tr>
</tbody>
</table>

It is clear that feeding cotton seed at this level had no substantial effects on sperm production or semen quality and the fructose and citric acid concentrations in the seminal plasma were not decreased. Since these compounds are produced by the seminal vesicles and are androgen dependent, testosterone production was presumably not impaired by the gossypol in the cotton seed meal.

There is evidence that ruminants can detoxify gossypol, putatively by binding to protein in the rumen (2) and this may account for the lack of an effect of the cotton seed meal. We conclude that CSM is safe to use as a protein supplement for rams.

OBJECTIVE ASSESSMENT OF SPERM MORPHOLOGY USING IMAGE ANALYSIS


Computer Imaging Group, Applied Physics Department, Chisholm Institute of Technology and Andrology Laboratory, Department of Pathology, University of Melbourne, Department of Obstetrics & Gynaecology, The Royal Women's Hospital, Melbourne.

Of all aspects of semen analysis, sperm morphology is the most prone to subjective errors of interpretation. An image analysis system has been developed to allow objective morphological classification of fixed (methanol, 30%) and stained (haematoxylin, 10%) human spermatozoa. The sperm images were obtained from an oil immersion microscope (magnification ×1000) using a video system and transmitted to a personal computer for analysis. A grey scale histogram of the sperm images was examined and a threshold level evaluated. This threshold level was used to set pixels of values greater than threshold to white and all other pixels to black thus producing a binary image which was then coded using straight line segments which made up of the 8 major directions of the compass - the so called chain code method. By using the Fourier transforms of the x and y projections of the chain code, features such as perimeter, length, area, symmetry, eccentricity (ratio of major to minor axes: an indication of ovality), minimum enclosing box and bending energy (a measure of the curvature of the object) are extracted. The technique is sensitive to individual differences in normal sperm populations and is able to classify abnormal sperm into predetermined class. Results on 170 sperm images, classified by a professional observer into the head classes - round (absent y acrosomes), normal, elongated (tapered and pyriform) and large, showed clear separation on plots, for example, of head width versus length and eccentricity versus length. The analysis system is being used to investigate the relationship between sperm morphology and human fertilization rates in vitro. It is concluded that this method could be developed into a practical system for routine clinical semen analysis.

THE DIFFERENTIAL FERTILITY METHOD: A MATHEMATICAL MODEL TO ESTIMATE THE IMPACT OF VARICOCELE ON MALE FERTILITY FROM INFERTILITY DATA

David J. Handelman

Dept of Medicine, University of Sydney and Andrology Unit, Royal Prince Alfred Hospital, Sydney, NSW

In principle, patients having any fixed condition that lowers fertility should accrue progressively in infertility clinics to a greater extent than patients with any incidental condition unrelated to fertility. For example, if varicocele, a relatively common condition, causes subfertility it should be present at a higher prevalence among men in infertile unions than in the general male community and this excess should increase progressively with longer durations of infertility whereas neutral indicators unrelated to fertility (eg left-handedness) would remain equally prevalent at all times among infertile men as among the general male community. A mathematical model based on life-table methods has then been developed and evaluated in simulations and to estimate the impact of varicocele on male infertility. The model includes terms for time (t, months) and 3 parameters to represent an asymptotic estimate of the prevalence of varicocele in the general male community (P1), the average fecundability of couples in the general community (P2) and the estimate of fertility of men with varicocele relative to men without varicocele (P3). An expression derived for the proportion of men with varicocele presenting with infertility at various durations is fitted to empirical data by an iterative, weighted, non-linear regression technique. The infertility database examined, corrected for representations, is that from 1379 couples evaluated at the Male Fertility Clinic at Royal Prince Alfred Hospital. The resulting estimates for the 3 parameters (+ estimated SE) are indicated

P1 = 22.0 + 2.4 + P2 = 0.201 + 0.031/cycle P3 = 0.948 + 0.015

The results for P1 and P2 agree well with independent estimates from other data. The parameter P3 quantitates the impact of varicocele demonstrating a small (~5%) effect size on fertility while the significant difference from unity indicates that varicocele is associated with male subfertility. Attempts to fit this model to large infertility databases from Melbourne (Dr H W G Baker) and Edinburgh (Dr RA Elton & TB Hargreave) however indicated no measurable effect of varicocele on male fertility. It is concluded that the differential fertility method may be useful to estimate the impact of fixed factors on time-dependent fertility. This model is appealing since it provides community-based estimates which are however evaluated from conveniently available infertility clinic data. Present limitations include the assumption of constant fecundability in the model and application is sensitive to inaccurate data ascertainment and referral bias. Using the differential fertility method it has been demonstrated for the first time that (a) varicocele is associated with male infertility more than by chance alone and (b) the effect of varicocele on male fertility is small.

Supported by the Wellcome Trust and NHMRC
Pulsatile release of prostaglandin (PG) F, at luteolysis in pregnancy is to be successfully established, pulsatile, but not basal, PGF release at the end of gestation is, however, a prerequisite of PGFM and normal parturition. In this study, basal and OT stimulated release of PGFM in ewes was investigated in S ewes at 20-25 (early), 60-65 (mid) and 105-110 days gestation. Blood samples were obtained from the left and right utero-ovarian vein (UOV) and carotid artery (CA) every 20 min for 2h prior to gestation. OT was administered via a jugular vein in early pregnant ewes and via a uterine artery in mid and late pregnant ewes. Blood samples were collected from each UOV and a CA at frequent intervals following administration of OT. Plasma concentrations of PGFM and PGF2α were determined by radioimmunoassay.

Basal UOV plasma PGFM concentrations were not significantly different in early and mid gestation (0.46 ± 0.07 nM, respectively) but increased significantly to 2.01 ± 0.11 nM in late pregnancy.

This work was funded by the NH&MRC, Australia.

130

Y.M. Hodgon, A. Torney, S. Averill, R. Rogers and D.M. Dr Kretser.

Department of Anatomy, Monash University, Clayton, Victoria 3168, and MRC, Prince Henry's Hospital, Melbourne, Victoria 3004.

Bovine inhibin is composed of two subunits designated A and B for both of which the gene sequence is now known (1). This study has employed the technique of in situ hybridization to determine whether inhibin mRNA is expressed in the fetal gonad. Ovaries and testes were removed from bovine fetuses obtained from the abattoir and embedded in O.C.T. compound. Frozen sections were cut on a cryostat and hybridized with 32P-labelled cDNA probes. cDNA probes complementary to the An, Ac and B subunits of inhibin were used (1). A fourth unrelated fragment of plasmid DNA (pBR 322) was used as a negative control.

Both probes to the A subunit of inhibin (An and Ac) were hybridized by the developing seminiferous cords of testes from fetuses with crown-rump length (C-R) ranging from 45-90 cm. Hybridization was abolished by pretreatment of sections with ribonuclease (RNase). Probe B and mBR were not hybridized by any sections of fetal testis. Ovarian sections from fetuses with C-R lengths of 10-50 cm hybridized probes to the An, Ac and B subunits over the cortical layer containing the primordial germ cells. Ovaries from larger fetuses (C-R length 62 and 69 cm) containing medullar follicles hybridized both probes to the inhibin subunits over the granulosa layer. Pretreatment with RNase abolished hybridization of these probes. The bacterial probe was not hybridized by the fetal ovarian sections.

In conclusion, this study demonstrates that the inhibin genes are expressed in fetal life. Expression in the male gonad is restricted to the A subunit only while the female gonad expresses both subunits.

1. Forsge et al. (1986) PNAS 83: 3091-3095.

131

B.M. Bindon, L.R. Piper, M.A. Hillard, R.D. Nethery and G. Uphill

CSIRO Division of Animal Production, Armidale, NSW 2350

Several studies confirm that the Booroola Merino ovary is more sensitive to hFSH (see 1). It has always been assumed that this meant that the Booroola ovary is more sensitive to endogenous gonadotrophins. However, there are important qualitative differences between hFSH and hFSH-S. Therefore, experiments compared Booroola with a random-bred Merino (C) and the CSIRO 'T' flock on the basis of their ovulation response to several FSH preparations. In all tests the ewes were synchronized with progestagen sponges (Repromap, Upjohn), then given FSH as subcutaneous injections twice daily for four days beginning on Day 11 after sponge insertion. The total dose of FSH was administered in decreasing doses in the ratio of 4; 3: 2; 1 over the four days of treatment. Sponges were removed on the morning of the third day. Ovulation rate was assessed by laparoscopy six days after oestrus. The doses (mg) of FSH tested were 4, 8, 16 (FSH-'P'), 2, 4, 8 (FSH-ovine), 16, 32, 64 (FSH-bovine) and 6, 12, 24 (for FSH-porcine). Tests I-3 were carried out concurrently and shared the same control (i.e., zero FSH) groups. There were 8-10 ewes per dose.

<table>
<thead>
<tr>
<th>Test</th>
<th>FSH</th>
<th>Sheep genotype</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(Burns-Biotec)</td>
<td>Booroola (B)</td>
<td>3.4±1.0</td>
<td>3.6±1.0</td>
<td>4.5±1.0</td>
<td>11.6±1.0</td>
</tr>
<tr>
<td>FSH-'P'</td>
<td>Control (C)</td>
<td>0.8±1.1</td>
<td>1.0±1.1</td>
<td>3.5±1.1</td>
<td>9.5±1.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(Heriot)</td>
<td>B</td>
<td>3.4±1.2</td>
<td>3.3±1.2</td>
<td>3.4±1.0</td>
<td>3.3±1.1</td>
</tr>
<tr>
<td>FSH-ovine</td>
<td>C</td>
<td>0.8±1.1</td>
<td>1.5±1.0</td>
<td>2.2±1.0</td>
<td>5.6±1.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>(ASRB)</td>
<td>B</td>
<td>3.4±1.0</td>
<td>4.2±1.0</td>
<td>5.4±1.0</td>
<td>12.4±1.0</td>
</tr>
<tr>
<td>FSH-bovine</td>
<td>C</td>
<td>0.8±1.1</td>
<td>1.0±1.1</td>
<td>1.0±1.1</td>
<td>1.8±1.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>(Heriot)</td>
<td>B</td>
<td>2.0±1.2</td>
<td>3.5±1.3</td>
<td>3.6±1.3</td>
<td>7.9±1.3</td>
</tr>
<tr>
<td>FSH-porcine</td>
<td>C</td>
<td>1.5±1.1</td>
<td>1.8±1.1</td>
<td>4.3±1.1</td>
<td>8.7±1.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>CSIRO 'T'</td>
<td>0.2±1.5</td>
<td>0.7±1.3</td>
<td>0.7±1.3</td>
<td>12.6±1.2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test</th>
<th>FSH</th>
<th>Sheep genotype</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(Burns-Biotec)</td>
<td>Booroola (B)</td>
<td>3.4±1.0</td>
<td>3.6±1.0</td>
<td>4.5±1.0</td>
<td>11.6±1.0</td>
</tr>
<tr>
<td>FSH-'P'</td>
<td>Control (C)</td>
<td>0.8±1.1</td>
<td>1.0±1.1</td>
<td>3.5±1.1</td>
<td>9.5±1.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(Heriot)</td>
<td>B</td>
<td>3.4±1.2</td>
<td>3.3±1.2</td>
<td>3.4±1.0</td>
<td>3.3±1.1</td>
</tr>
<tr>
<td>FSH-ovine</td>
<td>C</td>
<td>0.8±1.1</td>
<td>1.5±1.0</td>
<td>2.2±1.0</td>
<td>5.6±1.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>(ASRB)</td>
<td>B</td>
<td>3.4±1.0</td>
<td>4.2±1.0</td>
<td>5.4±1.0</td>
<td>12.4±1.0</td>
</tr>
<tr>
<td>FSH-bovine</td>
<td>C</td>
<td>0.8±1.1</td>
<td>1.0±1.1</td>
<td>1.0±1.1</td>
<td>1.8±1.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>(Heriot)</td>
<td>B</td>
<td>2.0±1.2</td>
<td>3.5±1.3</td>
<td>3.6±1.3</td>
<td>7.9±1.3</td>
</tr>
<tr>
<td>FSH-porcine</td>
<td>C</td>
<td>1.5±1.1</td>
<td>1.8±1.1</td>
<td>4.3±1.1</td>
<td>8.7±1.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>CSIRO 'T'</td>
<td>0.2±1.5</td>
<td>0.7±1.3</td>
<td>0.7±1.3</td>
<td>12.6±1.2</td>
<td></td>
</tr>
</tbody>
</table>

There is no evidence in Tests 1-4 that the Booroola ovary was more sensitive to FSH than the ovary of control Merinos. If anything, Tests 2 and 4 suggest the converse may be true. In Test 4 the moderately prolific 'T' Merino had a significantly higher ovulation response to dose 3 of FSH than either Booroola or C Merinos. The exceptional prolificacy of the Booroola does not appear to be the result of increased sensitivity to FSH.

THE INFLUENCE OF NUTRITION ON THE METABOLIC CLEARANCE RATE OF PROGESTERONE IN OVARIECTOMISED EWES

R.A. Parr, I.F. Davis, M.A. Miles, T.J. Squires and G.J. Simpson
Animal Research Institute, Department of Agriculture and Rural Affairs, Werribee, Victoria, 3030

We recently demonstrated that high levels of nutrition after joining were associated with reduced peripheral progestosterone (P4) concentrations and increased embryonic mortality (1). It was uncertain if this response was caused by changes in blood production rate (BPR) or metabolic clearance rate (MCR) of P4. The aim of this study was to determine if the MCR of P4 in ewes was influenced by nutrition.

Fifteen ovariectomised Merino ewes were individually penned in an animal house and fed a daily maintenance (M) ration of pelleted lucerne hay and barley. After a 12 day adjustment period all ewes had both jugular veins cannulated and 2 days later each ewe received an iv. infusion of P4 (840 μg/h for 4.5h). Blood samples were collected prior to and during infusions and the blood P4 concentrations of the final 3 samples, taken at 15 min intervals were used to calculate P4 MCR (2). Ewes were then placed on 1/4M (n=5), M (n=5) or 2M (n=5) rations for 5 days, after which time the infusion and blood sampling procedures were repeated. The mean (+/- s.e.m.) MCR of P4 in all ewes at the first infusion was 9.8+/-.01 1/h/kg.

TABLE 1 Mean (+/- s.e.m.) liveweight change, blood P4 concentration and MCR of ewes at second infusion.

<table>
<thead>
<tr>
<th>NUTRITION GROUP</th>
<th>n</th>
<th>LIVEWIGHT CHANGE (kg)</th>
<th>BLOOD P4 (μg/ml)</th>
<th>MCR (1/h/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/4M</td>
<td>4</td>
<td>-2.25+/-.04</td>
<td>7.9+/-.21</td>
<td>5.5+/-.03</td>
</tr>
<tr>
<td>M</td>
<td>4</td>
<td>+0.50+/-.02</td>
<td>2.3+/-.05</td>
<td>15.6+/-.51</td>
</tr>
<tr>
<td>2M</td>
<td>5</td>
<td>+2.40+/-.03</td>
<td>1.7+/-.03</td>
<td>16.8+/-.22</td>
</tr>
</tbody>
</table>

* Ewes not sampled at 2nd infusion due to cannula failure
a,b; Means with different superscripts differ. significant (P<0.05)

The mean MCR of P4 was directly related to level of nutrition. The kinetics of steroid metabolism are described by the equation BPR×MCR. Therefore these data support the hypothesis that the inverse relationship between nutrition and P4 is caused by changes in P4 MCR and not by changes in secretion.

This work is supported by the WRFT of the Aust.Wool Corp.

REDUCED UTERINE WEIGHT IN OVARIECTOMISED EWES IMMUNISED AGAINST CORTISOL OR OESTROGEN

N.R. Adams and S. Atkinson
CSIRO Division of Animal Production, Wembley, WA 6014

Heavy ovariectomised ewes have a lower concentration of oestrogen receptors in the uterus than light ewes (1). We postulate that this relationship may be mediated by differential secretion of adrenal steroids. In the present study, we immunised ovariectomised ewes against cortisol or oestrogen, to determine whether the adrenal gland produces enough steroids to affect the uterus.

Four groups of 10 Merino ewes were immunised using Freund's complete adjuvant, against human serum albumin (HSA), cortisol conjugated to HSA at carbon 3, cortisol conjugated to HSA at carbon 21, or a mixture of oestradiol and oestrone conjugated to HSA at carbon 3. At 9 weeks the ewes were ovariectomised, at 12 weeks they were re-immunised, and 10 days later they were killed by an overdose of nembutal. The uterus was weighed, minced, and the concentrations of protein, RNA and DNA were measured. Oestrogen receptors could not be measured accurately in the presence of cross-reacting antibodies.

The two groups immunised against cortisol gave a similar response, and the results have been pooled. As seen in Table 1, immunisation against either cortisol or oestrogen increased the weight of the adrenal glands (P<0.05) and decreased the weight of the uterus (P<0.05). There were no differences between groups in the concentration of protein in the uterus (overall mean 38.1 ± 1.3 mg/g), or the ratio of uterine RNA/DNA (overall mean 0.37 ± 0.01).

Table 1. Uterine pituitary adrenal and body weights of ewes immunised against cortisol or oestrogen

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Uterus wt (g)</th>
<th>Pituitary wt (g)</th>
<th>Adrenal wt (g)</th>
<th>Li'we wt (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSA-imm.</td>
<td>10</td>
<td>10.8±0.6</td>
<td>0.56±0.05</td>
<td>3.18±0.14</td>
<td>45.9±1.4</td>
</tr>
<tr>
<td>Cortisol-imm.</td>
<td>20</td>
<td>11.4±0.4*</td>
<td>0.64±0.04</td>
<td>3.72±0.02</td>
<td>47.8±0.9</td>
</tr>
<tr>
<td>Oestrogen-imm.</td>
<td>10</td>
<td>10.6±0.6*</td>
<td>0.66±0.05</td>
<td>3.69±0.18</td>
<td>44.8±0.8</td>
</tr>
</tbody>
</table>

* P<0.05, ** P<0.01 different from control (HSA)

Immunisation affected the weight of the adrenal gland, and presumably affected adrenal function. Immunisation against either cortisol or oestrogen gave a similar response, so it is not possible to say which steroid is involved. The similar values for protein and RNA/DNA indicate that the uterus had reached an equilibrium at its new size before the ewes were killed. The decline in uterine weight in the immunised ewes suggests that in the normal ovariectomised ewe, steroid secretion by the adrenal gland is responsible for maintaining uterine weight.

ADRENAL GLANDS ALTER UTERINE OESTROGENS IN OVARIECTOMISED EWES

S. Atkinson and N.R. Adams

CSIRO Division of Animal Production, Wembley, WA 6014

Immunising ewes against cortisol or oestradiol results in a decrease in uterine weight and an increase in the weight of the adrenal gland (3). This finding implies that the adrenal gland secretes sufficient steroids to affect the uterus. To further investigate these effects, ovarioiectomised Merino ewes were either adrenalectomised, administered glucocorticoid-like preparations, or remained as controls.

The adrenalectomised ewes (ADRX, n=6) were administered mineralocorticoid replacement therapy and were monitored daily for plasma glucose and Na+/K+ concentrations. ADRX ewes were killed 9 days after adrenalectomy, while the ewes administered glucocorticoid-like preparations (GLUC, n=6) were killed after 3 weeks of drug therapy. The control ewes (n=6) were killed simultaneously with the treatment groups. The ewes were adrenalectomised, ovarioiectomised and killed under general anaesthesia. Uterine tissues were homogenised before measuring oestadiol receptors and tissue concentrations of oestadiol-17β. Recovery of extraction of tissue oestadiol was 64.6% and the values presented were not corrected for this loss.

The ADRX ewes had significantly higher (P<0.01) concentrations of cytosolic oestrogen receptors in the uterus than did the GLUC ewes (Table 1). These results indicate that the adrenal gland affects the concentration of both oestadiol and its receptors in the uterus of ovarioiectomised ewes. The hormonal pathways responsible for these effects need to be determined.


Table 1. Concentrations of cytosolic oestrogen receptors and tissue oestadiol in the uteri of control, adrenalectomised (ADRX) and ewes given exogenous glucocorticoids (GLUC)

<table>
<thead>
<tr>
<th>Group</th>
<th>Oestrogen Receptors (pmole/mg protein)</th>
<th>Tissue Oestadiol (pg/g uterus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.75±0.06</td>
<td>21.96±3.9</td>
</tr>
<tr>
<td>ADRX</td>
<td>0.95±0.06*</td>
<td>6.61±1.0**</td>
</tr>
<tr>
<td>GLUC</td>
<td>0.6±0.02*</td>
<td>10.96±4.5</td>
</tr>
</tbody>
</table>

* different from controls P<0.01, ** P<0.001

A brief elevation of plasma progesterone concentration following a silent ovulation marks the onset of puberty in ewe lambs (1). This 'progesterone priming' seems essential for the establishment of normal cyclicity. Progesterone priming (PP) also facilitates the formation of functional CL in prepubertal ewes following PMSG induced ovulation (2). Without progesterone priming ovulation is induced, but the CL are short-lived. The present report details results of LH patterns and ovarian activity following FSH administration to ewe lambs with and without prior progesterone priming.

Fifty four prepubertal Corriedale ewes aged between 24-28 weeks, average weight 24.5 ± 2 kg were penned in an open-sided shed and fed a standard lamb grower ration. Half of the ewe lambs were primed with injections of 10 mg progesterone (Propergin, Intervet) daily for 10 days. The remainder served as controls. The animals were allocated to treatment groups as follows:

Group 1: To determine LH pulse rate frequency. Intensive blood sampling (20 min intervals for 6 h). Control (C), 6th day of PP (P6) and 2 days after finish of PP (P+2).

Group 2: Treated with 10 mg FSH-β (Burns-biotech, USA) over 4 days, ovariectomy at recorded intervals to assess ovarian activity, before (CF, PF), 1 day after (CFH, PFH) and 8 days after (CF 8, PF 8) initiation of FSH treatment

Group 3: Six hourly blood sampling after initiation of FSH treatment (CS, PS) to detect the LH surge. Each treatment group contained 5 ewe lambs.

Results of intensive bleeding showed that LH concentrations were below 0.5 ng/ml throughout the sampling period, and that LH pulse frequency was very low (4, 4 and 7 pulses/30 h in C, P6 and P + 2). Ovarian activity was similar in the C and PP groups before and during the FSH administration, but significantly more functional CL were present in the PP group 8 days after initiation of FSH treatment (3.4 vs 0.4, p < 0.05). The LH surge occurred later and was better synchronised in the PS group (39.0 ± 1.7 h vs 22.8 ± 4.8 h) and was of greater magnitude (40.8 ± 5.6 ng/ml vs 20.9 ± 6.4 ng/ml).

Progesterone priming may exert its effect in part by delaying and synchronising the LH surge during FSH administration.


DERANGED PLASMA GONADOTROPHIN CONCENTRATIONS IN SHEEP WITH GONADAL HYPOPLASIA

D.R. Galloway, P.J. Wright and I.J. Clarke*
Department of Veterinary Clinical Sciences, University of Melbourne, Werribee 3030;
* Medical Research Centre, Prince Henry's Hospital, Melbourne 3004, Victoria.

Plasma hormone concentrations were assessed in 5 rams and 3 ewes with bilateral gonadal hypoplasia from a Poll Dorset flock in which gonadal hypoplasia appeared in outbreak form in 1985. Histological examination of affected testicles revealed a Sertoli-cell-only picture. The hypoplastic ovaries lacked oocytes and follicular structures. Five normal rams and 2 ewes in seasonal anoestrous served as controls.

Blood samples were taken every 10 min for 6 h through indwelling jugular canulas. Plasma concentrations of luteinising hormone (LH) were determined on all samples. The concentrations of follicle-stimulating hormone (FSH) were determined on plasma pools derived from samples taken over the last 3 h.

Table 1. Plasma hormone concentrations (mean ± SEM) in sheep with normal or hypoplastic gonads.

<table>
<thead>
<tr>
<th>Sheep with</th>
<th>Hormone Plasma Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicles</td>
<td></td>
</tr>
<tr>
<td>Normal (n=5)</td>
<td>0.07 ± 0.06 &lt; 0.09</td>
</tr>
<tr>
<td>Hypoplastic (n=5)</td>
<td>2.29 ± 0.88* 17.04 ± 5.79***</td>
</tr>
<tr>
<td>Ovaries</td>
<td></td>
</tr>
<tr>
<td>Normal (anestrous) (n=2)</td>
<td>0.29 ± 0.03 2.00 ± 2.00</td>
</tr>
<tr>
<td>Hypoplastic (n=3)</td>
<td>12.43 ± 2.46*** 54.33 ± 5.48***</td>
</tr>
</tbody>
</table>

Significantly different from controls * P < 0.05 *** P < 0.001

The marked elevation of FSH concentrations associated with testicular hypoplasia (Table 1) probably reflects the absence of inhibin production by Sertoli cells which are not supporting spermatogenic activity. The small but significant elevation in LH is probably due to a failure of oestrogen production by the Sertoli cells leading to a deficiency in the inhibitory influence of testicular steroids on LH secretion despite testosterone production by the Leydig cells.

In the affected ewes the greatly increased LH and FSH concentrations (Table 1) reflect the failure of inhibitory feedback from the ovaries on gonadotrophin secretion when the oocytes and follicular cells are absent.

Disturbed plasma hormone concentrations in sheep with gonadal hypoplasia have not previously been described. The results provide a basis for the development of a diagnostic test that could be used in young animals.

A SENSITIVE IN VITRO BIOASSAY FOR PREGNANT MARE'S SERUM GONADOTROPHIN

V.W.K. Lee, A.R. Billett and M.P. Johnston
Division of Biological and Health Sciences, School of Sciences, Deakin University, Geelong, Vic. 3217.

A dose-related increase in inhibin secretion from rat granulosa cells in vitro by pregnant mare's serum gonadotrophin (PMSG) treatment has been shown previously (1). This observation raises the question of a bioassay for PMSG. We report here the characterization of a sensitive in vitro bioassay for PMSG based on secretion of progesterone from rat granulosa cells.

Granulosa cells were obtained by puncturing the follicles of ovaries from diethylstilbestrol treated immature female rats (2). The cells were washed, suspended in serum-free McCoy's 5a medium containing 10% foetal bovine serum and dispensed into 48-well culture plates at 500-1000 cells per well in 0.1 ml culture medium and incubated for 24 h with varying concentrations of PMSG (0-3000 mIU/ml). PMSG preparations were obtained from Organon Laboratories ('Polligon') and Herriot Agencies ('Prognescol') and bioassayed. In addition, serum samples collected from pregnant mares were also assayed in serial dilutions for PMSG bioactivity. In these samples pretreatment with 5% (w/v) polyethylene-glycol (PEG) was essential before addition to the granulosa cell culture medium. The media, removed after 24 h incubation, were assayed for progesterone concentrations by radioimmunoassay (RIA). Parallel line statistics were used for the calculation of biopotencies.

The linear dose-response range for the bioassay was 50-1000 mIU/ml. This linear dose-response range was at least 10,30 and a 1000-fold more sensitive than in vivo bioassays in mice, rats and sheep respectively. The indices of precision (1) for 10 consecutive bioassays ranged from 0.05 to 0.17. There was no departure from parallelism between the Polligon and Prognescol samples examined. To determine whether the bioassay could be applied to serum samples, gelding plasma was added to Prognescol preparations (PRO-05A and PRO-08A) in the granulosa cell culture medium. The addition of 5 or 10 ul of gelding plasma to Prognescol samples enhanced biopotencies (at least 2-10 fold) compared to Prognescol with no gelding plasma present. This "interfering" effect of plasma was abolished if the gelding plasma was pretreated with 5% PEG. Similarly, serum samples from pregnant mares were bioassayed after treatment with PEG and biopotencies ranged from 65 to 124 U/ml in 7 samples examined.

In conclusion, a sensitive and precise in vitro bioassay for PMSG, based on progesterone secretion from granulosa cells, has been described. This quick and economical assay system is recommended for use in the determination of PMSG bioactivity in serum samples prior to purification, in column fractions during purification, or in studies involving structure-function relationships of the PMSG molecule.

Relaxin has been implicated in the pre-partum changes which occur in uterine cervix and pubic symphysis in mammalian species. The definition of a precise physiological role for relaxin in sheep, however, remains enigmatic. Bassett and Phillips (1) reported the presence of relaxin in ovine ovaries at similar concentrations as found in porcine ovaries. Subsequently relaxin-like immunoreactivity and bioactivity have been demonstrated in peripheral plasma of pregnant ewes. Previous studies, however, have not utilized specific isolation techniques in their analysis of relaxin-like activity (RLA) but have relied upon direct immuno or bioassay procedures. In the present study, we applied a selective isolation procedure (2) to identify ovine RLA.

Following extraction and reverse-phase HPLC, immunoreactive components were identified in ovary, placenta and semen. Multiple peaks of RLA were observed in ovarian and placenta extracts and these peaks displayed similar retention times to authentic porcine relaxin. Immunoreactivity in the HPLC fractions was assessed using a number of anti-relaxin sera in either radioimmuno assays or dot-blot assays. When these fractions were assayed for bioactivity, no consistent relaxin-like effect could be identified. The major peak of RLA isolated from placenta was sequenced through 16 cycles on an automated protein sequencer. Only one sequence was revealed which unambiguously matched the sequence of ubiquitin. The full 76 amino acid sequence of ubiquitin shows no homology with relaxin. This protein appears to co-isolate with relaxin when standard techniques are employed. Ubiquitin occurs in all tissues in all species examined, thus, relaxin isolated previously may also contain this protein. The immunoreactivity data obtained in the present study is consistent with this suggestion. The status of relaxin in sheep, thus, remains tenuous and requires further investigation.

UNDERNUTRITION INCREASES THE INHIBITORY EFFECT OF OESTRADIOL ON THE PLASMA CONCENTRATIONS OF FSH AND LH IN EWES

K.E. Davis, F.R. Wright, and I.J. Clarke

Department of Veterinary Clinical Sciences, University of Melbourne, Werribee 3030; Medical Research Centre, Prince Henry's Hospital, Melbourne 3004, Victoria, Australia

We have investigated the endocrine basis of the relationship between nutrient status and reproductive function in ovariectomized ewes during seasonal anoestrus.

Mature Corriedale ewes, ovariectomized 6 months previously, either received small oestradiol implants (1) s.c. (OE ewes, n=23) 5 months before the start of the study, or remained untreated (0 ewes, n=21). The ewes received maintenance (M) or sub-maintenance (L) nutrition for 93 days from mid-July to mid-October. Plasma concentrations of LH and FSH were determined on days 1 and 93 of the study from blood samples taken each 10 min for 6h via jugular venous cannulae. For each ewe, the difference between the hormone concentrations obtained on each occasion was determined, the treatment group means of these differences calculated, and the significance of differences between these means tested.

The liveweights of all groups were similar on Day 1 (49.4±4.0 kg, means±s.e.m.), and on Day 93 for M and L nutrition groups were 51.6 ± 5.0 and 47.0±4.0 kg, respectively. The frequencies of LH pulses on Day 1 in OE ewes were 2.2±0.5 and 4.7±0.6 pulses/6h, and were not affected by nutritional treatment. Undernutrition reduced the plasma concentrations of LH and FSH and the LH pulse amplitudes in OE ewes, and increased plasma FSH concentrations in O ewes.

Table 1. Plasma hormone concentrations in ewes on day 1 (D1) and differences after receiving maintenance (M) or sub-maintenance (L) diets for 93 days. Values are means (s.e.m.).

<table>
<thead>
<tr>
<th>Ewes</th>
<th>FSH (ng/ml)</th>
<th>LH (ng/ml)</th>
<th>LH pulse amp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D1 diff</td>
<td>D1 diff</td>
<td>D1 diff</td>
</tr>
<tr>
<td>OE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 10</td>
<td>6.10(1)</td>
<td>2.4(1)</td>
<td>4.2(1)</td>
</tr>
<tr>
<td>L 15</td>
<td>6.50(1)</td>
<td>1.8(1)</td>
<td>3.9(1)</td>
</tr>
<tr>
<td>OE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 10</td>
<td>3.0(1)</td>
<td>3.0(1)</td>
<td>3.0(1)</td>
</tr>
<tr>
<td>L 15</td>
<td>3.0(1)</td>
<td>3.0(1)</td>
<td>3.0(1)</td>
</tr>
</tbody>
</table>

These data indicate that undernutrition increases the inhibitory effect of oestradiol on plasma gonadotrophin concentrations. This could result from an increase in the sensitivity of the hypothalamic-pituitary axis to oestrogen, or from a reduced clearance rate of oestradiol. The increased plasma FSH concentrations in O ewes on L nutrition could reflect its increased secretion or decreased clearance.

The mechanism determining the high ovulation rate (OR) in Booroola ewes was studied by examining the OR after unilateral ovariectomy (ULO) or administration of exogenous oFF (1).

In Experiment 1, Booroola F/+ (n=18) and +/+ (n=18) ewes had a progestagen pessary inserted for 14 days with an injection of PMSG on the twelfth day. At pessary withdrawal all ewes underwent laparotomy and one ovary was removed from 9 ewes in each group. Ovulation rate was determined by laparoscopy 7 days later. Results analysed by 2-way ANOVA showed compensation of ovulation rate in both F/+ and +/+ Booroola ewes following ULO (Table 1).

Table 1. Plasma hormone concentrations in ewes on day 1 (D1) and differences after receiving maintenance (M) or sub-maintenance (L) diets for 93 days. Values are means (s.e.m.).

<table>
<thead>
<tr>
<th>Grp</th>
<th>LH Diff</th>
<th>FSH Diff</th>
<th>LH Pulse Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>OE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M  10</td>
<td>2.0(1)</td>
<td>3.0(1)</td>
<td>1.0(1)</td>
</tr>
<tr>
<td>L  15</td>
<td>4.0(1)</td>
<td>2.0(1)</td>
<td>5.0(1)</td>
</tr>
</tbody>
</table>

The oestrous response of Booroola Mesing ewes to unilateral ovariectomy and ovine follicular fluid administration

R.C. Frya, B.M. Bindonb, L.R. Piperb and L.P. Cahill4.

Animal Research Institute, Werribee, Vic. & C.S.I.R.O., Armidale, N.S.W.

We have demonstrated that both Booroola F/+ and +/+ ewes exhibit a similar decrease in OR in response to treatment with exogenous steroid-free oFF.

EFFECT OF GLUCOCORTICOIDS ON THE PREOVULATORY LH SURGE AND OVULATION RATE IN THE EWE.

D.J. Phillips*, R.C. Fry*, I.J. Clarke*, A.R. Egan* & L.P. Cahill#

#Animal Research Institute, Werribee; *Medical Research Centre, Prince Henry's Hospital, Melbourne; **School of Agriculture & Forestry, University of Melbourne, Parkville.

Short-term glucocorticoid treatments have been reported to inhibit the preovulatory LH surge and affect ovulation in pigs (1), cows (2) and mares (3), but not in ewes (4). We examined the long-term effects of dexamethasone (dex) in oestrous and anoestrous ewes.

In experiment 1, 22 anoestrous Corriedale ewes were given either 0.25 mg dex (Decadron, Intervet) or 1 ml saline (i.m.) daily, commencing at the insertion of the pessaries were removed and the ewes given an lm. injection of 400 IU PMSG (Folligon, Intervet). Ovulation rate (OR) was recorded by laparoscopy 7 days later. Another 22 anoestrous ewes received the same dex treatments as above. After 12 days they were given 100 mg i.m. of oestradiol benzoate in oil (Intervet) and jugular blood samples taken half-hourly for 24 hours to monitor plasma LH levels. Experiment 2 involved 90 oestrous Corriedale ewes given either saline, 0.25 mg or 2 mg dex daily in a similar regime to experiment 1, except the ewes received either 0 (n=15) or 1000 IU PMSG (n=15) at pessary removal. Vasectomized rams were used in experiment 2 from the time of pessary removal.

In experiment 1, no differences were found between treatments in the timing or magnitude of the oestradiol-induced LH surge, nor in the timing of the LH surge and affect ovulation in pre-pubertal gilts.

TABLE 1: OR and proportion of ewes ovulating for dex treatments.

<table>
<thead>
<tr>
<th>SEASON</th>
<th>DOSE PMSG (IU)</th>
<th>DOSE DEX</th>
<th>PROPORTION OVULATING</th>
<th>OR/EWE OVUL. (MEAN ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anoestrous (Expt. 1)</td>
<td>400</td>
<td>0.25 mg/day</td>
<td>11/11</td>
<td>1.6 ± 0.2 ± 0.3a</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0</td>
<td>11/11</td>
<td>1.6 ± 0.2 ± 0.3a</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>13/15</td>
<td>1.2 ± 0.1 ± 0.2b</td>
</tr>
<tr>
<td>Oestrus (Expt. 2)</td>
<td>0</td>
<td>0.25 mg/day</td>
<td>15/15</td>
<td>1.3 ± 0.1 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2 mg/day</td>
<td>15/15</td>
<td>1.0 ± 0.1 ± 0.2b</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0</td>
<td>15/15</td>
<td>3.3 ± 0.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.25 mg/day</td>
<td>15/15</td>
<td>1.8 ± 0.2 ± 0.2b</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>2 mg/day</td>
<td>15/15</td>
<td>2.2 ± 0.4 ± 0.3</td>
</tr>
</tbody>
</table>

These results indicate that long-term glucocorticoid treatments up to 2 mg/day have no significant effect on natural or induced ovulation in ewes.


In conclusion, this study indicated that elevations in plasma cortisol levels in gilts induced by physical contact with boars are unlikely to stimulate puberty attainment by enhancing pituitary responsiveness to GnRH because of an increase in direct stimulation of LH release.

ACUTE INTRACAROTID INFUSION OF CORTISOL REDUCES PITUITARY RESPONSIVENESS TO EXOGENOUS GnRH IN PREPUBERTAL GILTS.

G.P. Pearce*, A.M. Paterson** and P.E. Hughes***

* Animal Science Group, School of Agriculture, University of Western Australia, Nedlands, W.A. 6009. ** Animal Production Division, Dept. of Agriculture, South Perth, W.A. 6151. *** Muresk Institute of Agriculture, Northam, W.A. 6401.

Maximum efficacy of boar-induced precocious puberty attainment in the gilt requires physical contact with the boar (1), which has been shown to induce rapid elevations in plasma cortisol levels in recipient gilts (2). Acute elevations in plasma cortisol have been shown to enhance the release of luteinizing hormone (LH) in response to exogenous gonadotrophin releasing hormone (GnRH) in male pigs (3).

The present experiment was designed to investigate the influence of acutely elevated plasma levels of cortisol on the endogenous and GnRH-induced release of LH in pre-pubertal gilts. A total of 24 gilts were fitted with indwelling jugular vein and carotid artery catheters at 160 days of age and allocated to treatment in a 2 x 2 factorial design involving carotid infusion of cortisol (10mg in 40 mls saline) or saline alone with and without i.v. injection of synthetic GnRH (Gonadorelin, Intervet, Australia). Plasma samples were taken every 15 mins. from 14.00 to 18.00 and hourly thereafter until 22.00 and concentrations of LH and total corticosteroids were measured.

Analysis of variance revealed that the LH response to GnRH injection was reduced by cortisol infusion (P<0.05, Fig. 1). However, the secretion of LH was greater during infusion of cortisol alone than during infusion of saline alone (P<0.054). Plasma cortisol levels were elevated above baseline in cortisol infused gilts only (P<0.01). Thus acutely elevated plasma levels of cortisol reduced pituitary responsiveness to exogenous GnRH challenge, but marginally increased endogenous LH release in prepubertal gilts.

Figure 1.

In conclusion, this study indicated that elevations in plasma cortisol levels in gilts induced by physical contact with boars are unlikely to stimulate puberty attainment by enhancing pituitary responsiveness to GnRH but may be involved in direct stimulation of LH release.

The role of ovarian oxytocin in stimulating the uterine luteolytic factor, prostaglandin (PG) F2α is now well established in the ewe (1-3). However, there is little information on the responsiveness of the uterine PGF2α-secretory system to an oxytocin-stimulus over the time of luteal regression. The 10 ewes used in this experiment were randomly assigned to two groups of 5 ewes. On Days 15, 16, 17 of the oestrous cycle the ewes were given iv injections of either 0.25 or 1.0 IU oxytocin. Blood samples were collected into heparinized syringes at -10, -5, 0, 2.5, 5, 7.5, 10, 15, 30, 45, 60 min around the time of the oxytocin injection. Plasma samples were stored at -15°C and analysed for 13, 14-dihydro 15-keto prostaglandin (PG) F2α (PGFM) by a direct radio-immunoassay. The ewes were run with teaser rams to detect behavioural oestrus.

The intra and interassay coefficient of variation was 15% and 21% respectively and the limit of detection was 75 pg/ml. Haemolysed samples gave falsely high values and these were not included in the analysis. Two ewes showed an increase in plasma PGFM levels before oxytocin treatment; these values were not included in the analysis of data. In ewes given 0.25 IU oxytocin no detectable PGFM response was observed on the day of oestrus and only 2 out of 5 ewes showed a rise of PGFM levels on Day -1 before oestrus. Two days before oestrus 3 of the 5 ewes showed an increase in plasma PGFM concentrations with the levels rising to a mean maximum of 180 pg/ml at 15-45 min post injection. Similar results were obtained in ewes given 1 IU oxytocin. There was no oxytocin-induced PGFM response on the day of oestrus. On Day -1, the results were inconsistent with the PGFM concentrations increasing to a mean maximum of 128 ± 18 (SE) pg/ml in only 3 of 5 ewes. There was a marked rise 2 days before oestrus in all ewes with the levels rising to a maximum of 300 ± 32 pg/ml after the oxytocin injection. A small PGFM rise (100 pg/ml) was observed in 2 ewes on Day -3. These data when combined with previous results(3), suggest that the oxytocin-induced PGFM response rises to a maximum at 2 days before oestrus. Thereafter the response declines and on the day of oestrus there was no detectable increase in plasma PGFM concentrations to an oxytocin challenge.

References
Physiology and Pharmacology and Anatomy of Pregnancy

Steroidogenesis in the corpus luteum (CL) is mediated by receptors, the CL of ewes during pregnancy and during the oestrous cycle were examined for oxytocin receptors.

Corpora lutea were obtained from an abattoir and were used on the same day for oxytocin radioreceptor assays according to a published procedure (2). Luteal tissue was incubated with $^{3}H$-oxytocin at 20°C and the bound oxytocin was separated from the unbound by PEG precipitation. Specific oxytocin binding to luteal tissue taken from ewes during the oestrous cycle was not observed. However, in pregnant ewes oxytocin receptors were found in the CL, appearing at a foetal head length of approximately 0.6 cm (30-40 days of pregnancy) and persisting up to a head size of 10 cm, the largest examined so far. The affinity constant $K_a$ of the CL receptor is 1-2 x $10^{-9}$ M, a value similar to that for the uterus, but the receptor number ($R_t$; see Table) is much lower than our estimate of $1771 \pm 174$ fmol/mg protein (SD, n=4) for the uterus. Our results suggest that a direct oxytocin action on the ovine CL may be possible during pregnancy but not during the oestrous cycle. This difference may explain the conflicting published results.

EVIDENCE FOR DIRECT PHOTOPERIODIC DRIVE IN THE EWE

S.A. Cutler,1 G. Evans1, R.J. Scaramuzzii2 & J.A. Downing2

1 Department of Animal Husbandry, University of Sydney, NSW 2006
2 CSIRO Division of Animal Production, Blacktown, NSW 2148

Several studies have shown that a steroid dependent mechanism, under photoperiodic control, is involved in the inhibition of luteinising hormone (LH) secretion during anoestrus. This study investigated the possibility of a direct effect, independent of gonadal steroid secretion, of photoperiod on the gonadotrophin releasing hormone (GnRH) pulse generator which is responsible for LH release.

Six Merino crossbred ewes were ovariectomised and housed in a controlled environment room. Three ewes had silastic implants containing oestradiol-17β inserted subcutaneously (+E), while the other three had empty implants inserted (-E). They were subjected to alternating 16-week periods of long days (16L:8D) (LD) and short days (8L:16D) (SD) for 84 weeks. Serial venous blood samples were collected every 8 weeks (+E) and the plasma was assayed for LH. The LH secretion profiles were analysed for pulse frequency, amplitude and basal levels. These parameters are recorded in Table 1.

Table 1. LH pulse frequency (FRQ), amplitude (AMP) and basal levels (BAS) measured in plasma of ewes at the 8 sampling times ($*$SEM, n=3)

<table>
<thead>
<tr>
<th>Daylength</th>
<th>FRQ</th>
<th>AMP</th>
<th>BAS</th>
<th>FRQ</th>
<th>AMP</th>
<th>BAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-E</td>
<td></td>
<td></td>
<td>+E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. SD</td>
<td>8.6±1.8</td>
<td>0.9±0.2</td>
<td>1.2±0.3</td>
<td>7.0±0.5</td>
<td>0.8±0.3</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>2. SD</td>
<td>9.0±1.0</td>
<td>0.6±0.1</td>
<td>1.5±0.5</td>
<td>5.7±0.5</td>
<td>0.7±0.4</td>
<td>0.7±0.5</td>
</tr>
<tr>
<td>3. LD</td>
<td>4.0±1.5</td>
<td>0.6±0.1</td>
<td>2.5±0.5</td>
<td>2.9±0.6</td>
<td>1.5±0.4</td>
<td>0.2±0.2</td>
</tr>
<tr>
<td>4. LD</td>
<td>7.3±1.2</td>
<td>0.8±0.1</td>
<td>2.8±0.6</td>
<td>1.7±0.9</td>
<td>2.4±0.4</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td>5. SD</td>
<td>7.9±2.2</td>
<td>0.7±0.2</td>
<td>1.9±0.6</td>
<td>9.3±0.7</td>
<td>1.2±0.2</td>
<td>2.2±0.5</td>
</tr>
<tr>
<td>6. SD</td>
<td>5.0±0.5</td>
<td>0.6±0.1</td>
<td>2.2±0.3</td>
<td>8.3±1.3</td>
<td>0.6±0.1</td>
<td>1.9±0.6</td>
</tr>
<tr>
<td>7. LD</td>
<td>4.3±1.3</td>
<td>0.8±0.1</td>
<td>3.3±0.8</td>
<td>3.0±1.7</td>
<td>2.9±0.3</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>8. LD</td>
<td>3.7±1.4</td>
<td>0.7±0.2</td>
<td>2.7±0.8</td>
<td>2.6±0.6</td>
<td>2.5±0.4</td>
<td>0.2±0.1</td>
</tr>
</tbody>
</table>

All +E ewes displayed a marked seasonal variation between the SD and LD periods in LH pulse FRQ, AMP and BAS (P<0.05). The -E ewes showed a decrease in pulse frequency between sampling 2 and 3 (P<0.05) and a tendency towards relatively high frequency in SD periods and low frequency in LD periods. The BAS of the -E ewes varied between LD and SD periods (P<0.05), though the pattern was dissimilar to that of animals with oestrogen implants. LH pulse AMP did not vary in -E ewes.

Although based on a small number of animals, this study provides further evidence (2) that photoperiod has a direct effect on the GnRH pulse generator. However, the changing pattern of LH secretion is less extreme in the absence of steroid negative feedback. The synergistic actions of direct photoperiodic drive and steroid negative feedback on the GnRH pulse generator may precipitate seasonal breeding patterns.

STERIOD SECRETION RATES IN ANDROSTENEDIONE-IMMUNE EWE S WITH AN AUTOTRANSPLANTED OVARY

B.K. Campbell,¹ R.J. Scaramuzzi ² and J.A. Downing ²

¹Department of Animal Husbandry, University of Sydney, Sydney, NSW; ²CSIRO, Division of Animal Production, Prospect, NSW.

Although immunization against androstenedione (A4) increases ovulation rate, the precise pattern of ovarian steroid secretion is unclear. An experiment to measure the temporal pattern of steroid secretion was carried out in conscious ewes in which the left ovary and its vascular pedicle had been autotransplanted to the neck.

Aged Merino ewes with transplants were divided into control (C; n=5) and immunized groups (IM; n=6). Treated ewes were immunized (2ml s.c.) with Fecundin, 1 and 4 weeks before the start of blood sampling. Ovarian and jugular venous blood was collected every 10 min at two stages of the follicular phase (21-27h and 38-42h after 125 μg of prostaglandin 'Estrumate'; PG) and during the mid-luteal phase (8h at 15 min intervals). The ewes were monitored regularly for luteal function and pre-ovulatory LH surges. Hormone levels and anti-A4 titres were assayed by RIA and ovarian secretion rates of oestradiol-17β (E2), progesterone (P4) and A4 determined.

Following the booster injection, P4 increased simultaneously with titre in IM ewes, reaching 30 ng/ml at the time of PG injection when mean titre was 1:10,000. All ewes responded to PG with LH surges 42 to 72 h later. Two IM ewes had a second LH surge within 3-4 days at a time when peripheral P4 levels were 2-3 ng/ml. The frequency of steroid and LH pulses was greater in IM ewes but this difference was significant only during the luteal phase. The secretion rate of A4 was 6-10 times greater (19-37 ng/min) in IM ewes at all sampling stages. Progesterone secretion rates were 3 times greater (16 ng/min) during the luteal phase in IM ewes. The amplitude of E2 pulses was significantly reduced in IM ewes (4.8 vs 2.1 ng/min at +24h and 6.5 vs 2.8 ng/min at +40h for C and IM respectively) during the follicular but not the luteal phase. In IM ewes the overall secretion rate of E2 was lower (3.5 vs 2.5; +24h and 5.0 vs 3.2 ng/min; +40h for C and IM respectively) during the follicular phase and higher (0.2 vs 0.4 ng/min for C and IM respectively) during the luteal phase but these differences were not significant. Analysis of bound and free steroid using polyethylene glycol showed that all peripheral and ovarian venous A4 and 86% of peripheral P4 was bound in IM ewes compared to no binding (<0.1%) in C ewes. Similarly, 50% of ovarian venous E2 was bound in IM ewes compared to 15% in C ewes.

We conclude that immunization against A4 increases the secretion rate of A4 and P4 but not of E2. The enhanced plasma binding of P4 and E2 in A4-immune ewes may interfere with the biological action of these steroids. The importance of these changes to the mechanism of action of Fecundin remains to be established.
INHIBIN SECRETION BY THE SHEEP OVARY DURING THE LUTEAL AND FOLLICULAR PHASES OF THE OESTROUS CYCLE AND FOLLOWING STIMULATION WITH FSH

C. G. Tzonis, D. T. Baird and A. S. McNeilly

Department of Obstetrics & Gynaecology and *MRC Reproductive Biology Unit, Centre for Reproductive Biology, University of Edinburgh, 37 Chalmers Street, Edinburgh, Scotland, EH3 9EW, UK.

Inhibin from various species has recently been isolated, sequenced and cloned by several groups (1). In the female, although inhibin is synthesized by the granulosa cells of the ovarian follicle and accumulates in the follicular fluid, little is known about the secretion rate of inhibin from the ovary in vivo or the factor (s) regulating its secretion into the peripheral blood.

We have investigated this question in the sheep by using an extremely sensitive bioassay for inhibin, utilizing sheep pituitary cells in culture, which is able to measure inhibin in sheep plasma (2). Four Finnish Landrace x Merino ewes which had the left ovary autotransplanted to the neck were used in this study. On day 12 of the cycle premature luteal regression was induced with an injection of 100 µg cloprostenol (PG) and ovarian vein blood collected 4-hourly for 72h. These same 4 ewes were used in the ensuing cycle, this time infusing HIN-OFSH-S14 at 10 µg/h for 48h immediately after an injection of PG and sampled as above. Three blood samples were collected before PG in both cycles.

The results show that in the control and FSH-infused cycles the inhibin secretion rate (SR) during the luteal phase (~2h before PG) was 27-63 U/min. After PG injection, in the control cycle the inhibin SR declined rapidly to reach 3-5 U/min at 48h after the injection of PG. In contrast, in the FSH-infused cycle after PG injection, the inhibin SR increased slightly and remained elevated at 42-50 U/min at 60h. In the late follicular phase the oestradiol (E2) SR was greater in the FSH-infused than the control cycles indicating more follicular growth and maturation.

These data demonstrate that, 1) the high inhibin SR observed during the luteal phase suggests that the sheep corpus luteum secretes inhibin, 2) in the control cycle, the SR of inhibin declines in parallel to the fall in FSH concentration 3) prevention of the follicular phase drop in FSH concentration by infusion of exogenous FSH, stimulates secretion of inhibin from the ovary. We conclude, therefore that the fall in FSH concentration during the late follicular phase is due to rising level of oestradiol and results in reduced inhibin secretion.


Supported by a grant from the Medical Res. Council (UK), G 426375.
SYNERGISM BETWEEN OESTRADIOL AND INHIBIN IN THE CONTROL OF FSH SECRETION IN THE EWE.

G.B. Martin*, C.A. Price** & R. Webb**

* MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, U.K. ** AFRC Institute of Animal Physiology & Genetics Research, Edinburgh Research Station, Roslin, Midlothian EH25 9PS, U.K. ** Present address: Animal Science Group, School of Agriculture, University of W.A., Nedlands, WA 6009.

Recent work has shown that inhibin and oestradiol (E2) are the major components of the ovarian feedback loop controlling FSH secretion in the ewe (1,2). However, these studies did not assess the relative importance of the two hormones or whether their effects were interactive or additive. We therefore studied the dose-responses to inhibin and E2 and tested the hypothesis that these hormones act synergistically to inhibit the secretion of FSH.

We used 48 Welsh Mountain ewes in a factorial design with 3 levels of E2 (0, 1 or 2 cm long) and 4 levels of steroid-free bovine follicular fluid (bFF: 0, 0.4, 0.8 or 1.6 ml sc every 8 h) containing 4.5 kU inhibin per ml (3). Treatments were begun on the day of ovariectomy and, 1 week later, blood plasma was sampled every 3 h for 27 h and assayed for FSH and E2 (1,2).

In ewes treated with 0, 1 and 2 cm implants, the concentrations of E2 were 2.0±0.9 (mean±sem), 3.3±0.2 and 4.9±0.3 pg/ml, respectively. Analysis of variance revealed a synergistic interaction between E2 and inhibin on mean plasma FSH concentrations (P<0.01). This was most evident in the effect of the 2 cm implant on the slope of the inhibin dose-response curve (Fig. 1). Plasma FSH concentrations typical of intact ewes were restored by combinations of the lowest doses of E2 and bFF. The high dose of E2 was insufficient to control FSH secretion in the absence of bFF, but the highest dose of bFF reduced FSH concentrations to undetectable levels independently of E2.

We conclude that FSH secretion in the ewe is primarily controlled by the synergistic action of oestradiol and inhibin.

Figure 1. Effect of oestradiol and bovine follicular fluid (bFF) on plasma concentrations of FSH in ovariectomized ewes. Each point is the mean from 4 ewes and the SED is 21.8 ng/ml. The level for intact ewes is the mean (±sd) of all 48 ewes before ovariectomy.

SYNERGISM BETWEEN OESTRADIOL AND INHIBIN IN THE CONTROL OF FSH SECRETION IN THE EWE.

G.B. Martin*, C.A. Price** & R. Webb**

* MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, U.K. ** AFRC Institute of Animal Physiology & Genetics Research, Edinburgh Research Station, Roslin, Midlothian EH25 9PS, U.K. ** Present address: Animal Science Group, School of Agriculture, University of W.A., Nedlands, WA 6009.

Recent work has shown that inhibin and oestradiol (E2) are the major components of the ovarian feedback loop controlling FSH secretion in the ewe (1,2). However, these studies did not assess the relative importance of the two hormones or whether their effects were interactive or additive. We therefore studied the dose-responses to inhibin and E2 and tested the hypothesis that these hormones act synergistically to inhibit the secretion of FSH.

We used 48 Welsh Mountain ewes in a factorial design with 3 levels of E2 (0, 1 or 2 cm long) and 4 levels of steroid-free bovine follicular fluid (bFF: 0, 0.4, 0.8 or 1.6 ml sc every 8 h) containing 4.5 kU inhibin per ml (3). Treatments were begun on the day of ovariectomy and, 1 week later, blood plasma was sampled every 3 h for 27 h and assayed for FSH and E2 (1,2).

In ewes treated with 0, 1 and 2 cm implants, the concentrations of E2 were 2.0±0.9 (mean±sem), 3.3±0.2 and 4.9±0.3 pg/ml, respectively. Analysis of variance revealed a synergistic interaction between E2 and inhibin on mean plasma FSH concentrations (P<0.01). This was most evident in the effect of the 2 cm implant on the slope of the inhibin dose-response curve (Fig. 1). Plasma FSH concentrations typical of intact ewes were restored by combinations of the lowest doses of E2 and bFF. The high dose of E2 was insufficient to control FSH secretion in the absence of bFF, but the highest dose of bFF reduced FSH concentrations to undetectable levels independently of E2.

We conclude that FSH secretion in the ewe is primarily controlled by the synergistic action of oestradiol and inhibin.


AUSTRALIAN SOCIETY FOR REPRODUCTIVE BIOLOGY

Minutes of 18th General Meeting

Held at 5:39 pm, Tuesday September 2, University of Queensland.

18.1 Attendance and apologies Approximately 100 persons attended. Apologies were received from T. Robinson, D. Lindsay, J. Yovich, J. Findlay, P. Quinn and B. Stone.

18.2 Minutes of 17th AGM. These were circulated in the book of Abstracts. They were accepted as a true record (proposed L. Martin, seconded G. Stone).

18.3 Matters arising There were no matters arising other than those covered by agenda items.

18.4 Treasurer's Report A financial statement was circulated and was printed in the last Newsletter.

The Treasurer noted that the major expenditure increases over 1984/85 were in increased printing costs and in student travel subsidies. He noted that a number of members were unfinancial as of August 31st, and proposed that these should receive the next newsletter and then get removed from the mailing list, as they represented a drain to the Society in terms of capitation fees to the secretariat.

After pointing out that early payment of fees had not proved a meaningful incentive and that the Committee had earlier resolved to eliminate the rebate for such payment, the Treasurer moved (seconded B. Miller) that the Annual Ordinary Subscription be set at $15.00. In discussion to this motion the Treasurer indicated that the proposed fee would adequately cover capitation fees to F.A.S.T.S. (see 18.12), and the motion was carried. The Treasurer also moved (seconded B. Setchell) that the cost of supplying the 1986 book of abstracts to non-members be set at $15.00: this was carried without discussion.

18.5 Chairman's Report A Copy of the report was circulated in the last Newsletter. He noted that there was increasing diversity of work being reported at the Annual Conference, and that this reflected the increasing maturity of the Society. The Chairman noted with regret the death of John Elkington shortly before the meeting: John had been active on the Local Organising Committee.

18.6 Secretary's Report A final report on the Boden Conference held in early 1986 had been received with thanks. Potential topics for 1988 were sought. A list of unfinancial members and also of those who had moved without informing the Society that they had changed their address was to be placed on view for the rest of the meeting. The Secretary also called for nominations for the Goding Lecturer of 1987.

18.7 New Members A list of proposed new members had been circulated with the book of abstracts. In addition, four prospective members who had been accidentally omitted from the list through no fault of their own were read out to the members present. There were no objections, and the nominees were accepted as Ordinary Members of the Society (proposed J. Cummins, seconded B. Miller).

18.8 Programme Organising Committee Report This was read by L. Purvis on behalf of the Chairman, D. Lindsay. A total of 134 abstracts had been received, of which nearly a third were late. No papers were rejected: 36 were selected for oral presentation with priority given to candidates for the Junior Scientific Award, leaving only 5 slots for others. The new format for a covering page for abstracts worked better than in previous years and allowed for rapid transfer to computer files. The report was accepted with thanks to the Committee (proposed L. Martin, seconded I. Clarke).

153
Organising Committee.

The Chairman reported on correspondence from the Gene Transfer Symposium (144). Registrants for ASRB were 176. The Chairman wished to place on record the warm welcome given by the Organising Committee. Jan Commins (ASRB - Chairman); Peter Chenoweth (ASRB); John Elkington (ASRB); Brian Hirschfield (ADS); Sally Jubb (ASRB); Len Martin (ASRB/EFA); Mike Waters (EFA); Margaret Williamson (EFA) and A.C.T.S. as secretariat.

18.10 Chairman; Peter Chenoweth spoke on preparations for the meeting. The postgraduate student AGM is to be timetabled. The AGM is to be brought forward to 4.30 pm if possible.

Motions. Further discussion expressed some sympathy for the position of post-doctoral fellows as well as post-graduate students. It was noted that poor presentations cost the meeting time and money. It was agreed that the Secretary ask the POC to enforce instructions on the format of presentations (In Instructions to Authors). It was felt that a 2–3 minute summary by the presenting author should be followed by a discussion period allocated at the discretion of the chairman.

GE had been asked by members for more rigid refereeing of Abstracts, and pointed out that poor presentations cost the meeting time and money, and that there may be space restrictions for posters at some meetings. It was agreed that the Secretary ask the POC to enforce the refereeing guidelines and to consider the quality and correct format of Abstracts.

The postgraduate student AGM is to be timetabled.

4.13 POC Report.

Gareth Evans tabled a letter he had written to David Lindsay, outlining complaints and criticisms received at the Brisbane meeting. No report had been received from the POC since the Brisbane meeting, but GE and DL had discussed the matter by telephone.

The major criticism seemed to be the grouping to topics. DL said that it was difficult to please everyone, given that there were time and space restrictions at all meetings. However, in discussion, it was decided to ask the POC to suggest a first and seconds choice topic title, so that they (the authors) could have the opportunity to indicate what they perceived to be the major subject matter of their work. In future years it may be possible to set topics for sessions more rigidly.

DL had indicated that the requirement to give students priority for oral presentations had restricted the POC's options. GE said he would point out that the intention was that students be given priority only if they had not presented an oral earlier at the previous two meetings. This would allow them the opportunity to present an oral at least once during their studies.

It was agreed that poster session chairmen and presenters should be given instructions on the format of presentations (Instructions to Authors). It was felt that a 2–3 minute summary by the presenting author should be followed by a discussion period allocated at the discretion of the chairman.

GE had been asked by members for more rigid refereeing of Abstracts, and pointed out that poor presentations cost the meeting time and money, and that there may be space restrictions for posters at some meetings. It was agreed that the Secretary ask the POC to enforce the refereeing guidelines and to consider the quality and correct format of Abstracts.

The postgraduate student AGM is to be timetabled.

The AGM is to be brought forward to 4.30 pm if possible.

John Rodger is to be volunteered to chair a sub-committee to award the Junior Scientist prize. It was agreed that $250 would be awarded to the Junior Scientist in 1987 (proposed CN, seconded IC).

The appointment of the next POC was deferred to the next meeting.
Minutes of the Annual Student Meeting held at 1.00 pm Wednesday 3rd September 1986

Attendance - approximately 30 students

1. All students found the cost of the Annual Dinner prohibitive and 90% of the students at the meeting did not attend.

2. The poster sessions were very successful in terms of time allocation and presentation and similar arrangements would be preferred at future conferences.

3. Concern was raised re the timing of the Annual General Meeting each year, i.e. just prior to the Annual Dinner. Those students who did attend the dinner found there to be no time to get changed etc between the end of the meeting and the departure of buses for the dinner. It was proposed that perhaps an alternative time could be arranged for the Annual Meeting, e.g. one lunch-time.

4. The student social evening was thought to be a success and it would be ideal if it is incorporated as an event as part of the ASRB Annual Conference.

5. Concern was raised re the time of the post conference excursions. They should be organised after the end of the conference, and not coincident with the last ASRB session.

6. It was proposed that a programme for the ASRB and ESA meetings should be enclosed in satchels (or displayed on a board) to remove the necessity of buying conference proceedings, e.g. where students are not financial members of ESA.

7. It was strongly suggested to have the date and time of the Postgraduate Annual Meeting affixed and circulated to all the students. It was suggested that Tuesday lunch time is a suitable time for the event.

8. Early registration was found to be more appropriate and preferable than at the opening ceremony.

Chairmen

1969-1973  T.J. Robinson  
1973-1977  C.W. Emmens  
1977-1981  D.M. de Kretser  
1981-1983  N.W. Moore  
1983-1985  J.K. Findlay  
1985-1987  B.M. Bindon

Life Members

C.W. Emmens  
T.J. Robinson

Junior Scientist Award

1981  P.J. Lutjen  
1982  R.J. Rodger & C.B. Gow  
1983  S.P. Flaherty  
1984  C. O'Neill  
1985  B.J. Waddell  
1986  L.J. Wilton