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August 1993

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THE AUSTRALIAN SOCIETY FOR REPRODUCTIVE BIOLOGY INC.

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Price of the Proceedings to Non-members : $25 plus postage.
Details of the scientific programme for the associated meetings of the Endocrine Society of Australia, and the New Zealand Society of Endocrinology can be found in the Combined Societies Conference Handbook available at Registration.

This also contains information on programmes of other Societies meeting in Dunedin this week.
MONDAY AUGUST 23

Session 1 (Oral - concurrent): GAMETOGENESIS AND GONADAL CELL INTERACTIONS

Chairman: Dr. Gail Risbridger
Time: 0845 - 1000  Venue: Archway 3 Lecture Theatre
1. Cummins, J.M., Meloni, B.P. and Jequier, A.M.
   Human sperm and testis mitochondrial DNA
2. Swan, M.A. and Gill, J.
   Electron microscopy of avian spermatogenesis and diagnosis of male infertility: use of laparoscopic biopsy
3. Hayes, T.M., de Kretser, D.M. and McFarlane, J.R.
   Microtubule associated proteins in the rat testis
4. Kim, Y.H., Temple Smith, P.D., de Kretser, D.M., Stanton, P.G. and McFarlane, J.R.
   Isolation and partial characterisation of rat sperm tail fibrous sheath proteins
5. Jolly, P.D., Tisdall, D.J., Heath, D.A., Lun, S. and McNatty, K.P.
   Evidence of apoptosis in bovine granulosa cells
   Indomethacin blocks the immunosuppressive activity of rat testicular macrophages in-vitro

Session 2 (Oral - concurrent): ENDOCRINE, NUTRITIONAL AND LACTATIONAL INTERACTIONS

Chairman: Dr. Michael D’Occhio
Time: 0845 - 1000  Venue: Archway 4 Lecture Theatre
   Nutritional effects of luteinising hormone (LH) secretion in rams after exposure to oestrous ewes
8. Walkden-Brown, S.W., Boukhliq, R., Fisher, J.S. and Martin, G.B.
   Does the nutrition of the ram influence its behavioural and endocrine response to oestrous ewes?

MONDAY AUGUST 23 (cont.)

   Dietary fatty acids stimulate gonadotrophin secretion in the ram
10. Smith, J.E., Cruickshank, G.J., Parr, J. and Konlechner, J.A.
    Effect of dietary protein intake on the changes in levels of FSH produced by injections of follicular fluid in ovariotomised ewes
    Effects of ovariectomy and/or weaning on plasma LH in postpartum Bos indicus cows
12. Curlewis, J.D., MacGregor, D., Gale, J., Loudon, A.S.I. and Brinklow, B.R.
    Effect of phase two lactation on prolactin in marsupials

Session 3: STATE OF THE ART LECTURE

Dr. John M. Hutson

Hormonal control of testicular descent and the cause of cryptorchidism
Chairman: Assoc. Prof. David Handelsman
Time: 1030 - 1100  Venue: Archway 4 Lecture Theatre

Session 4 (Oral - concurrent): SPERM MOTILITY AND IN-VITRO FERTILIZING ABILITY

Chairman: Dr. John Smith
Time: 1100 - 1200  Venue: Archway 4 Lecture Theatre
    Isolation and characterisation of anionic proteins and peptides from bovine seminal plasma and their effects on sperm motility
    Assessing the changes in translational and rotational velocities of bull sperm under aerobic and anaerobic conditions after cold shock using the TTLV laser machine
15. Upseti, G.C., Brown, K., Oliver, J.E. and Smith, J.F.
    Modification of ram semen diluent (RSD-1): organic nutrient(s) requirement for ram spermatozoal motility
16. Stojanov, T., Maxwell, W.M.C., Rhodes, S.L. and Evans, G.
    Insemination of in vitro matured sheep oocytes with low numbers of fresh sperm

Session 5 (Poster - concurrent): IMPLANTATION AND PLACENTATION

Chairman: Dr. Brendan Waddell
Time: 1100 - 1200  Venue: Gazebo Lounge, Student Union
17. Walton, C.E., Chen, Q. and Nancarrow, C.D.
    DNA amplification of an oestrogen-associated oviducal glycoprotein sequence from primates
MONDAY AUGUST 23 (cont.)

Is uterine function compromised in heifers synchronized with a long-duration progesterone treatment?

19 Sakoff, J.A., Murdoch, R.N. and Dunstan, R.H.
Changes in the polyunsaturated fatty acid composition of the uterus during induction of the decidua cell reaction in pseudopregnant mice

20 Herrmann, B., O'Neil, C. and Batyye, K.M.
The activity of PAF-acetylhydrolase within the uterus during the peri-ovulatory and preimplantation phases

21 Lee, R.S.F., Tervit, H.R. and Peterson, A.J.
Pregnancy-specific proteins in the uterine fluid of sheep around the time of implantation

22 Lee, C.S., Gogolin-Ewens, K. and Brandon, M.R.
Localisation of a pregnancy-associated molecule (SBU-59) in sheep

23 O'Leary, P.C., Sunley, A.K. and Waddell, B.J.
Endocrine interaction of the human trophoblast and decidua: effects of decidual secretions on trophoblast steroidogenesis

24 Regnault, T.H., Scaramuzzi, R.J., Sriskandarajah, N. and Oddy, V.H.
Depression of insulin response to a glucose load in pregnant ewes with high circulating placental lactogen levels

Session 6:

GODING LECTURE
Professor G.C. Liggins
Cortisol: a fetal hormone for all seasons

Chairman: Prof. Rex Scaramuzzi
Time: 1200 - 1300
Venue: Castle 2 Lecture Theatre

Session 7 (Oral):

SERONO-ASRB JUNIOR SCIENTIST AWARD
Chairman: Prof. Brian Satchell
Time: 1400 - 1600
Venue: Archway 4 Lecture Theatre

25 Godfrey, S.L., Walkden-Brown, S.W., Martin, G.B., Gherardi, S.G., Lindsey, M.J. and Porter, B.L.
Immuno-castration of adult cashmere bucks with Vaxstrate

26 Markey, C.M., Jequier, A.M. and Meyer, G.T.
Morphological damage to the ram testis and epididymis following ischaemia: possible implications in male infertility

27 Barker-Gibb, M.L. and Clarke, I.J.
Chronic oestradiol treatment of ovariectomized ewes in the non-breeding season does not affect the density of NPY terminals in the hypothalamus

MONDAY AUGUST 23 (cont.)

28 Bonello, N., Norman, R. and Brännström, M.
Cytokines and plasminogen activator activity in the rat ovary at ovulation

29 Hill, J.L., Walker, S.K. and Nancarrow, C.D.
The role of an oestrus-associated oviducal glycoprotein in the early development of the sheep embryo

30 Spindler, R., Renfree, M.B. and Gardner, D.K.
Nutrient uptake during embryonic diapause and reactivation in Tammar wallaby blastocysts

31 Tatham, B.G., Dowsing, A.T. and Trounson, A.O.
Enucleation by centrifugation for bovine nuclear transplantation

32 Lane, M. and Gardner, D.K.
In situ removal of embryo-toxic ammonium generated by the metabolism and breakdown of amino acids in culture media

Session 8: (Oral - concurrent):

NUTRITION AND FERTILITY
Chairman: Dr. Graeme Martin
Time: 1630 - 1800
Venue: Archway 3 Lecture Theatre

33 Walkden-Brown, S.W., Restall, B.J., Norton, B.W., Scaramuzzi, R.J. and Martin, G.B.
Nutrition affects testicular size in male goats without changing LH, FSH or testosterone concentrations

34 Brown, B.W.
Gonadotrophin and testosterone concentrations and testicular growth in rams supplemented with lupins from birth to puberty

35 Rhodes, F.M., Fitzpatrick, L.A., Kinder, J.E. and Entwistle, K.E.
Changes in ovarian morphology associated with dietary intake in Bos indicus heifers

36 Smith, J.F., Parr, I. and Konlechner, J.A.
Effect of level of dietary protein intake on the plasma levels of progesterone in ovariectomised ewes treated with CIDR® devices

37 Wright, P.J. and Clarke, I.J.
The frequency of release of LH in anoestrous Corriedale ewes is influenced by nutrient status but not by post-partum status

Session 9 (Oral - concurrent):

ENDOCRINE REGULATION OF REPRODUCTION IN THE MALE
Chairman: Dr. Simon Maddocks
Time: 1630 - 1800
Venue: Archway 4 Lecture Theatre

38 O'Connor, A.E., de Kretser, D.M. and Wreford, N.G.
Human recombinant inhibin partially reverses the hemo-castration induced rise in serum FSH in the rat
TUESDAY AUGUST 24 (cont.)

39 Tilbrook, A.J., de Kreuser, D.M. and Clarke, I.J.
Effect of stage of breeding season on the pituitary actions of inhibin and testosterone to control
FSH secretion in rams

40 D'Occchio, M.J., Garozzo, S.M., O'Connor, A. and Wreford, N.G.
Tests of morphology and sperm production in bulls receiving the gonadotrophin releasing hormone
agonist Decaprist

41 Auclair, D., Sowerbutts, S.F. and Setchell, B.P.
Active immunisation against oestradiol-17β in developing ram lambs can have marked effects on
the structures and functions of the testes

42 Jongman, E.C., Hemsworth, P.H. and Galloway, D.B.
The effect of sexual stimulation on testosterone in boars in two different mating systems

TUESDAY AUGUST 24

Session 10 (Oral - concurrent) : OVARIAN STEROIDOGENESIS
Chairman: Dr. Robert Norman
Time: 0800 - 0900
Venue: Archway 3 Lecture Theatre

43 Downing, J.A., Scaramuzzi, R.J. and Joss, J.
Steroid secretion in ewes following an ovarian arterial infusion of sheep growth hormone

44 Rabiiee, A.R., Gooden, J.M., Miller, B.G. and Lean, I.J.;
Evaluation of transovarian uptake of metabolites and progesterone secretion

45 Lopes, T. and Waddell, B.J.
Influence of prolactin and LH on ovarian progestin secretion during late pregnancy in the rat

46 Bruce, N.W.
Corpora lutea of previous cycles are a source of progestins in the rat

Session 11 (Oral - concurrent) : CRYOPRESERVATION AND
OOOCYTE DEVELOPMENT
Chairman: Dr. Leanda Wilton
Time: 0800 - 0900
Venue: Archway 4 Lecture Theatre

47 Payne, S.R., Oliver, J.E. and Upreti, G.C.
The role of antifreeze proteins in the freezing of ram spermatozoa

48 Valcic, M.C., Lacham-Kaplan, O. and Trounson, A.O.
Pronuclei formation and embryo cleavage following electrofusion of round spermatids with
oocytes from the mouse

Session 12
ASRB SPECIAL LECTURE
Dr Paul Wasserman
Regulation of mammalian fertilization by gamete receptors and binding proteins
Chairman: Dr. Jim Cummins
Time: 0900 - 1000
Venue: Castle 2 Lecture Theatre

Session 13 (Oral - concurrent) : ASRB/ESA COMBINED SESSION :
INHIBIN, ACTIVIN AND FOLLISTATIN
Chairman: Assoc. Prof. Jock Findlay (ASRB) and Dr. John France (ESA)
Time: 1030 - 1200
Venue: Archway 1 Lecture Theatre

ESA Keelan, J., Song, L. and France, J.T.
An immunoblotting study of inhibin and activin production by the human placenta in vivo and in vitro

51 de Kreuser, D.M., Foulds, L.M., Hancock, M., Miller, S., McFarlane, J., Goss, N. and Jenkin, G.
Isolation of activin A from ovine amniotic fluid and persistence of inhibin in amniotic fluid after
foetal orchidectomy

ESA Cameron, V.A., Sawchenko, P.E. and Vale, W.W.
Effects of steroids on activin receptor expression in rat brain and pituitary

52 Farnworth, P.G., Robertson, D.M. and Schwartz, J.
Activin A regulates production of follistatin (FS) by ovine anterior pituitary cells in vitro

53 Kleemann, D.O., Grosser, T.L., Bindon, B.M., Russell, D. and Findlay, J.K.
Effect of the FECO gene and age on plasma inhibin concentrations in merino rams

ESA Flemming, J.S., Galloway, S.M., Greenwood, P.J., Penty, J.M., Tisdall, D.J. and
Montgomery, G.W.
Genetic and structural analysis of the βA - inhibin gene in sheep carrying the Booroola FECO
mutation

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TUESDAY AUGUST 24 (cont.)

Session 14 : (Oral • concurrent) : FOLLICULOGENESIS AND OVULATION
Chairman: Dr. Ken McNatty
Time: 1030 - 1200
Venue: Archway 2 Lecture Theatre

54 Downing, J.A., Scaramuzzi, R.J. and Joss, J.
The direct effect of insulin on ovarian steroid secretion in ewes with an autotransplanted ovary

Ovarian follicular insulin-like growth factor binding proteins and follicular status in dairy cattle

56 Roberts, R.D.
Insulin-like growth factor binding proteins in the ovary of the domestic hen (Gallus domesticus)

57 O'Shea, T.O., Baxter, G. and Webb, R.
Factors in follicular fluid that delay or hasten oestrus

58 Brannstrom, M., Seamark, R., Robertson, S. and Norman, R.
The rat ovary produces cytokines during ovulation

59 Sernia, C., Bathgate, R. and Gemmell, R.T.
Methionin and arginine vasopressin in the corpus luteum of the brush-tail possum (Trichosurus vulpecula)

Session 15 (Miniposter • concurrent) : GAMETES AND EMBRYOS IN CULTURE
Chairman: Dr. Jeremy Thompson
Time: 1030 - 1200
Venue: Archway 4 Lecture Theatre

Laparoscopic recovery of ovarian oocytes from slaughtered or living sheep

61 Fry, R.C., Simpson, T.L., Squires, T.J., Miles, M.A. and Niall, E.
Oocyte recovery is influenced by needle type and aspiration pressure

62 Shannon, P. and Vishwanath, R.
The effect of optimal and sub optimal concentrations of sperm on the fertility of fresh and frozen bovine semen

63 Sánchez-Partida, L.G., Zupp, J.L., Maxwell, W.M.C. and Setchell, B.P.
Effect of levels of egg yolk and compatible solutes on the post-thaw motility of ram spermatozoa

64 Sánchez-Partida, L.G., Sowerbutts, S.F., Maxwell, W.M.C. and Setchell, B.P.
Effect of levels of taurine and levels of glycerol on the post-thaw motility of ram spermatozoa

65 Marshall, J.T.A. and Nancarrow, C.D.
Changes in saccharide-binding sites on the surface of ram spermatozoa following freeze-thawing

66 McGowan, L.T., Wells, R.W., Pugh, P.A., Bell, A.C.S. and Tervit, H.R.
Culture conditions affect the freezability of in vitro produced cattle embryos

HARRISON LECTURE
Professor Eberhard Nieschlag
Hormonal male contraception: a challenge to endocrine research
Chairman: Assoc. Prof. David Handelsman
Time: 1200 - 1300
Venue: Castle 2 Lecture Theatre

STATE OF THE ART LECTURE
Dr. John F. Cockrem
Seasonal breeding - are New Zealand birds different?
Chairman: Dr. Terry Fletcher
Time: 1400 - 1430
Venue: Archway 4 Lecture Theatre

ANNUAL GENERAL MEETING
Chairman: Prof. Rex Scaramuzzi
Time: 1430 - 1600
Venue: Archway 4 Lecture Theatre

MRC LECTURE
Prof. Yves Comnarnous
Structure-function relationships and mechanisms of action of pituitary and placental gonadotropins
Chairman: Dr. David Robertson
Time: 1630 - 1730
Venue: Castle 2 Lecture Theatre
WEDNESDAY AUGUST 25

Session 20 (Miniposter - concurrent) : SPERM MOTILITY AND ARTIFICIAL INSEMINATION
Chairman: Dr. R. Vishwanath
Venue: Archway 3 Lecture Theatre
Time: 0830 - 1000

69 Al-Somai, N., Vishwanath, R., Shannon, P. and Molan, P.C.
High molecular weight protein aggregates in bovine seminal plasma produce cationic peptides that affect bull sperm motility

70 Upreti, G.C., Oliver, J.E., Munday, R. and Smith, J.F.
Effect of diluent type on motility of ram spermatozoa stored for varying periods at 15°C

71 Upreti, G.C., Board, K.R., Oliver, J.E. and Smith, J.F.
Modification of ram semen diluent (RSD-1): effect of lipids on ram spermatozoa motility

72 Chainmaysuhlur, A., Sánchez-Partida, L.G., Maddocks, S. and Setchell, B.P.
Quail yolk and coconut extract in diluents for storage of ram semen at 30 and 5°C

73 Hill, J.L. and Nancarrow, C.D.
Absence of binding of an ovine oestrous-associated oviducal glycoprotein to ram spermatozoa

Transcervical insemination of merino ewes

75 Macmillan, K.L. and Taufa, V.K.
Pregnancy rates to first insemination in dairy cattle previously treated with progesterone during late dioestrus and with or without oestradiol benzoate

Session 21 (Oral - concurrent) : UTERINE REMODELLING AND IMPLANTATION
Chairman: Dr. Mike Legge
Venue: Archway 4 Lecture Theatre
Time: 0830 - 1000

76 Harper, G.M., Gooneratne, A.D., Molloy, C. and Bullock, D.W.
Purification of a stage-specific lectin receptor from the sheep uterus

77 Hurst, P.R., Gibbs, R.D., Clark, D.E. and Myers, D.B.
Decrease in fibrillar collagen types I, III and V during rat implantation

78 Clark, D.E. and Hurst, P.R.
Decidualisation and the remodelling of laminin during natural embryo implantation in the rat

79 Macpherson, A.M. and Rogers, P.A.W.
Human endometrial endothelial cell proliferation during the menstrual cycle

Endothelin secretion in vitro by cultured human endometrial epithelial cells: effect of transforming growth factor-β1 and interleukin-1α

81 Abberton, K.M., Rogers, P.A.W., Taylor, N. and Critchley, H.O.D.
Endometrial oestrogen and progesterone receptor expression in women with menorrhagia

WEDNESDAY AUGUST 25 (cont.)

Session 22 : STATE OF THE ART LECTURE
SA3

Dr. Leeanda J. Wilton
Diagnosis of genetic diseases in preimplantation embryos
Venue: Archway 4 Lecture Theatre
Time: 1030 - 1100

Session 23 (Miniposter - concurrent) : EMBRYO DEVELOPMENT AND LACTOGENESIS
Chairman: Dr. Colin Nancarrow
Venue: Archway 4 Lecture Theatre
Time: 1100 - 1230

82 Spindler, R., Renfree, M.B. and Gardner, D.K.
Nutrient uptake during embryonic diagnose and subsequent reactivation in mouse blastocysts

83 Bowen, J.M., Pugh, P.A. and Thompson, J.G.
Development of in-vivo and in-vitro derived ovine embryos in media supplemented with human serum or fatty acid-free bovine serum albumin

84 Rhodes, S.L., Stojanov, T., Evans, G. and Maxwell, W.M.C.
Effect of type and concentration of serum on the development of in vitro matured and fertilised sheep embryos

85 Szell, A.Z. and Newton, P.J.
The effects of co-culture with oviduct cells and the addition of glutamine on the development of sheep embryos in vitro

Survival after transfer of fresh or frozen bovine embryos produced in vitro in a cell- and serum-free medium

87 Thompson, J.G. and Bell, A.C.S.
Partitioning of glucose carbon in post compaction ovine embryos

88 Elke, G., Zupp, J.L., Seamark, R.F. and Setchell, B.P.
Fertilising ability of spermatozoa from rams subjected to intermittent scrotal insulation and development of the resultant embryos in vitro

89 Knight, T.W. and Scott, I.
Time and incidence of foetal mortality in Alpacas

90 Kent, J.C. and Harmen, P.E.
Calcium, phosphate and citrate in sows milk during lactogenesis Ii
WEDNESDAY AUGUST 25 (cont.)

Session 24 (Poster - concurrent) : FOLLICULAR DYNAMICS AND OESTROUS SYNCHRONY

Chairman: Dr. Peter Jolly
Time: 1100 - 1230 Venue: Marama Hall

91 McMillan, W.H., Hall, D.R.H., Oakley, A.P. and Morris, C.A.
Follicular dynamics in cows from a herd with a history of twin-calving

92 McMillan, W.H., Oakley, A.P. and Hall, D.R.H.
Follicular dynamics, ovulation and oestrus in beef cows suckling either one or two calves

93 McMillan, W.H., Hall, D.R.H. and Oakley, A.P.
Follicle and ovulatory patterns in roemney ewes teased by rams during late anoestrus

94 Macmillan KL., Taufa, V.K and Burke, C.R.
Effects of oestradiol on synchrony patterns in cattle treated with an intravaginal progesterone device

95 McMillan, W.H., Hall, D.R.H. and Evans, P.H.
Oestrous synchrony, ovulation rate and embryo survival in recipient ewes commencing synchrony treatment in either mid or late cycle

96 McDougall, S., Macmillan, K.L. and Williamson, N.B.
Effect of treatment of non-cycling, lactating dairy cows with progesterone and/or oestradiol

97 Scott, J.C. and McLeod, B.J.
The effects of exogenous progesterone on ovulation and pregnancy in Alpacas

98 Fisher, M.W., Meikle, L.M., MeLeod, B.J. and Shackell, G.H.
GnRH-induced accessory corpora lutea in red deer and sheep
HORMONAL CONTROL OF TESTICULAR DESCENT AND THE CAUSE OF CRYPTORCHIDISM

JM Hutson
Royal Children’s Hospital, Surgical Research Unit, Flemington Road, Parkville, Victoria, 3052, Melbourne, Australia.

Testicular descent occurs in two separate morphological and hormonal stages. In the first stage the gubernaculum enlarges to anchor the testis near the inguinal region as the embryo grows. In the second phase the gubernacular migrates from the groin to the scrotum. Non-androgenic hormones control the first phase with conflicting evidence suggesting that mullerian inhibiting substance (MIS) may be the active hormone. The second phase is controlled by testosterone, but it has been proposed recently that androgens act indirectly via the nervous system. Calcitonin gene-related peptide (CGRP) is a neuropeptide that has been found in the genitofemoral nerve spinal nucleus, and it has been proposed that CGRP may act as a second messenger for androgens to stimulate gubernacular migration. The evidence for this proposal will be presented.

The cause of undescended testes is multifactorial. True hormonal deficiencies such as in androgen synthesis or action and in the persistent mullerian duct syndrome do cause undescended testes. However, these hormonal anomalies are rare, and most children have no recognisable hormonal cause for their undescended testes. The hypothesis that the genitofemoral nerve and CGRP may mediate the action of androgens on gubernacular migration suggests that anomalies of the genitofemoral nerve or its content of CGRP may be a common cause of undescended testes.
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SEASONAL BREEDING - ARE NEW ZEALAND BIRDS DIFFERENT?

J.E. Cockrem
Department of Physiology and Anatomy, Massey University, Palmerston North, New Zealand.

The New Zealand avifauna includes many unique species, including such distinctive birds as the kiwis (Apteryx sp.) and the kakapo (Strigops habroptilus). Do New Zealand birds also show unique features in their patterns of seasonal breeding? In order to answer this question the breeding seasons of three species and then of the entire New Zealand avifauna are considered, and a model to explain the physiological control of the timing of breeding in New Zealand birds is presented.

The main egg-laying season of kiwis extend from early winter until mid-summer, and is longer in the North Island brown kiwi (July-December) than the little spotted kiwi (September-December). Both male and female North Island brown kiwis have annual cycles of plasma levels of estradiol, and males have an annual cycle of testosterone levels. Plasma steroid levels rise in April and May, indicating that the reproductive system is being switched on at this time. In contrast to kiwis, the yellow-eyed penguin (Megadyptes antipodes) has a short period of egg-laying in early spring (September and early October), with a correspondingly short period of elevated plasma steroid levels in males and females. A third species, the kakapo, is a large, flightless parrot that lays eggs in mid-summer (January and February). Data on the months in which eggs are laid have been combined for all native New Zealand birds, showing a broad peak of egg-laying from August to February. The pattern of egg-laying for species that have been introduced to New Zealand (largely from the Northern Hemisphere) is very similar to that for the native birds, suggesting that the pattern of seasonal breeding by New Zealand birds is strongly influenced by the local conditions rather than being distinctive to the native species.

A speculative model to explain the physiological control of the timing of breeding in New Zealand birds uses the suggestion made by Nicholls et al. (1) that long daylengths that stimulate gonadal growth in birds also initiate processes that lead to photorefractoriness and gonadal regression. It is proposed that the annual reproductive cycles of New Zealand birds effectively start in April. Daylength is decreasing at this time and the period of photorefractoriness that was initiated by the long daylengths of summer is ending. This refractory period can start before the summer solstice (e.g. yellow-eyed penguin) or may not occur until well after the solstice (e.g. kakapo). In the North Island brown kiwi gonadal growth probably commences in April, with very little negative steroid feedback on the hypothalamus. In the little spotted kiwi gonadal growth probably starts later and steroid feedback is much stronger. The yellow-eyed penguin does not initiate significant gonadal growth until daylengths increase after the winter solstice; in this species steroid feedback is strong and must be overcome by photoperiodic stimulation. The kakapo also requires photoperiodic stimulation to overcome steroid negative feedback. The varied breeding seasons of other New Zealand birds can also be explained using this model. It is concluded therefore that the basic physiological mechanisms that control the timing of seasonal breeding in New Zealand birds are similar to those of other birds.


DIAGNOSIS OF GENETIC DISEASES IN PREIMPLANTATION EMBRYOS

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Genetic diseases affecting fetuses are currently diagnosed at 6-16 weeks gestation by amniocentesis or chorionic villus sampling. Diagnosis in the preimplantation embryo has the advantage that unaffected embryos can be selected for transfer circumventing the need for termination of affected fetuses. Biopsy of cells from preimplantation embryos can be done either at the blastocyst or 4-8 cell stage. Blastocysts can be collected by uterine lavage although recovery rates are low, or by culture of embryos produced by in vitro fertilization (IVF). One or two cells can also be removed from 8-cell embryos. This does not compromise pre- or post-implantation development in mice or in vitro development of human embryos. Only a small number of biopsied human embryos have been transferred but it appears that they are unaffected by the biopsy procedure.

One of the limitations of preimplantation diagnosis is the small amount of material available for analysis. To date, preimplantation diagnosis has been predominantly used for sexing of embryos of patients who carry X-linked diseases such as muscular dystrophy, haemophilia etc. Female, and hence unaffected, embryos are selected for transfer. Initially embryo sexing was done by amplifying highly repeated fragments of DNA specific to the Y chromosome using the polymerase chain reaction (PCR). Cells which did not exhibit a Y-specific band on a gel were presumed to come from female embryos. This approach has a risk of misdiagnosis and chromosome dosage cannot be determined even when simultaneously amplifying sequences from the X and Y chromosomes.

The use of chromosome specific probes detected by fluorescent in situ hybridisation (FISH) has circumvented many of these problems. FISH is performed on single metaphase or interphase nuclei, is rapid and efficient and chromosome dosage can be determined. To date, FISH has been used successfully for embryo sexing in patients with X-linked diseases. It is also applicable to the detection of aneuploidies such as Down syndrome. In conjunction with chromosome painting, a technique which labels the entire chromosome, it may enable detection of structural chromosomal anomalies such as translocations.

Detection of single gene defect diseases by embryo biopsy remains a challenge as only one or two cells are available for analysis. The absence of a human B-globin trans gene has been detected by PCR amplification of 1-5 cells biopsied from mouse blastocysts. In humans, this approach has a high failure rate particularly when single cells are used. However diagnosis of single gene defects is possible in the preimplantation embryo. A child free of cystic fibrosis was recently born to a couple who carry the mutation which causes the disease. The child resulted from an embryo biopsied at the 8-cell stage. As more efficient PCR protocols are developed the prospects for preimplantation diagnosis of other single gene defect diseases are encouraging.
HUMAN SPERM AND TESTIS MITOCHONDRIAL DNA

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Many ageing and degenerative disorders are related to deletions to the mitochondrial genome (mtDNA) that cause disordered oxidative phosphorylation and uncontrolled free radical release (1,2). Some forms of human infertility due to spermatogenic failure may also be associated with mitochondrial disorders, resulting in accelerated testicular ageing, with progressive spermatogenic and endocrine decline, vascular insufficiency of the testis and free-radical-induced damage to spermatozoa (Cummins et al., submitted). Such damage typically inhibits sperm function by lipid peroxidation, however advancing paternal (as well as maternal) age is also associated with genetic damage in offspring (3). Human spermatozoa carry oxidative DNA breakage products that can be decreased with ascorbate therapy (4). Nevertheless, infertile couples where the male has very severe semen defects are increasingly being treated by sperm microinjection of oocytes in vitro, despite little understanding of aetiology or genetic background. We have therefore started a study of male reproductive tissue mtDNA in relation to male infertility.

Aliquots (200µl) of frozen normal (n=3), asthenozoospermic (n=1) and teratozoospermic (n=2) semen samples were lysed and the extracted mtDNA was subjected to 30-32 cycles of PCR using established primers and protocols (5). One additional fresh, normospermic sample was studied after Percoll density gradient fractionation, and material was also obtained from a testicular biopsy specimen from a 36 year old man with spermatogenic failure. The primers chosen amplified a small and a large sequence (398 and 5349 bp respectively). The larger of these spans a common site for age-related deletion events in muscle and brain tissues (5).

We have successfully amplified both sequences from all samples. By serial dilution we have managed to amplify mtDNA from as few as c50 spermatozoa. On this limited series we have not yet detected any deletions from spermatozoa. However, the testis material revealed the presence of the common deletion (5), consistent with the premature aging hypothesis. We intend to increase the extent of the mtDNA that we can amplify by suitable choice of primers, to increase resolution to single spermatozoa and to screen a wide range of fertile and infertile men. If idiopathic male infertility is indeed linked with mitochondrial disorders it may therefore be maternally inherited. This would explain some puzzling demographic and genetic aspects of this syndrome. In addition, it may open the way for appropriate redox therapy for some forms of male infertility (1).

ELECTRON MICROSCOPY OF AVIAN SPERMATOGENESIS AND DIAGNOSIS OF MALE INFERTILITY: USE OF LAPAROSCOPIC BIOPSY.

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Successful captive breeding of non-domestic species of birds is essential for the future survival of many species. Rare and valuable parrots caged as true pairs are sometimes infertile for unknown reasons. When the female lays infertile eggs it is assumed that the male is infertile or unable to impregnate. For the purpose of studying spermatogenesis and diagnosing male infertility, we describe a technique in which a sample of avian testicular tissue is biopsied under laparoscopy and examined by light and electron microscopy. The birds biopsied, a Barbary dove (Streptopelia risoria), a rainbow lorikeet (Trichoglossus haematodus) and a scaly-breasted lorikeet (Trichoglossus chlorolepidotus) were all of proven fertility and were not rare as we wished to gain experience with the technique before assessing rare and valuable males.

Using 1 mm cup forceps, the biopsy samples obtained were approximately 3 mm, with a small piece of capsule attached. This was more than sufficient for analysis. The Barbary dove was re-examined 10 days after biopsy, when the testes were found to have healed.

When the biopsied testicular tissue was examined by light and electron microscopy, active spermatogenesis was present in all three species. The testicular spermatocytes of each species had elongated cylindrical sperm-heads. Condensing nuclei were surrounded by a longitudinal manchette. Acrosomes were cone-shaped. The Barbary dove had only a narrow subacrosomal space with a small amount of dense material adjacent to the nucleus, while the lorikeets had more extensive subacrosomal space containing a dense fibrous rod.

In the rainbow lorikeet, cytoplasmic canal formation was similar to that in mammals: from an alignment of vacuoles along the axoneme. This occurred before neck attachment. The sperm-tail did not attach to the nucleus to form the neck attachments until after considerable outgrowth from the spermatid.

In comparison to mammals, the early stages of spermatogenesis had some unusual features in all three species examined here: spermatogonia and spermatocytes had wide cytoplasmic continuity and typical intercellular bridges developed late in spermatogenesis. Initially centriole pairs were arranged in parallel and later assumed the more usual perpendicular orientation.

MICROTUBULE ASSOCIATED PROTEINS IN THE RAT TESTIS.


Microtubule associated proteins (MAPs) are known to initiate and stabilise the polymerisation of microtubules. MAPs have been seen as decorating microtubule arrays using electron microscopy, but their role as effectors of microtubule function remains inconclusive. The most abundant sources of MAPs are neural tissue and the testis. Numerous MAPs from neural tissue have been reported in the literature; these include three isotypes of MAP-1 (A, B & C, later identified as cytoplasmic dynein), MAP-2, MAP-4 and a heterogeneous group of related proteins collectively known as Tau. The testis possesses a variety of microtubular networks (eg. the axoneme, the manchette, meiotic and mitotic spindles and Sertoli cell cytoskeletal microtubules) and MAP-1A, B & C, MAP-4 and Tau have all been identified in testsis. In addition, Boekelheide et al. (1) reported the presence of up to sixteen uncharacterised heat stable MAPs greater than 80kDa in rat testsis. The aim of this study was to further characterise and identify the MAPs present in the rat testsis.

HPLC gel filtration profiles of homogenates from rat brain and testsis were assayed by ELISA using an antiserum which specifically recognises MAP-2 and Tau (anti-MAPs) and a monoclonal antibody to Tau (Tau-1). The gel filtration profiles from the brain extract contained 2 peaks corresponding to MAP-2 (>200 kDa) & Tau (80kDa) while in the testsis, multiple peaks could be identified. Using the Tau-1 antibody a single peak corresponding to Tau could be found in the testsis extract, however, the anti-MAPaps antiserum identified peaks >200 kDa (MAP-4), 80kDa (Tau), and numerous peaks were present in the 30-50 kDa range, which may represent novel MAP-2, 4 and Tau related proteins.

Using the taxol-induced assembly method for purification of microtubules (2) we isolated MAPs from both adult rat brain and testicular tissues. The homogenates were made to 20μM taxol (a plant derived alkaloid known to stabilise microtubules) and to 1mM GTP (necessary for in vitro polymerisation) and put through one cycle of temperature dependent assembly. Using the criterion of co-assembly with tubulin during polymerisation and analysis on 8% SDS-PAGE, more than twenty-five heat-stable testicular MAPs greater than 30kDa could be identified. Similar analysis of brain MAP extracts reveals only three to four heat stable MAPs.

These data demonstrate that the testsis may contain numerous novel MAPs which do not occur in brain tissue and that some of these putative testicular MAPs may share the homologous microtubule binding domain found on the C-terminus of MAP 2, 4 and Tau. Further studies are underway to isolate and identify these novel MAPs.


ISOLATION AND PARTIAL CHARACTERISATION OF RAT SPERM TAIL FIBROUS SHEATH PROTEINS.


The fibrous sheath (FS) in the principle piece of the tail of most mammalian spermatozoa is composed of two structural elements: the longitudinal columns and numerous transverse ribs which surround the outer dense fibres (ODF). The isolation and partial biochemical characterisation of rat sperm FS has been previously reported (1,2) suggesting the FS to be keratin-like with extensive disulfide linkages (3). The major protein components as revealed by SDS-PAGE are reported as approximately 75 kDa, 25 kDa and 14 kDa together with several other minor components (1,2). The aim of this study was to extend this work, by isolating and characterising the individual protein components of the FS.

Rat sperm were obtained from the cauda epididymides of 12 adult Sprague-Dawley rats. The sperm filtered through a Nitex screen were decapitated by sonication and layered through a sucrose step gradient. Isolated sperm tails were treated in several steps with Triton X-100, DTT, and urea. Finally the solubilised sperm tail suspensions were centrifuged through a sucrose step gradient and the insoluble pellet fixed for electron microscopy.

The residual contamination of ODF or other sperm tail components in the FS preparation was minimal as judged by electron microscopy. SDS-PAGE of the isolated FS resolved at least 20 different proteins of varying abundance. The range and molecular size distribution of these 20 proteins were similar to that reported by Oko (2), except the band at 14.5 kDa by Oko was much less abundant our preparation of rat FS. The most abundant proteins were a double band at 80-87 kDa, a 66 kDa, and 28.5 kDa bands. The minimal contamination of ODF in this preparation as compared with that of Oko may explain the differences in abundance of proteins. Using electroelution following SDS-PAGE we have isolated 10 of the most abundant proteins with a high degree of purity. Preliminary results of amino acid analysis on 6 of the isolated rat FS proteins show that these proteins have a similar composition of amino acids, abundant in aspartic acid, glutamic acid, glycine, and to a lesser extent tyrosine. Further work is being conducted to obtain amino acid sequence data for these proteins, and will provide further information on the homology between these proteins.

Evidence of Apoptosis in Bovine Granulosa Cells

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Apoptosis or 'programmed cell death' is an active genetically governed process of selective cell death implicated in the mechanisms of ovarian follicle atresia and luteolysis (1,2). Apoptosis is associated with the activation of an endogenous endonuclease which cleaves chromatin between nucleosomes producing DNA fragments of integer multiples of 180-200 base pair subunits in size, which resolve into a characteristic ladder-like pattern on agarose gel electrophoresis (AGE). The aim of this study was to determine whether apoptotic cell death occurs in granulosa cells (GC) from bovine follicles 4-6 mm in diameter, classified morphologically as healthy or atretic.

Follicles were dissected from ovaries collected postmortem from 8 cows in the luteal stage of the oestrous cycle. Individual follicles (n=30) were classified by size and as healthy or atretic according to previously defined morphological criteria (3,4). Granulosa cells were collected and snap frozen to -70°C in Ca²⁺/Mg²⁺-free buffer containing EDTA (2 mM). Number of cells recovered was determined and expressed as a percentage of the maximum number of cells (%Gmax) previously recorded for a follicle of that size (3). Follicular fluid was also collected and levels of oestradiol-17β determined by RIA (4). Total DNA was isolated from GC from each follicle and purified by treatment with ribonuclease-A (100 µg/ml), phenol/chloroform extraction and ethanol precipitation, then quantitated by fluorescent assay. DNA (500 ng) from each sample was labelled at 3'-ends with 50 µCi of [α³²P]dATP (6000 Ci/mmol) using 25 U terminal transferase and 5 mM CoCl₂ for 60 min at 37°C (1). Labeled DNA was purified by ethanol precipitation using 20 µg glycogen as carrier and fractionated for electron microscopy.

These results suggest that apoptosis occurs in bovine GC and that degree of internucleosomal DNA fragmentation is most evident in cells from follicles that are severely atretic. Further work is being conducted to obtain amino acid sequence data for these proteins, and will provide further information on the homology between these proteins.
INDOMETHACIN BLOCKS THE IMMUNOSUPPRESSIVE ACTIVITY OF RAT TESTICULAR MACROPHAGES IN-VITRO

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Macrophages have been recognised as a prominent cellular component of the testicular interstitial tissue of several species including man, monkey, rat and boar (1). They represent some 20% of the cell population and have an intimate relationship with Leydig cells in the interstitium. We have previously shown that rat testicular macrophages (TM) do not stimulate lymphocyte (Le) proliferation in culture, and in fact secrete factors which inhibit the proliferation of lymphocytes stimulated in vitro by the mitogen Concanavalin A (Con A) (2). These results suggest that the TM could contribute to the immunologically privileged status afforded the testis. Subsequent studies have indicated that the poor Le proliferation response observed when TM are cultured in vitro with Le may be partly due to the low basal production of interleukin 1 by TM and their inability to produce this cytokine in response to stimulation by factors such as Lipopolysaccharide (LPS) (3,4). However this does not explain the suppressive nature of factors secreted by TM into culture media. The production of prostaglandins (PG) by activated macrophages has been shown to inhibit lymphocyte function (5). PG is produced by an increase in cellular CAMF levels during macrophage activation and may be blocked by indomethacin, an inhibitor of prostaglandin synthase. In the present study we have examined the possibility that prostaglandin production by TM may be responsible for the immunosuppression exhibited by these cells in vitro.

Rat peripheral blood lymphocytes were isolated using standard protocols (2) and cultured at 10^6 cells/ml in 96 well round bottomed culture plates. TM were isolated by enzymatic digestion of the testis (3) and then cultured as in (3) but with the addition of indomethacin (DM) at 10ug/ml to some cultures in order to inhibit PG production. After 48 hours the TM conditioned medium (CM) was collected, centrifuged and frozen until use. Some of the CM collected was then dialysed removing molecules <100000MW, such as PG. Lymphocytes were optimally stimulated with the mitogen Concanavalin A (Con A) at 10ug/ml and concurrently cultured with the CM or dialysed CM (20% of the total culture volume). After 48 hours of culture, 3H-thymidine incorporation was used to measure the lymphocyte proliferation.

Indomethacin treated cultures and the dialysed media of TM proved to be significantly less suppressive than untreated CM. The CM from TM treated with LPS and indomethacin, and the dialysed CM from LPS stimulated TM cultures, did not significantly reduce lymphocyte proliferation from that of the control. That the presence of indomethacin in culture, or the removal of small molecular weight compounds from CM should remove the immunosuppression previously observed suggests that PGs are involved in the immunosuppression encountered in vitro. The presence of PGs may also down-regulate cytokine production by TM (6) and hence provide a basis for immune privilege in the testis. PGs may also play a role in regulating Leydig cell steroidogenesis, and this may provide a basis for physiological TM-Leydig cell associations.

REFERENCES


NUTRITIONAL EFFECTS ON LUTEINIZING HORMONE (LH) SECRETION IN RAMS AFTER EXPOSURE TO OESTROUS EWES.

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The secretion of LH pulses in rams is increased by nutritional supplements (1) and by the introduction of ewes (2). In goat bucks, the magnitude of the response to does is greater for bucks on a 'High' plane of nutrition than for those on a 'Low' plane (3). We tested whether rams show a similar interaction between the nutritional and social stimuli.

Twenty, 2 year old Merino rams grazing spring pasture were penned indoors and allocated to 2 nutritional groups ('High' or 'Low') following stratification by scrotal circumference and condition score. The Low rams received 500 g hammer milled outen hay per day while High rams received 1 kg of the hay plus 750 g lupin grain per day for 35 days. After 21 days, the rams were penned in groups of 4 and from 1860 hours blood was sampled every 20 minutes for 12 hours before and after the introduction of oestrous ewes (4 per pen). Number of mounts per hour were recorded for each ram.

The High rams had greater condition score (2.9 vs 1.45, P < 0.001) and scrotal circumference (3.14 vs 2.69 cm, P < 0.001) than Low rams on Day 35. High rams increased their condition score (2.45 to 2.9, P < 0.005) but not scrotal circumference (3.10 to 3.14 cm), while both decreased in Low rams (2.55 to 1.45, P < 0.001 and 3.13 to 2.69 cm, P < 0.001). There was no difference between the groups in the frequency of LH pulses before the ewes (Fig. 1). The presence of oestrous ewes increased the frequency of LH pulses in both groups (P < 0.001) and resulted in a higher frequency for High rams than Low rams (P < 0.01). Pulse amplitude was greater for High rams than Low rams before and after ewes (P < 0.01), but there was no effect of the presence of the ewes. Mean LH concentration did not differ between the groups at any stage. However, over all rams, it increased after the introduction of the ewes (P < 0.01). The average number of mounts per hour was the same for both ram groups (2.6 High vs 2.5 Low).

There was no relationship between mounting activity and LH secretion.

Figure 1. LH secretion in rams before (D) and after (■) the introduction of oestrous ewes. ** P < 0.01: Between treatments, histograms of the same type are different.

The presence of oestrous ewes increases the frequency of LH pulses in rams and the magnitude of the change depends on their nutrition. The difference in responsiveness of the rams to oestrous ewes may be due to decreased responsiveness of the LH pulse generator to social stimuli when an animal is in a catabolic nutritional state as the main effect of our diets was decreased condition and testis size of the Low rams.

DOES THE NUTRITION OF THE RAM INFLUENCE ITS BEHAVIOURAL AND ENDOCRINE RESPONSE TO OESTROUS EWES?

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The LH response of rams and goat bucks exposed to oestrous females (the "female effect") varies with the nutritional status of the male (1,2). To determine the major nutrients involved in this variation we examined the endocrine and behavioural responses to oestrous ewes of mature Merino rams given dietary treatments designed to mimic the major nutritional components of a 750g lupin grain supplement.

High and low control groups were fed a maintenance diet (Maint) or Maint + 750 g/d of lupin grain. Three other groups were fed Maint and received abomasal infusions of glucose (180 g/d), casein (200 g/d) or the same amount of both glucose and casein (GC), while the final group received GC+volatile fatty acid salts (VFA, acetate 127 g/d, propionate 64 g/d) in vegetable oil (60 g/d) as an oral supplement to the Maint diet. Dietary treatments were applied for 5 weeks in April-May (n=5/treatment). Liveweight and scrotal circumference were monitored weekly and blood was sampled for LH on days 35 and 36. After the first sample on day 36, 30 oestrous ewes were penned in front of the pen so that each ram had access to 2-3 ewes through the front of the pen which enabled the passage of heads only. During exposure to oestrous ewes the frequency of investigative (sniffs, nudges, licks), reproductive (flehmen, pawing, vocalisation) and aggressive (male and female directed) ram behaviours was recorded.

![Figure 1](image_url)  
Figure 1. a) LH concentration in rams (mean ± sem, n=30) before and during exposure to oestrous ewes. b) Effect of diet on LH pulse frequency (mean ± sem, n=5) in rams before (day 35) and during (day 36) exposure to oestrous ewes.

The diets induced significant changes in liveweight and testicular size (3) Exposure to oestrous ewes increased LH pulse frequency (3.7 ± 2.2 pulses/12h, p<0.01), pulse amplitude (5.5 ± 4.2 ng, p<0.05) and mean LH concentration (2.1 ± 1.1 ng/ml, p<0.001, Fig. 1a) with 21/30 rams exhibiting an increase in the number of LH pulses. There was no effect of dietary treatment on LH secretion before or during ewe contact (Fig. 1b) and no significant association between LH secretion and change in liveweight or scrotal circumference. Similarly, there was no effect of diet on any behavioural variable. However change in LH pulse frequency following exposure to ewes was significantly correlated with the frequency of investigative (r=0.66, p<0.001) and reproductive (r=0.56, p<0.01) but not aggressive behaviours in individual rams.

We conclude: a) full contact with oestrous ewes is not necessary to induce the female effect in rams; b) supe-maintenance ram nutrition does not always enhance the endocrine response to oestrous ewes; c) The magnitude of the endocrine response to ewes is associated with the frequency of investigative and reproductive behaviours expressed by rams under conditions where mating and mounting are precluded.


DIETARY FATTY ACIDS STIMULATE GONADOTROPHIN SECRETION IN THE RAM

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We previously showed that supplementation of a maintenance diet with lupins stimulates gonadotrophin secretion in rams. This effect is associated with increased blood concentrations of glucose and fatty acids and could not be reproduced by intravenous glucose infusion (1). This experiment tested whether fatty acids could stimulate gonadotrophin secretion in rams.

Twenty four Merino rams were housed indoors and fed a diet consisting of 900 g of hammer milled hay and 100 g lupins (6.17 MJ/d and 19.76 g CP/d) for 2 weeks before they were randomly allocated to a control or a treated group. The control rams continued on the same diet. The treated rams received, in their diet, a mixture of sodium acetate (127 g/d), sodium propionate (64 g/d) and vegetable oil (60 g/d) for 11 days. The estimated metabolisable energy intake and protein intake were 11.83 MJ/d and 19.76 g CP/d for the treated group. Blood was sampled every 20 min for 24 h before the start and at the end of the treatment period. All samples were assayed for LH and two-hourly samples were assayed for FSH, glucose and insulin. Data were log transformed before analysis and pre-treatment values were used as covariates.

Fatty acid supplementation did not affect food intake. Mean LH pulse frequency and FSH concentrations were similar in both groups before treatment started. However, at the end of the treatment period, rams fed the fatty acid supplement had a higher LH pulse frequency (p<0.05) and FSH concentrations (p<0.05) than the control rams. No changes in LH pulse amplitude or basal concentration were observed between or within groups. Plasma concentrations of glucose were relatively constant throughout the day and between and within group differences were not statistically significant. Maximum insulin concentrations were observed after feeding and then declined to reach a plateau 6 to 8 hours afterwards. Neither the decline nor the plateau of insulin were statistically different between the control and the supplemented rams.

The present study represents the first successful attempt to stimulate gonadotrophin secretion in intact rams with semi-purified nutrients. Glucose and/or insulin do not appear to play an intermediary role as peripheral blood concentrations were not affected by the treatment. Therefore, the stimulation of gonadotrophin secretion in lupin-fed rams may be mediated via a biological pathway associated with the synthesis or utilisation of fatty acids. Future research in this area should focus on the effect on GnRH secretion of individual fatty acids and their oxidative metabolites (i.e. aspartate and glutamate).

EFFECT OF DIETARY PROTEIN INTAKE ON THE CHANGES IN LEVELS OF FSH PRODUCED BY INJECTIONS OF FOLLICULAR FLUID IN OVARIECTOMISED EWES.

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Increased dietary protein results in increased ovulation rate (1). Interference with the inhibitory feedback mechanisms on FSH release have been suggested as a possible mechanism. However, no effect of protein was found in studies on oestradiol feedback (2). The effect of protein Intake on "inhibin" feedback is reported in this trial.

Twenty four ovariectomised ewes fitted with abomasal catheters and fed on a low protein (12%) diet were allocated to four groups. Two of these groups were given abomasal infusion of protein (Alacen 100 gm/ewe/day) for 7 days and the other 2 groups given infusions of equivalent volumes of water (1 l/day). On day 3 of infusion, injections of charcoal stripped ovine follicular fluid (oFF-containing 14 u/ml inhibin activity) were given at 10:00 h and 22:00 h and again on day 4 at 10:00 hr. One group in each of the infusion treatments received either a high (3x2.5ml) or low (3x0.5ml) dose of oFF. Blood samples for FSH assay (RIA) were taken at 2 hr intervals between 09:00 h and 21:00 h on the day before and for days 1 and 2 of infusion then for 40 h from 07:00 h on days 3 and 4 and again from 09:00 h to 15:00 h on days 5 to 8.

FSH levels ranged from 6.0 to 24.0 ng/ml for individual ewes and data has been analysed as variation from the mean value for the day before infusions commenced (expressed as a percentage).

There was a significant (P<0.005) effect of dose of oFF on the depression of plasma FSH concentration with the high dose rate reducing FSH levels, to 69% of pretreated values, following the third injection. The first significant decrease in FSH was recorded 8 h after the 1st injection of oFF and pre-treatment levels were regained on day 7. No significant depression in FSH levels was seen with the low dose and protein infusion had no effect at either level of oFF.

The reduction in plasma FSH levels to 70% of pretreatment levels confirms the previously reported effects of oFF. That this depression and subsequent recovery was not influenced by dietary protein intake indicates that the mechanism by which nutrition effects ovulation rate is not via the disturbance of inhibin negative feedback mechanisms.


EFFECTS OF OVARIECTOMY AND/OR WEANING ON PLASMA LH IN POSTPARTUM BOS INDICUS COWS

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The inhibitory effects of suckling on postpartum ovarian activity are mediated by central inhibition of LH (1) involving modulation by ovarian factors as well as mechanisms that are independent of the ovaries (2,3). The aim of this study was to determine the relative importance of ovary-dependent control mechanisms and ovary-independent suckling effects in the suppression of LH secretion in the postpartum Bos indicus cow.

Twenty grade Brahman cows (4-6% Bos indicus) were allocated to groups either ovariectomised (Ovx) and their calves weaned (OW, n=5), ovariectomised and suckled by their calves (OS, n=5), intact and their calves weaned (IW, n=5) or intact and suckled by their calves (IS, n=5). Ovariectomy and weaning occurred at 7-10 days postpartum. At weekly intervals, commencing 7-10 days postpartum, cows were bled by coccygeal venipuncture for plasma LH determination by RIA. The ovaries of IS and IW cows were examined weekly by ultrasonography to determine the time of first postpartum ovulation. Bodyweight (BWT) and body condition score (BCS) were recorded weekly.

There were no differences in BWT or BCS between groups in the first 5 weeks postpartum. Mean BCS (sem) at calving was 5±1 (1-9 scale). The mean interval (sem) from calving to first ovulation was less for weaned than in suckled cows (27±4 d vs 72±11 d, respectively; P<0.01). Plasma LH concentrations from 1-5 weeks postpartum are presented in Figure 1. At 2-3 days postpartum, mean plasma LH (sem) was 0.9±0.03, 0.9±0.03, 0.9±0.03 and 1.3±0.24 for IS, IW, OS and OW cows, respectively. Ovariectomy elevated plasma LH, the effect being greater in weaned than in suckled cows (P<0.01).

Figure 1. Mean LH (sem) for intact-Suckled (IS), intact-Weaned (IW), Ovx-Suckled (OS) and Ovx-Weaned (OW) cows from 1-5 weeks postpartum.

In summary, both suckling and ovarian factors inhibit secretion of LH, but ovarian factors exert a much greater inhibitory influence on LH secretion than suckling during the postpartum period of the Bos indicus cow.

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This work was partially supported by the Meat Research Corporation.
EFFECT OF PHASE TWO LACTATION ON PROLACTIN IN MARSUPIALS

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In marsupials, prolactin (PRL) profiles during lactation are characterized by a peak of PRL at birth, low concentrations during the first half of lactation (phase 2) and markedly elevated levels during the second half of lactation (phase 3). All previous studies of phase 2 lactation have not demonstrated a difference in PRL concentrations between lactating (L) and non-lactating (NL) animals. Nevertheless, it is known that PRL is essential during this period because treatment with bromocriptine terminates lactation. Here we describe 2 independent studies of PRL which were undertaken to re-evaluate PRL levels during this period and a third study in which we characterized the molecular forms of PRL detected by the available antisera against marsupial PRL.

Study 1 - Bennett’s wallaby. L (n=4) and NL (n=5) wallabies were sampled each week by venepuncture of the lateral tail vein during phase 2 lactation. PRL levels could be collected over a relatively prolonged period without causing stress to the animals. The jugular vein of 4 L (mean age of pouch young, 40±12 days) and 4 NL possums was surgically cannulated at least 1 week before sampling. On the day of the experiment, the animals were brought to the laboratory to disturb each animal so that a 1 m length of tube could be connected to the cannulae. Animals were then returned to their nest sites. With this arrangement, blood could be withdrawn without physical contact with the possum and, in most cases, the animals slept during the sampling period. Blood was collected at 20 min intervals for 4 h and plasma PRL levels later determined in the heterogeneous PRL RIA. At the onset of lactating, PRL concentrations were similar in the PRL RIA (3.4±0.2 and 3.7±0.2 ng/ml for NL and L groups) and in the first 2 h period there was no effect of lactation (P > 0.1). However during the sampling period, levels in the lactating animals increased gradually so that in the third and fourth hours of sampling, the effect of lactation was significant (P < 0.05). In the final sample, PRL levels were 3.1±0.3 and 6.4±1.8 ng/ml in NL and L groups respectively.

Study 3. The molecular forms of pituitary PRL were examined by SDS-PAGE/western blot. Three antisera (As 33/1-8, As G3/1 and anti-PRL-peptide (As S3)) were tested. Pituitaries from the red-necked wallaby, brushtail possum, koala and bandicoot were examined. In addition, for the brush-tailed phalanger, a range of physiological states (male/female, early/late lactation, pre-pubertal female) were also studied. In all cases, only one form of PRL, MW 23500, was detected. In contrast when pig pituitary was blotted with As S3, the expected pattern with two forms of PRL, MWs 23000 and 26000, was observed. These correspond to the non-glycosylated and glycosylated forms of pig PRL.

In conclusion, these studies suggest that early lactation does increase PRL concentrations in marsupials and that these differences are more evident when an homologous assay is used. Further, frequent sampling from undisturbed animals may be required to demonstrate these differences. Finally, the available antisera against marsupial PRL detect only one form of PRL, and so at present we conclude that changes in physiological state are unlikely to alter the processing of this hormone.

Proteins in bovine seminal plasma (BSP) exist in large aggregates of smaller units of molecular weight (MW) < 3.5 KDa (1). The smaller units are composed of cationic and anionic peptides of estimated MW 1 KDa (2). Anionic Proteins (APs) in BSP have been implicated in gonadotrophin release, sperm capacitation and acrosome reaction (3). The aim of this work was to isolate and study the effects of these APs and their low MW constituents on the motility of bull sperm. 10 ml BSP from fresh semen was loaded on a column of SP Sephadex C25, washed with 30% (v/v) acetic acid at pH 3 (30%AC). The unbound material was neutralised, its volume reduced to 10 ml, ammonium sulphate (AS) added to 1.7 M and then loaded on a Phenyl Superose column. Elution of the bound material was achieved by a descending gradient of 1.7 M AS followed by water and 30% ACN. The fractions eluted with water and 30% ACN were chromatographed on a Sephadex G25 column and eluted with 30% ACN in 0.02 M HCl, pH 1.7. Fractions eluted late with water and with 30%ACN gave one peak near the bed volume (ACNfrac). The fractions eluted earlier with water were mainly at the void volume (WF1) followed by a second peak with MW <5 KDa and > 1.5 KDa (WF2) and a peak near the bed volume of <1.5 KDa. On Superose 12, at neutral pH, WF1 and WF2 re-aggregated to a higher MW while ACNfrac did not. Table 1 shows the effect the these fractions on motility of washed sperm.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc (%)</th>
<th>Protein conc. (mg/ml)</th>
<th>% Motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>82 ± 4.0a</td>
<td>72 ± 3.0a</td>
</tr>
<tr>
<td>WF1</td>
<td>10</td>
<td>11.7</td>
<td>83 ± 2.5a</td>
</tr>
<tr>
<td>WF2</td>
<td>10</td>
<td>3.7</td>
<td>72 ± 3.0b</td>
</tr>
<tr>
<td>ACNfrac</td>
<td>10</td>
<td>2.2</td>
<td>84 ± 3.5a</td>
</tr>
<tr>
<td>BSP</td>
<td>10</td>
<td>43.0</td>
<td>80 ± 2.2a</td>
</tr>
</tbody>
</table>

The APs in BSP are aggregates (4) which under disaggregating conditions release low MW components with molecular weight < 5 KDa. Both WF1 and WF2 reduce motility of sperm on incubation however, WF2 was more detrimental to sperm motility compared to the ACNfrac. The activity of WF1 and WF2 could be due to a cationic patch on the molecule similar to the APs reported earlier (4). WF2 on the other hand, possibly has the right proportion of cationic and anionic peptides that fulfil the criteria for maximum activity. The lack of reduction of sperm motility in ACNfrac could be attributed to the competitive effect of the anionic material that counteracts/reduces the effect of the active cationic protein on sperm and thus plays a part in its protection. An acidic protein such as antiserumalplasmin reverses the effect of seminalplasmin, a cationic protein in BSP, on the sperm membrane needs to be established.

MODIFICATION OF RAM SEMEN DILUENT (RSD-1): ORGANIC NUTRIENT(S) REQUIREMENT FOR RAM SPERMATOZOA MOTILITY

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The organic nutrients in RSD-1 consist of glucose, fumarate, pyruvate and glutamate (1). RSD-1 was developed from a media that was optimized for the maintenance of sheep hepatocytes (2) and the organic nutrient requirements for spermatozoal motility were not evaluated during the initial development of RSD-1. This study was undertaken to assess the requirement for these organic components and other tricarboxylic acid (TCA) cycle intermediates for spermatozoal motility.

Semen was collected, diluted and motility parameters were measured as previously described (1). Under incubation at 38°C, the percentage motility and velocity of spermatozoa declined rapidly in the absence of all organic nutrients. After 1, 5, and 24 hours incubation at 38°C, the % motile spermatozoa and velocity score (indicated in brackets) for RSD-1 incubations were 80 (3.9), 70 (3.7) and 38 (1.9) respectively compared to 70 (3.6), 45 (2.8) and 4 (0.5) for incubations in deficient RSD-1 (ie. RSD-1 without any organic nutrient). Addition of glucose alone to deficient RSD-1, partially restored motility, increasing % motile and score to 24 (1.5) after 24 h incubation at 38°C. Fumarate, pyruvate or glutamate were ineffective in restoring velocity score (0.2 to 0.4) and percentage motile spermatozoa, increased only for fumaric (19%) after 24 h storage at 38°C.

Other TCA cycle intermediates (Citrate, isocitrate, α-ketoglutarate, oxaloacetate succinate, malate) alone did not improve spermatozoal motility. However a combination of glucose with oxaloacetate, or citrate or fumarate was more effective than glucose alone, but not different from RSD-1. Fumarate and oxaloacetate were more effective than citrate in restoring motility in presence of glucose. The % motile and velocity score for glucose, glucose + fumarate, glucose + oxaloacetate, glucose + citrate and RSD-1 were 51 (2.6), 63 (3.6), 60 (3.4), 54 (2.8) and 65 (3.1) respectively after 24 h incubation at 38°C. Additional improvements in motility parameters were not obtained when either acetate or other TCA cycle intermediates were added to the glucose + oxaloacetate combination.

The above studies have shown the involvement of glucose metabolism and TCA cycle in maintenance of sperm motility. Subsequent studies will examine the value of alternate sugars and optimization of concentrations required under conditions of storage.

Satisfactory in vitro fertilisation (IVF) of in vitro matured (IVM) sheep oocytes has been reported using 1-2 million fresh sperm/ml (1,2), although these authors did not examine lower numbers of sperm. This study examined the effect of dose of fresh sperm on fertilisation of IVM sheep oocytes.

Immature oocytes collected on 3 separate days, were cultured for 22hr at 39°C (5%CO₂ in air) in M199 + 10% FBS (foetal bovine serum), LH (10µg/ml), FSH (10µg/ml) and oestradiol (1µg/ml). Fresh ram semen (pooled ejaculates from 4 rams) was diluted 20-fold with h-SOF (Hepes-buffered SOF + 3mg/ml BSA (bovine serum albumin)) and washed twice (centrifugation at 800g, 6 min). After discarding the supernatant the semen pellet was resuspended with b-SOF (bicarbonate buffered SOF) + 20% SS (sheep serum) to the desired concentration of sperm. After 25 hr of culture, cumulus-expanded oocytes were randomly allocated to 6 groups in b-SOF + 20% SS that contained 0.075, 0.15, 0.3, 0.6, 1.2 or 2.4 million sperm/ml.

Eighteen hr after insemination presumptive zygotes were fixed and stained with 1% aceto-orcein, and examined for the presence of two pronuclei and a sperm tail as the criteria for fertilisation. The results are presented in Table 1.

Table 1. Effect of dose of sperm on fertilisation of oocytes.

<table>
<thead>
<tr>
<th>Dose of sperm (million/ml)</th>
<th>No. of oocytes</th>
<th>Fertilised/ inseminated (%)</th>
<th>Polyspermic/ fertilised (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.075</td>
<td>4/78 (9)*</td>
<td>0/4 (0)*</td>
<td></td>
</tr>
<tr>
<td>0.15</td>
<td>14/74 (19)*</td>
<td>0/14 (0)*</td>
<td></td>
</tr>
<tr>
<td>0.30</td>
<td>24/110 (22)*</td>
<td>0/24 (0)*</td>
<td></td>
</tr>
<tr>
<td>0.60</td>
<td>55/102 (53)*</td>
<td>1/55 (2)*</td>
<td></td>
</tr>
<tr>
<td>1.20</td>
<td>65/83 (78)*</td>
<td>2/65 (3)*</td>
<td></td>
</tr>
<tr>
<td>2.40</td>
<td>32/32 (100)*</td>
<td>5/32 (15)*</td>
<td></td>
</tr>
</tbody>
</table>

Proportions in columns with different superscripts differ (p<0.05)

The proportion of oocytes fertilised increased with increasing dose of sperm inseminated (linear, p<0.001), as did the proportion of polyspermic oocytes (linear, p<0.001). This study confirms that 1-2 million fresh sperm/ml are required to obtain satisfactory fertilisation of IVM oocytes, although a low level of polyspermic fertilisation is likely with doses higher than 1 million sperm/ml. In cases where the number of sperm is limited (as in sperm sexing by fluorescence activated cell sorting) some fertilised sheep oocytes may be obtained with less than 1 million sperm/ml of fertilisation medium.

References:


DNA AMPLIFICATION OF AN OESTROGEN-ASSOCIATED OVIDUCAL GLYCOPROTEIN SEQUENCE FROM PRIMATES

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The authors wish to thank Dr D. Spielman, Taronga Zoo, Sydney, NSW for collecting and supplying the primate blood samples.
IS UTERINE FUNCTION COMPROMISED IN HEIFERS SYNCHRONISED WITH A LONG-DURATION PROGESTERONE TREATMENT?

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Long-duration exogenous progesterone treatment can result in good synchrony but poor fertility. The reason for this sub-fertility is unknown but may involve compromise of: oocyte/embryo quality, sperm transport, corpus luteum (CL) function or uterine function. The aim of this study was to compare uterine function (and therefore indirectly CL function) in heifers treated to synchronise oestrus with either a short- or a long-duration progesterone treatment. Ninety seven 18-month old Hereford x Friesian heifers were treated with a progesterone-impregnated CIDR® device for either 10 or 19 days (10 D and 19 D groups). In addition, the 10 D group received a 10 mg oestradiol benzoate capsule at CIDR® insertion and a prostaglandin injection 6 days later. Each heifer received a single frozen-thawed embryo 7 days after the onset of oestrus (onset = d 0). On d 16, 33 heifers were slaughtered and their reproductive tracts flushed to recover conceptus material. The remainder were monitored for return to oestrus until d 25, and then pregnancy status assessed at d 40 (using ultrasound) and day 60 (slaughter). Two of the 46 heifers in the 10 D group had excess uterine tone at transfer, and neither were pregnant at day 60. Of the 8 heifers in the 19 D group with excess tone, 3 were pregnant. The factors which may explain excess tone are the failure of circulating progesterone to effectively suppress luteinising hormone secretion or uterine function. The later. Each heifer received a single frozen-thawed embryo 7 days after the onset of oestrus (onset = d 0). On d 16, 33 heifers were slaughtered and their reproductive tracts flushed to recover conceptus material. The remainder were monitored for return to oestrus until d 25, and then pregnancy status assessed at d 40 (using ultrasound) and day 60 (slaughter). Two of the 46 heifers in the 10 D group had excess uterine tone at transfer, and neither were pregnant at day 60. Of the 8 heifers in the 19 D group with excess tone, 3 were pregnant. The factors which may explain excess tone are the failure of circulating progesterone to effectively suppress luteinising hormone secretion and therefore allow follicle persistence and excess oestrogen production (1). There was no effect of synchrony treatment on pregnancy rate between day 16 and 60 (Figure 1). Two thirds of the heifers with recovered material were pregnant at day 16. Based on non-return rates to day 25, the pregnancy rate had fallen to 54%. By day 40, only 45% of heifers were pregnant and this had reduced to 36% pregnant by day 60. In summary, these data show that extended-duration progesterone treatment does not compromise either CL function or the ability of the uterus to support pregnancies following embryo transfer. The sub-fertility following AI is thus likely to be associated with impaired sperm transport or compromised oocyte/embryo quality.


CHANGES IN THE POLYUNSATURATED FATTY ACID COMPOSITION OF THE UTERUS DURING INDUCTION OF THE DECIDUAL CELL REACTION IN PSEUDOPREGNANT MICE

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Department of Biological Sciences, The University of Newcastle, N.S.W. 2308

The peri-implantation period of pregnancy is associated with a high incidence of reproductive wastage. This may be due to either poor embryo development or failure of the uterine endometrium to provide a suitable environment for implantation. The embryo-induced transformation of hormonally primed uterine stroma into decidual tissue in preparation for implantation is characterized by cellular proliferation and differentiation. Lipid metabolism and, in particular, membrane polyunsaturated fatty acid (PUFA) composition can modulate membrane fluidity which can subsequently influence carrier mediated transport systems, membrane-bound enzymes, receptor function, signal transduction mechanisms, prostaglandin production, and cell growth (1) all of which are associated with the decidual cell reaction (DCR) (2). In addition, intracellular fat stores provide the oxidative energy for these cellular functions.

In the present study, the PUFA composition of uterine tissue was analysed by GC-MS techniques at various times (20, 40, 80, 160, 320, 640, & 1280 min [21.3h]) following the induction of the DCR with Concanavalin A (Con A)(125ug/hom in 0.9% NaCl) (3) in the left uterine horn on day 4.5 of pseudopregnancy. The right uterine horn was not stimulated to undergo decidualization and was used as a control in all cases. The range of PUFAs extracted from whole uterine tissue included arachidonic acid C20:4(n-6), linoleic acid C18:2(n-6), C18:2 isomers (a) and (b), C20:2(n-6), C20:3(n-6), C20:3(n-3), C20:4 isomers (a) and (b), and C22:2(n-6), C22:4(n-6).

It was found that the PUFA relative abundance/mg of dry tissue in both the right (untreated) and left (treated) uterine horns changed significantly with time after stimulation. This may be due to altering hormonal conditions accompanying these stages of pseudopregnancy. However, the levels of the PUFAs in the left horn with the exception of the two C18:2 isomers, did not differ significantly from those in the right horn until 21.3h after stimulation with Con A, when levels in the left horn fell significantly (P<0.01) below those in the right horn. This decline in PUFA content in the left horn corresponded with significant increases in the weight of the horn, as opposed to the right uterine horn, due to growth changes associated with decidualization.

In conclusion, the results suggest that major changes in the PUFA composition of whole uterine tissue accompany the process of decidualization in pseudopregnant mice. These PUFAs may be utilized as a source of oxidative energy and/or involved in cellular membrane remodelling, including altered cell function during the DCR.

THE ACTIVITY OF PAF-ACETYLHYDROLASE WITHIN THE UTERUS DURING THE PERIOVULATORY AND PREIMPLANTATION PHASES.

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Platelet-activating factor (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine, PAF) is produced by gametes, embryos and the reproductive tract, and has been proposed to be involved in the establishment of pregnancy. PAF is thought to have a short half-life in vivo due to its rapid metabolism by the essentially ubiquitous enzyme, PAF:acetylhydrolase. This study investigated the activity of PAF: acetylhydrolase within the uterine fluid and endometrial tissue on various days of the reproductive cycle.

Eight-ten week old QS female mice had ovulation synchronised by i.p. injection of 2.5 i.u. PMSG (Day-2) and hCG (D 0). In a second group, females were placed with either fertile or vasectomised males until mating occurred. The uterus was removed at approximately 15:00h every day from D -2 to D +4. The presence of a vaginal plug represented D +1 of pregnancy or pseudopregnancy. The uterus was flushed with Tyrode’s solution. Immediately after flushing, the uterus was opened and the endometrial tissue scrapped from the uterus into Tyrode’s solution. The cells were disrupted by sonication. The protein concentration of both the flushings and scrapping were assayed (BioRad). The specific activity of PAF acetylhydrolase was assayed using a radiometric assay as previously described (1).

AH. Specific Activity (pmoles acetate released/mg protein/min) (mean ± SEM)

<table>
<thead>
<tr>
<th>Day of Cycle</th>
<th>Uterine Fluids</th>
<th>Endometrial Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2</td>
<td>259.50 ± 80.60</td>
<td>19.35 ± 2.49</td>
</tr>
<tr>
<td>-1</td>
<td>52.94 ± 12.73</td>
<td>18.33 ± 3.20</td>
</tr>
<tr>
<td>0</td>
<td>47.03 ± 9.43</td>
<td>15.33 ± 3.20</td>
</tr>
<tr>
<td>Day of Pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+1</td>
<td>63.95 ± 11.69</td>
<td>8.33 ± 1.52</td>
</tr>
<tr>
<td>+2</td>
<td>28.56 ± 7.38</td>
<td>9.50 ± 9.50</td>
</tr>
<tr>
<td>+3</td>
<td>14.04 ± 7.27</td>
<td>9.08 ± 1.70</td>
</tr>
<tr>
<td>+4</td>
<td>18.82 ± 6.62</td>
<td>11.01 ± 1.50</td>
</tr>
</tbody>
</table>

Acetylhydrolase showed a significant reduction (p < 0.01) in activity from the beginning of the cycle to the time of ovulation (D 0), and a further significant reduction (p < 0.001) from the 1st to the 4th day of pregnancy (day of implantation). During corresponding periods the activity in endometrial tissue did not change. The profile in the fluids and tissue of pseudopregnant animals was similar to pregnancy. The results suggest that coincident with two key reproductive events (fertilisation and implantation) in which PAF is claimed to play a role, PAF metabolism may be reduced resulting in its prolonged half-life and hence activity within the reproductive tract.

LOCALISATION OF A PREGNANCY-ASSOCIATED MOLECULE (SBU-59) IN SHEEP

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Our previous studies have shown that the monoclonal antibody, SBU-3 raised against trophoblast microvilli from the sheep placenta recognises a population of binucleate cells and the syncytiotrophoblast (1). Recently a monoclonal antibody, designated SBU-59, raised against purified SBU-3 glycoproteins was found to have a broader spectrum of reactivity in ovine placental tissue than SBU-3. The present studies examine the distribution of this glycoprotein in the placenta and other reproductive tissues of the sheep.

Pregnant sheep uteri at 29 to 130 days of gestation were collected from an abattoir. Tissues from placentomes and interplacentomal areas were fixed in 95% cold ethanol for immunohistochemical studies. Histological sections of 5 to 6 µm were stained with the indirect immunoperoxidase technique. In addition histological sections of foetal and cycling ovary, one cell oocyte, morula and blastocyst of sheep were stained as described above.

In early pregnancy the glycoproteins recognised by SBU-59 were confined to the binucleate cells of the placental region; positive staining also appeared in the maternal tissue as a result of the fusion of binucleate cells with maternal uterine epithelial cells. In addition to staining the binucleate cells in the placenta, SBU-59 also recognised a subpopulation of binucleate cells present in the interplacentomal areas. As trophoblast erosion of the interplacentomal uterine epithelium occurred (35-40 days) SBU-59 positive binucleate cells were found interspersed or fused with the interplacentomal uterine epithelium. SBU-59 molecules present on the apical surface of the uterine epithelial cells, the intercellular space of the uterine epithelial cells, and the luminal and epithelial cells of the endometrial glands located at the upper region of the stroma were probably secreted by these binucleate cells. It is also interesting to note that SBU-59 also stains the primary oocytes of sheep foetus, the primary oocytes of the ovary, and the trophoblast of early blastocyst.

It is concluded that the interplacentomal SBU-59 positive binucleate cells play an active role in the transitory erosion of the interplacentomal uterine epithelium. Furthermore, the positive SBU-59 staining of oocytes and preimplantation embryos suggests that this monoclonal antibody may be used to study cellular differentiation in early embryogenesis.


ENDOCRINE INTERACTION OF THE HUMAN TROPHOBLAST AND DECIDUA: EFFECTS OF DECIDUAL SECRETIONS ON TROPHOBLAST STEROIDOGENESIS.

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Endocrine interaction between the trophoblast and adjacent decidua is likely to play an important role in the establishment and maintenance of mammalian pregnancy. In humans, the decidua is a rich source of insulin-like growth factor binding protein-1 (IGFBP-1) and prolactin (PRL), but the biological role of these decidual products has not been fully explored. In the present study, we investigated the possibility that decidual secretions exert a local, paracrine effect on the steroidogenic activity of the villous trophoblast. Decidual IGFBP-1 may be particularly important in this regard, since IGF-I and -II have both been shown to affect trophoblast steroidogenesis. The steroidogenic activity of human trophoblast cells was measured in the presence of decidual culture medium (DCM), containing secretory products of decidual explants. Because any action of IGFBP-1 is likely to involve interaction with IGFs, the effects of DCM were examined in the presence and absence of exogenous IGF-I.

Human placenta were obtained after uncomplicated C-section at term. Trophoblast cells were isolated from villous fragments using trypsin/DNase dispersion and Percoll gradient purification as previously described. Cells (5x10⁶) were incubated for 24h in control medium (DMEM) alone or with the addition of 50% DCM (see below), 20 ng/ml IGF-I, or both treatments combined. All incubations were carried out on a rocking platform in an atmosphere of 5% CO₂ at 37°C. The DCM had been obtained previously from a 24-h incubation of term decidua parietalis fragments in DMEM. Following their initial 24-h incubation, trophoblast cells were centrifuged and the medium replaced with similar treatment medium containing 1.0 µg/ml of either progesterone (P₄) or androstenedione (Δ₄). Incubation was continued for 24h, and the media was removed and stored for subsequent RIA of progesterone (P₄) and oestradiol (E₂).

In the absence of added precursors, P₄ secretion was 0.8 ± 0.1 ng/3h (mean ± SEM, n=5 placentas), but no E₂ secretion was detectable. The figure shows rates of P₄ and E₂ synthesis in the presence of their respective precursors for all groups. In control incubations, synthesis of P₄ was 47 ± 14 ng/3h and that of E₂ was 625 ± 110 pg/3h. P₄ synthesis was unaffected by IGF-I alone, but increased in response to DCM (11%; P<0.05; ANOVA) and DCM/IGF-I (22%, P<0.05). Conversion of Δ₄-A→E₂ was also unaffected by IGF-I alone, but increased markedly in response to both DCM (3.1-fold; P<0.01) and DCM/IGF-I (2.9-fold; P<0.01).

In conclusion, these data demonstrate that secretory products of the decidua dramatically alter steroidogenic activity of trophoblast cells in vitro. Current studies are aimed at determining whether either of the major decidual products, IGFBP-1 and PRL, account for this stimulation.


Supported by the King Edward Memorial Hospital Research Foundation.
DEPRESSION OF INSULIN RESPONSE TO A GlUCOSE LOAD IN PREGNANT EWES WITH HIGH CIRCULATING PLACENTAL LACTOGEN LEVELS

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1CSIRO Division of Animal Production, Blacktown 2148; 2University of Western Sydney, Hawkesbury, Richmond 2753 and 3NSW Agriculture, Camden 2570

Ovine Placental Lactogen (oPL) is implicated in the reparation of maternal nutrients during pregnancy (1). Its mode and site(s) of action in the proposed sparing of glucose for fetal requirements, are ill-defined. One possible mechanism could be through reduced pancreatic sensitivity to glucose, as ewes carrying more than one fetus display a diminished insulin sensitivity when subjected to a glucose challenge (2).

Medium wool Merino ewes, 3-4 years old (45-60 kg live weight, 125 days post coitus), of three litter sizes, dry (D), single (S) and twin (T) were used. A dose of 0.4 g glucose/kg i.v. was injected 24 hours post-feeding. Blood samples were collected at -15, 0, 20, 35, 55, 95, 155 minutes after the dose. Data were analysed by repeated measures ANOVA. The glucose dose increased insulin concentration. Compared to D and S ewes, T ewes had a lower (P<0.05) insulin concentration peak (Figure 1). T ewes had a higher (P<0.001) level of oPL, 2494 ± 163 ng/ml (n=4), than S ewes 932 ± 94 ng/ml (n=11). Differences in insulin response pattern were also observed. T ewes, but not D and S ewes displayed a biphasic insulin response to the glucose dose, similar to that observed in D ewes given > 0.5 g/kg i.v. (T.R.H. Regnault, unpublished results).

These data suggest that oPL may reduce pancreatic β-cell sensitivity to glucose, thereby favouring non-insulin dependent uptake of glucose by placental tissues. Moreover we speculate that, while reducing peak insulin concentration, the glucose threshold for eliciting a biphasic response may also have been lowered in T ewes. Such a mechanism may facilitate glucose uptake by the gravid uterus, while also ensuring that glucose uptake occurs in insulin dependent tissues.


IMMUNO-CASTRATION OF ADULT CASHMERE BUCKS WITH VAXSTRATE®

Susan J. Godfrey1, Stephen W. Walkden-Brown2, Graeme B. Martin2, Stephen G. Gherardi1, Mark J. Lindsey3 and Ben L. Porter1

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Non-surgical castration has potential applications in the live export of feral goat bucks and the control of unwanted male behaviour in growing bucks. In this study, conducted during the breeding season in the southern hemisphere, we tested whether the commercial anti-GnRH vaccine, Vaxstrate® (Arthur Webster Pty Ltd, Castle Hill, N.S.W., batch No 2542), would reduce testosterone concentrations and testicular size in mature cashmere bucks.

Twenty cashmere bucks (1.5 - 4 yrs) were housed and acclimatised to individual pens and ad libitum feeding of commercial goat pellets (crude protein 17.5%, ME 11.6 MJ/kg) for one month. Bucks either remained as non-immunised controls (5 Entire, 5 Castrated) or were immunised with Vaxstrate® (1 ml sc, n=10). Immunised bucks were given booster immunisations after 2 (Vax 2, n=5) and 4 weeks (Vax 4, n=5). Castration and primary immunisation were performed on 21 January, 1993. Voluntary feed intake was measured daily while liveweight and scrotal circumference were measured weekly for 10 weeks following primary immunisation. Each fortnight (to week 8) jugular blood was sampled every 30 minutes for h and pooled aliquots were assayed for testosterone.

In Entire bucks scrotal circumference and testosterone concentrations increased with the onset of the rut (Fig. 1). Immunisation with Vaxstrate® significantly reduced testicular size and plasma testosterone concentrations (p<0.001, Fig. 1). Testosterone concentrations fell to castrate levels within 2 weeks of booster immunisation. Entire bucks exhibited a decline in voluntary feed intake after week 3. Average intakes of pellets (kg/day) over weeks 5 to 8 were: Entire, 1.36; Vax 2, 2.04; Vax 4, 1.80; and Castrated, 1.91 (sem ± 0.188). Liveweights increased throughout the experiment, but did not differ between treatments (initial 44.5 ± 2.5 kg, final 52.5 ± 2.5 kg, p>0.05).

Vaxstrate® provides effective immuno-castration following two vaccinations at either 2 or 4 week intervals. The results also confirm the autumn decline in voluntary feed intake in cashmere bucks (1) and suggest that it is testosterone dependent.

Supported by the MRC. We thank Mr A. Farnham and Mrs E.J. Speijers for their assistance.

MORPHOLOGICAL DAMAGE TO THE RAM TESTIS AND EPIDIDYMIS FOLLOWING ISCHAEMIA: POSSIBLE IMPLICATIONS IN MALE INFERTILITY

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Blood vessels supplying the testis and epididymis show a predisposition to sclerotic changes in mammals and these changes are also seen in both young and aged men. This study investigated the effects of such vascular anomalies using the ram as a model to determine if there is a link between arteriosclerosis of the spermatic artery and testicular pathology.

Spermatic arteries of 16 rams were partially occluded either unilaterally or bilaterally, using a silk ligature or 1cm length of polyethylene tubing. During an 8 week post-operative period semen samples were assessed using a Hamilton Thorn Research Motility Analyzer as an index of how testicular and epididymal function were affected by ischaemia. At the end of the 8 weeks rams were castrated and the testis and epididymides were prepared for histological analysis. Of the epididymal regions only the caput was analysed as this region is a common site of tubular obstruction which causes male infertility.

Morphometric results indicated that a range of ischaemic insult had been induced in the experimental testes and epididymides (n=22) and this was evidenced by a significant decrease (P<0.05) in the % spermatogenic epithelium, the height of the seminiferous tubule epithelium and the diameter of the tubule lumen compared to the contralateral (n=10) and control (n=5) testes. The decrease in seminiferous tubule diameter was accompanied by a decrease in the tubule diameter of the caput epididymis (r=0.61; P<0.01). The testicular biopsy score count (TBSC) averaged 5.81 ± 0.72 (SEM) in the experimental testes, indicating a general absence of spermatozoa and spermatid, in contrast to the contralateral (8.66 ± 0.08) and control (8.88 ± 0.16) testes which contained a full complement of spermatogenic cells. This score highlighted the focal damage that ensued within the testes as a result of ischaemic insult, a feature quite typical of many human pathologies. A histological description of the progression of testicular and epididymal damage due to increased degrees of ischaemia will be presented. One prominent feature of the induced oxidative damage was the accumulation of lipofuscin within various tissue compartments of the testes and epididymides indicating the involvement of reactive oxygen species in the tissues’ demise.

Sperm concentration declined by a range of degrees depending upon the severity of ischaemic damage and there was an exponential relationship between this measure and the TBSC (r=0.82; P<0.01). Testis weight also decreased with ischaemia and when plotted against both % spermatogenic epithelium (r=0.73; P<0.01) and the TBSC (r=0.73; P<0.01) a quadratic relationship was evident. These revealed a threshold weight below which the normal spermatogenic epithelium is structurally impaired. Further, there was a positive linear correlation between testicular weight and sperm output (r=0.82; P<0.01).

We hypothesize that similar morphological damage occurs in human males exhibiting arteriosclerosis of the spermatic artery and that this may be a significant contributing factor to their fertility.


CHRONIC OESTRADIOL TREATMENT OF OVARIECTOMIZED EWES IN THE NON-BREEDING SEASON DOES NOT AFFECT THE DENSITY OF NPY TERMINALS IN THE HYPOTHALAMUS

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In rats and rabbits, the effects of neuropeptide Y (NPY) on LH secretion are steroid dependent. In contrast, recent studies on sheep (1,2) have shown that gonadal steroids do not influence the effect of NPY on basal LH secretion, although differential effects have been noted around estrus (2,3). The present study aimed to investigate the effects of oestradiol (E) on the immunocytochemical expression of NPY terminals within the arcuate nucleus-median eminence (ARC-ME), medial preoptic area (MPOA) and organum vasculosum of the lamina terminalis (OVLT) of the Corriedale ewe during anoestrus when the negative feedback effect of E is maximal.

Twelve OVX ewes were divided into 2 equal groups. One group received a 3cm s.c. implant of E at least 8 days prior to sacrifice, and the other group remained untreated. Matched pairs of sheep from each group were decapitated, and their brains perfused with PBS and a modified Bouin’s fixative and then post-fixed in the same Bouin’s solution for 8 hrs. After cryoprotection in 25% sucrose, pairs of brains were sectioned, and the rostra-caudal extent of the ARC-ME, MPOA, and OVLT of each pair of sheep were immunostained under identical treatment conditions with a polyclonal antibody to NPY. The paraventricular nucleus (PVN) was also sampled as a control area. Pairs of sections (n=3-6, depending on nucleus/area) through each brain region were image analyzed by sampling bilateral areas (n=4-16) of each coronal section of the area under investigation, via a fixed sub-area thresholding technique. The mean % area and spatial density of immunostained terminals within each region was obtained for each pair of sections and totals obtained for each nucleus/area. There was no significant difference between E treated and OVX sheep in % immunostained area or density of NPY terminals within the ARC, ME, PVN, MPOA or OVLT. The data suggest that E does not influence the expression and/or release of NPY from terminals within the POA-OVLT (region of GnRH cell bodies) or ME (region of GnRH terminals) which is in line with previous evidence showing that NPY has the same effect on LH secretion in OVX, intact and OVX + E treated ewes.

Ovulation is initiated by the luteinizing hormone (LH) surge, involves plasminogen activator (PA) directed degradation of the preovulatory follicle wall (1) and has been suggested as being akin to an inflammatory process (2). Recent data indicates that cytokines, such as interleukin-1 beta (IL-1β) and tumour necrosis factor alpha (TNFα), derived from leukocytes infiltrating the preovulatory ovary, can promote ovulation (3,4) and these cytokines are known to at least partially govern the regulation of the PA response in non-ovarian systems (5).

The aim of this study was to investigate the effects of IL-1β and TNFα on ovulation induced PA activity of incubated preovulatory follicles and isolated perfused ovaries of the rat.

Preovulatory follicles of equine chorionic gonadotrophin (16IU) primed Sprague Dawley rats were incubated with ovine LH (1μg/ml), recombinant human IL-1β (0.1-10ng/ml), recombinant human TNFα (1-100ng/ml), IL-1β (1pg/ml-10ng/ml) ± LH or TNFα (1-100ng/ml) ± LH for 14 hours. Alternatively, follicles were incubated with LH (1μg/ml), IL-1β (10ng/ml) ± LH or TNFα (100ng/ml) ± LH for 6 hours. Whole rat ovaries were perfused for 8 hours with LH (0.1μg/ml), IL-1β (4ng/ml) ± LH or TNFα (40ng/ml) ± LH. PA activity in follicular and ovarian homogenates was determined by a labelled fibrinolytic assay. Additionally, secreted levels of the ovulatory mediators progesterone (P) and prostaglandin E (PGE) were measured by RIA to determine follicular viability. The presence of leukocytes in the follicle wall was confirmed using immunohistochemical localization.

IL-1β induced significant 2.5-fold and 4-fold rises in PA activity in 6 and 14 hour follicle incubations, respectively, and enhanced PA activity in perfused ovaries 4-fold. In each case and at each concentration, IL-1β and TNFα, alone, had no effect. Coincubation with LH and IL-1β significantly inhibited the LH-induced increase in PA activity, up to 80% in the 14 hour incubations and 95% in the perfusion studies, but not after 6 hour follicle incubations. TNFα in the presence of LH revealed the same inhibitory trend, but was not significant in the 14 and 6 hour follicle incubations, although inhibiting LH-induced PA activity by 95% in perfused rat ovaries. P and PGE results were as expected for viable follicles. Monocytes/macrophages and neutrophils were positively identified in the thecal layer of isolated follicles.

In summary, IL-1β dose and time-dependently inhibited the LH-induced increase in PA activity in rat preovulatory follicles while increasing P and PGE synthesis. The PA results were confirmed in the perfused rat ovary. Results of the perfused rat ovary indicate TNFα may also play a similar inhibitory role, but the follicle incubation results were less definitive. IL-1β and TNFα have been shown to promote ovulation (3,4), but this study suggests IL-1β (and possibly TNFα) may also be mediators of a negative-feedback loop designed to regulate the intensity of LH activation of PA at ovulation.

**References:**


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**THE ROLE OF AN OESTRUS-ASSOCIATED OVIDUCAL GLYCOPROTEIN IN THE EARLY DEVELOPMENT OF THE SHEEP EMBRYO**

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It is clearly established that in vitro culture of ovine embryos using synthetic oviduct fluid (SOF) and human serum (HS) results in several developmental abnormalities, including early blastocoele formation and decreased numbers of nuclei per blastocyst (1). Preliminary experiments involving the incorporation of a fraction (F1) of oviduct fluid (OF), enriched with an oestrus-associated oviducal glycoprotein (EOP), into the existing embryo culture system indicated that EOP significantly increased the number of nuclei per blastocyst (2). The present work examines more closely the effects of F1 on early embryo development.

OF was fractionated (F1,F2) by gel filtration using a Superose 12 FPLC column (Pharmacia). F1 contained predominantly EOP while F2 consisted largely of albumin. One cell sheep embryos were incubated in synthetic oviduct fluid (SOF) containing human serum (HS) (10% or 20%) in the presence and absence of F1 and F2. All embryos were incubated in 0.5 ml of the appropriate culture medium under paraffin oil at 39°C in a gas phase of 5% CO2, 5% O2 and 90% N2. Cleavage of embryos was examined by light microscopy after 24 h and blastocyst formation was assessed every 8 h from day 4 of culture. Newly formed blastocysts were removed from culture, placed in fixative for a minimum of 36 h and nuclei numbers assessed by aceto-orcein staining.

**Table 1:**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% divided embryos after 24 h</th>
<th>% formation of blastocysts from cleaved embryos (no.)</th>
<th>Day of blastocyst formation</th>
<th>Mean no. nuclei per blastocyst</th>
<th>Total no. embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOF+HS</td>
<td>71.9±4.6 b</td>
<td>55.8±19.9 a</td>
<td>4.3±0.1 b</td>
<td>43±6.2 b</td>
<td>70</td>
</tr>
<tr>
<td>SOF+HS+F1</td>
<td>71.9±4.6 a</td>
<td>70.3±33 a</td>
<td>5.3±0.1 a</td>
<td>50±4.1 a</td>
<td>78</td>
</tr>
</tbody>
</table>

Means with different superscripts differed significantly p<0.05

The number of embryos cleaving in the presence of F1 was significantly lower than in other treatments (Chi-square). However, the percent of blastocysts forming from cleaved embryos did not differ between treatments. Time taken for blastocyst formation and the mean number of nuclei per blastocyst were both significantly increased (ANOVA) in the presence of F1. These results suggest that F1 may delay the development of some one cell embryos when compared to the control culture system. The presence of F1 slows down the rate at which blastocyst formation occurs and this may be directly responsible for the increase in numbers of nuclei in blastocysts. Preliminary data suggest that the effect of F1 is concentration dependent and this aspect is presently being investigated. In conclusion, these results strongly imply that EOP may play an important role in the regulation of early embryo development.

NUTRIENT UPTAKE DURING EMBRYONIC DIAPAUSE AND REACTIVATION IN TAMMAR WALLABY BLASTOCYSTS

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The tammar wallaby, Macropus eugenii, maintains a diapausing blastocyst in the uterus while suckling young. Blastocyst reactivation can be induced by removal of the suckling young. Reactivation in the wallaby blastocyst, as estimated by uridine and thymidine incorporation (1,2) and mitosis (3), occurs around 4 to 5 days after removal of pouch young (RPY), while blastocyst diameter does not alter significantly until day 8 after RPY (4). Synthesis of cellular material and ion transport, necessary for cell proliferation and blastocyst expansion respectively, require considerable utilisation of metabolites. It is therefore expected that substrate uptake increases over the period of reactivation. While it has been shown that glucose incorporation has increased by day 5 after RPY (5), it is not known which substrate(s) is used to provide the energy required during reactivation, or at what stage metabolic reactivation is initiated.

Glucose and pyruvate uptake by individual blastocysts was assessed on selected days after RPY, using ultramicrofluorescence (6). Blastocysts were incubated individually at 37°C for 3h in 1μl drops of phosphate buffered salt solution containing 0.5 mM glucose and pyruvate, supplemented with 2 mg/ml BSA. Serial nanolitre samples of medium were taken every 30 minutes for metabolite analysis and linear rates of nutrient uptake determined.

<table>
<thead>
<tr>
<th>Days after RPY</th>
<th>n</th>
<th>Glucose uptake (pmol/embryo/h)</th>
<th>Pyruvate uptake (pmol/embryo/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>23.4 ± 1.0</td>
<td>8.0 ± 1.7</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3.0 ± 1.0</td>
<td>3.2 ± 1.3</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>11.4 ± 4.3</td>
<td>1.1 ± 2.0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>8.4 ± 2.1</td>
<td>6.0 ± 3.0</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>11.6 ± 7.3</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>174.0 ± 28.4*</td>
<td>84.0 ± 27.1</td>
</tr>
</tbody>
</table>

Uptakes are mean ± sem. *Significantly different to day 0, P<0.05.

In contrast to previous work (5), no increase in glucose uptake before expansion of the blastocyst was evident. There was no significant increase in glucose uptake until day 10 (P>0.05) despite the increased mitotic activity of blastocysts from day 4 after RPY (3). It is therefore conceivable that the reactivating wallaby blastocyst uses an alternative energy source, such as amino or fatty acids for the resumption of cell activity, while glucose is utilised during the expansion of the blastocyst.


ENUCLEATION BY CENTRIFUGATION FOR BOVINE NUCLEAR TRANSPLANTATION

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Nuclear transplantation (NT) has the potential to produce large numbers of practically identical progeny. Before reprogramming of a donor nucleus can take place the recipient oocyte must have its genetic material removed or destroyed. Traditionally micromanipulation, a highly skilled, expensive and time consuming technique was used to enucleate metaphase II (M II) oocytes. We report the use of centrifugation in a Percoll gradient to rapidly enucleate large numbers of oocytes for use in successful production of NT embryos. Enucleation by centrifugation relies on the separation of oocytes into fragments in such a way that the M II plate breaks from the oocyte as it is stretched apart.

Bovine oocytes aspirated from atretic collected ovaries were matured in TCM 199 plus FCS and hormones for 22 hours. Cumulus cells were removed by pipetting in 0.5% hyaluronidase and oocytes which had extruded the first polar body selected (176/278; 63%). Enucleation occurred at 24 hours post maturation (hpm), group 1 oocytes were enucleated via micromanipulation. Group 2 oocytes had the zona removed in 0.5% Pronase and 20 oocytes were centrifuged in 0.4 ml tubes in 100 μl each of 7.5, 30 and 45% iso-osmotic Percoll (1) containing 10 μg/ml cytochalasin B at 5000 g for 4 seconds. Group 3 oocytes were centrifuged at 15000 g for 2 minutes before having the zona removed and centrifuged as in group 2.

Enucleation of micromanipulated and centrifuged oocytes was checked by staining the biopsied karyoplast or a sample of the fragments with Hoechst 33342 and viewing via epifluorescent microscopy at 32 hpm. Enucleated oocytes were reconstituted with blastomeres from in vivo produced frozen/thawed bovine embryos via micromanipulation (group 1) or by aggregation to a fragment using 100 μg/ml phytohemagglutinin (groups 2 and 3).

Electrofusion was achieved using a BTX ECM 200 and Optimizer with Zimmerman’s fusion media. After manual alignment an AC pulse of 8V for 5 seconds was followed by fusion via a single DC pulse of 77V (1.75 kV/cm) for 40 μs, fusion was assessed 15 minutes later. Fused oocytes from groups 2 and 3 were placed in a false alginate zone and all NT embryos were cultured in SOF plus amino acids under mineral oil with 4 embryos per 30 μl drop changed every 48 hours (2). Cleavage was assessed 40 hours post fusion and development to morula day 6 post fusion.

<table>
<thead>
<tr>
<th>Group</th>
<th>Enucleation (%)</th>
<th>Fusion (%)</th>
<th>Cleavage (%)</th>
<th>Morula (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24/50 (48)</td>
<td>15/24 (63)</td>
<td>8/15 (53)</td>
<td>1/8 (13)</td>
</tr>
<tr>
<td>2</td>
<td>68/76 (90)</td>
<td>11/16 (69)</td>
<td>3/11 (27)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3</td>
<td>69/76 (89)</td>
<td>18/21 (86)</td>
<td>6/18 (33)</td>
<td>1/11 (9)</td>
</tr>
</tbody>
</table>

TEM studies of centrifuged oocytes show the stratification of cytoplasm into 5 phases according to density of its components. Lipid being least dense is found at the upper pole, followed by membrane bound vesicles, smooth ER, a large organelle free region containing the MI plate and at the lower pole mitochondria are concentrated.

Enucleation results in a higher enucleation rate than micromanipulation (89% vs 48%). It is partially due to the emission of identifiable nuclear fragments which possess an extrusion cone containing the MI plate. Electrofusion rates were similar between groups (63% to 86%). Development of NT embryos produced in groups 1 and 3 was similar (13% vs 9%). Group 2 fragments were darker than the opaque group 3 fragments which contain less lipid and organelles, suggesting that successful NT may rely on a cytoplasmic factor. Full developmental capacity is currently being tested via transfer of NT embryos into recipient cows. Enucleation by centrifugation enables the rapid production of large numbers of enucleated oocyte fragments which can be used for the successful production of bovine nuclear transplantation embryos.

IN SITU REMOVAL OF EMBRYO-TOXIC AMMONIUM GENERATED BY THE METABOLISM AND BREAKDOWN OF AMINO ACIDS IN CULTURE MEDIA

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Mouse embryo development in culture is significantly increased by the inclusion of specific amino acids in the medium (1). However, amino acids are metabolised by embryos and break down at 37°C to produce ammonium in the culture medium at a rate of 100μM every 24h. Ammonium is detrimental to mouse embryo development in vitro at a concentration as low as 75μM. During extended culture periods it is necessary to renew the medium every 48h if amino acids are included in the formulation. However, preimplantation embryos produce an autocrine factor(s) that stimulates development (2,3). Renewing the medium may therefore deprive the embryo of the beneficial effect(s) of this factor(s).

The aim of this study was to develop a method of enzymatically removing ammonium from the culture medium. The proposed mechanism of removal was based on the following reaction:

Glutamate dehydrogenase (plus ADP as an enzyme activator)

$$\text{NH}_2^+ + \text{NAD}^+ + \alpha\text{-ketoglutarate} \rightarrow \text{Glutamate} + \text{NAD}^+ + \text{H}^+$$

Initially, zygotes (C57BL/6 x CBA/Ca) were cultured in groups of 10 in 20μl drops of medium mMTF (1) under paraffin oil 5% CO₂ in air at 37°C. At the 2-cell stage the individual assay components of the cocktail were titrated into the medium by a 1μl injection of a x20 stock. Blastocyst formation and cell number were assessed after 96h of culture. Neither α-ketoglutarate nor glutamate dehydrogenase had an effect on blastocyst formation or cell number (P<0.01) at concentrations from 0.03-0.2mM. In contrast, ADP inhibited both blastocyst development (P<0.05) and cell number (P<0.01) at a concentration as low as 0.08μM. The concentration of glutamate in the culture medium, as determined after the conversion of ammonium was determined using microfluorometry and found to be around 0.25mM. Glutamate at this concentration significantly increased blastocyst formation (P<0.05).

Only ADP, an activator of glutamate dehydrogenase, was inhibitory to embryo development. This observed inhibition is consistent with the known detrimental effects of adenine nucleotides on embryos. In the absence of ADP the reaction still progressed, although at a slower rate, and converted 0.3mM ammonium in 3h. The final enzyme cocktail was therefore: NADH (0.12mM), α-ketoglutarate (0.4mM) and glutamate dehydrogenase (0.375U). Addition of this cocktail after 48h of culture in the presence of ammonium significantly stimulated cleavage (p<0.01) at concentrations from 0.03-0.2mM. In contrast, ADP inhibited both blastocyst development (P<0.05) and cell number (P<0.01) at concentrations of glutamate from 0.03-0.2mM. In contrast, ADP inhibited both blastocyst development (P<0.05) and cell number (P<0.01) at concentrations of glutamate from 0.03-0.2mM.

The endocrine and spermatogenic functions of the testis are thought to be controlled by gonadotrophins. We describe an experiment in which differential nutrition during the non-breeding season induced a large change in testicular size in goat bucks without concomitant changes in peripheral concentrations of LH, FSH or testosterone.

Murine castrate bucks were fed ad libitum diets of High (CP 17.6%, ME 8.3 MJ/kg) or Maintenance (CP 6.5%, ME 6.6 MJ/kg) quality for 16 weeks starting in early July at Wollongbar (29°S 153°E) (n=6/diet). Every week live weight was measured, testicular weight estimated from scrotal circumference and three plasma samples collected at 40 min intervals were poured for hormone assay. During weeks -1, 1, 4 and 13 relative to the onset of dietary treatments, blood was sampled every 20 minutes for 8 hours and assayed for LH and testosterone. Data was analysed by repeated measures ANOVA.

The High diet induced large increases in liveweight and testicular weight (p<0.001, Fig. 1). However there was no effect of diet (main effect of interaction with week) on weekly concentrations of LH, FSH or testosterone which remained at low concentrations throughout the experiment (LH 2.6±0.24 ng/ml; FSH 0.86±0.68 ng/ml; testosterone 0.7±0.4 ng/ml). Data from the pulse bleeds supported the weekly measurements with no effects of diet on LH pulse frequency (1.0±0.16 pulses/8h), mean LH concentration (2.6±0.20 ng/ml) or testosterone pulse frequency (1.3±0.15 pulses/8h). However mean testosterone concentration (overall mean 1.1±0.17) was significantly elevated in High bucks at week 13 (2.7±0.45 v. 1.4±0.21 ng/ml, p<0.05).

Figure 1. Liveweight (a) and testicular weight (b) in bucks fed High (e) or Maintenance (e) diets.

The change in testicular weight induced by nutrition in this experiment (51%) was much greater than that occurring during the seasonal cycle in bucks on a maintenance diet (17%) (1). However, in contrast with the seasonal pattern, it was not associated with clear or sustained changes in either gonadotrophin or testosterone concentrations which remained at the low levels typical of the non-breeding season (1). These data suggest that nutrition may affect testicular weight, and therefore sperm production, without affecting testosterone secretion, and that this effect may be independent of changes in gonadotrophin concentrations.

GONADOTROPIN AND TESTOSTERONE CONCENTRATIONS AND TESTICULAR GROWTH IN RAMS SUPPLEMENTED WITH LUPINS FROM BIRTH TO PUBERTY

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During early life in rams, the marked rise in plasma FSH levels influences the development of Sertoli cells (1) and consequently spermatogenesis. Since supplementation of adult rams with lupin grain produces an increase in plasma FSH concentrations (2), it is possible that such supplementation of ram lambs early in life may enhance their subsequent sperm producing capacity.

Two groups of Merino ram lambs (n=15 per group) were reared in adjoining paddocks until they were 14 months of age. Group A served as non-supplemented controls. From 2 weeks before parturition until weaning at 12 weeks of age, the ewes in Group B were supplemented with lupin grain at a rate of 500g per ewe per day, fed twice weekly; from weaning to puberty (28 to 30 weeks of age), the lambs in this group were fed lupin grain at a rate of 10g/kg body weight per day, fed twice weekly. At frequent intervals, the lambs were weighed to assess growth rates, estimates of testicular weights (TW) were obtained using an orchidometer and blood (5ml) was sampled from a jugular vein for measurement of FSH. Around puberty, jugular blood (5ml) was obtained from each lamb at 20min intervals for 12h for measurement of plasma LH and testosterone (T) concentrations.

Lams in Group B grew at a faster rate (mean 207g/day) than control lambs (197g/day) from birth to puberty, with the result that the animals in the former group had a significantly greater mean body weight than those in the latter group, both at puberty and at 14 months of age (mean ± s.e.m. 37.0±0.7 v's 32.9±0.9, t = 4.20; 52.4 ± 0.7 v's 46.9 ± 0.8, t = 5.09, respectively, P<0.001).

Lupin supplementation of rams early in life advances peak FSH secretion, increases body growth rate and testicular weight, with likely beneficial effects on the sperm producing capacity of the animals.


CHANGES IN OVARIAN MORPHOLOGY ASSOCIATED WITH DIETARY INTAKE IN BOS INDICUS HEIFERS

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The effect of level of dietary intake on follicular dynamics has been examined in Bos taurus heifers (1), however the sequential changes which occur in the ovary in the period preceding the onset of nutritional anoestrus have not been reported. The aim of this study was to investigate the effects of dietary restriction on ovarian function in Bos indicus heifers.

The ovaries of 17 post-pubertal maiden Brahman heifers were examined daily, using a linear ultrasound scanner equipped with a 7.5 MHz transducer (Aloka 210 DX). The growth and regression of individual follicles >5mm, and the diameter of the corpus luteum (CL) were recorded (2). Twelve heifers were randomly allocated to a restricted diet (TRT) in order to produce a decrease in liveweight until the onset of anoestrus. The other five heifers remained on an unrestricted diet (CIL). The linear rate of change of maximum follicle and CL diameter, and initial size were compared between groups, using a random coefficients regression model (3).

Mean (±sem) LWs at the start of the study period were 318.8±3.96 and 316.2±3.72 kg for CIL and TRT heifers, respectively. A mean reduction in LW of 250g. was observed during the treatment period in the TRT heifers; during the same period LW of the CIL group increased by 67±7.49 kg (21.2%). There was a significant difference between the groups in the rate of change in follicle and CL size (P<0.001) associated with the decrease in LW in the TRT animals, although there were no differences in initial diameters (Fig 1).

Mean plasma FSH levels for rams in Group B peaked 10-20 days earlier than that for Group A and remained at an elevated level for 10-20 days longer. There was no significant difference between the 2 groups in the number of animals exhibiting LH or T pulses in 12h. Around puberty, mean TW for Group B was significantly greater than that for Group A (155.6g ± 14.4 v's 119.4 ± 9.9, t = 2.07, P<0.05). At 14 months of age, mean TW for the 2 groups did not differ significantly but a greater proportion of rams in Group B (75%), than in Group A (53%), had TW ≥ 250g.

Data suggest that lupin supplementation of rams early in life advances peak FSH secretion, increases body growth rate and testicular weight, with likely beneficial effects on the sperm producing capacity of the animals.


This study was partially funded by the Meat Research Corporation.
EFFECT OF LEVEL OF DIETARY PROTEIN INTAKE ON THE PLASMA LEVELS OF PROGESTERONE IN OVARIECTOMISED EWES TREATED WITH CIDR® DEVICES

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Studies into the mechanisms of nutritional influences on ovulation rate (1) have suggested that increased protein intake stimulates the level of liver steroid metabolism (2). These trials were conducted to examine the effects of dietary protein intake on circulating levels of plasma progesterone.

Trial 1. Twenty four ovariectomised ewes fitted with abomasal catheters and fed on a low protein diet (100 g/d) were allocated to four groups. Two groups were given abomasal infusions of protein (Alacen 100g/ewe/d) for 16 days while the other groups received infusions of equivalent volume of water (1 l/d). Ewes in one group of each of the infusion treatments were treated with new CIDR® devices on day 4 and these were removed 11 days later. The other groups were treated with CIDR devices that had previously been inserted into ewes for 14 days (used). Daily blood samples for progesterone assay (RIA) were taken at 11.30 h each day of the trial and at 2 h intervals for 8 h on 2 days following both insertion and removal of devices.

Trial 2. Involved 10 ewes in a cross-over design where those infused with protein during the first infusion period were retreated with the same CIDR devices (5 new - 5 used) while receiving water infusions.

In trial 1 there were significant effects of CIDR type on the pattern of plasma progesterone profiles. Mean values 8 h after insertion were 3.7±0.28 ng/ml and 1.05±0.10 ng/ml for new and used devices respectively, while on days 4 and 11 values were 1.7±0.2 and 0.90±0.1 for new and 0.65±0.06 and 0.45±0.06 for used devices. Within 2 h of device removal base levels of 0.15 ng/ml were recorded in all groups. There was no significant effect of protein treatment. However, between ewe variation within groups was very high with some ewes having levels 2-3 times that of others.

Trial 2 produced similar patterns for the different types of devices as in trial 1. However, there was an interaction with protein treatment which had the effect of depressing the levels seen in ewes treated with new CIDRs. Mean levels at 8 h after insertion were 3.1±0.3 and 4.8±0.4 for the protein and water group respectively while for days 4 and 11 values were (1.5±0.3 v 2.2±0.3) and (0.9±0.2 v 1.3±0.2). These differences were significant in 3 of the 5 ewes. Measurement of CIDR progesterone contents indicated no between animal or nutritional effects. Pre- and post-treatment values were 345 mg and 150 mg for new and 110 mg and 75 mg for used devices.

These results indicate that the absorption of progesterone from CIDR devices is virtually identical for all animals and that different circulating levels reflect the animal's metabolism. Within individual animals, the level of protein intake may modify the clearance rate of progesterone and this supports the suggestion of altered steroid metabolism being involved in the nutritional control of ovulation rate.


THE FREQUENCY OF RELEASE OF LH IN ANOESTROUS CORRIEDALE EWES IS INFLUENCED BY NUTRIENT STATUS BUT NOT BY POST-PARTUM STATUS

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The responsiveness of ewes to measures to induce ovarian cyclicity reflects their depth of anoestrus. The depth of anoestrus may be affected by nutrition and by post-partum status. We have studied the influence of these factors on the pulsatile release of LH in ewes during the anoestrous season as part of our investigations of the endocrine basis of the depth of anoestrus.

The frequency of release of plasma LH was determined during December in mature anoestrous Corriedale ewes that were i) 11-23 days post partum and of body condition score 2-3 (Group CPP, n=19), ii) non-post partum and of condition score ≥3 (CN, 12) and iii) of condition score ≤2 (CR, 9). Blood samples for plasma LH determination were taken each 10 min for 12 hr. CN and CR ewes were ovariectomised 2 days later and implants (0.5cm) containing oestradiol were placed s.c. Blood samples were taken 12 days later, the implants removed and blood samples taken again 14 days later.

Plasma LH patterns in ewes (ovary-intact - OV-I, ovariectomised - OVx, OVx with oestradiol implant s.c - OVx-imp) during the anoestrous season

<table>
<thead>
<tr>
<th>GROUP STATUS</th>
<th>Number of ewes with plasma LH pulse frequency/12 hr of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>CPP</td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>5</td>
</tr>
<tr>
<td>OV-I</td>
<td>2</td>
</tr>
<tr>
<td>OVx-imp</td>
<td>1</td>
</tr>
<tr>
<td>OVx</td>
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</tbody>
</table>

Plasma LH pulse frequency in i) ovary-intact ewes was similar in CPP and CN but lower (P<0.05) in CR ewes, in ii) ovariectomised ewes with oestradiol implants was lower in CR ewes than in CN ewes, and iii) in ovariectomised ewes without implants was similar in CN and CR ewes.

These results suggest that the increased depth of anoestrus in ewes of reduced nutrient status may be associated with a greater degree of sensitivity of the hypotalamic-pituitary axis to the inhibitory effects of oestradiol on the secretion of LH than in ewes of better nutrient status.
HUMAN RECOMBINANT INHIBIN PARTIALLY REVERSES THE HEMICAstration INDUCED RISE IN SERUM FSH IN THE RAT.

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Testicular hypertrophy is well known to occur following hemicastration in the neonatal period. This hypertrophy is thought to be mediated by elevated FSH levels resulting from a reduction in inhibin levels following hemicastration. It has recently been shown that follicular fluid can inhibit both the hemicastration induced rise in FSH and the consequent testicular hypertrophy in prepubertal rats (1). We have recently demonstrated that human recombinant inhibin (hRI) has little effect on serum FSH levels in young (<20 days) intact animals. We were therefore interested to explore the effect of neonatal hemicastration on testicular development and the influence of hRI in the young hemicastrated animals.

Newborn male rats were hemicastrated or sham operated (n=10) under hypothermia induced anaesthesia. Animals were killed by decapitation and trunk blood collected at 5, 10, 15, 20, 50 and 90 days. Serum FSH and inhibin were measured by specific radioimmunoassay. Elongated sperm production (ESC) was assessed at 50 and 90 days. In a separate experiment, hemicastrates, sham and control groups (n=7) received a subcutaneous injection of 100ng hRI/g body weight or vehicle on day 10. Six hours after injection, animals were killed and testis weight, serum FSH and inhibin was measured.

Testicular weight in hemicastrated animals was significantly increased (1.3-1.5 fold) from day 5 to 90 (p<0.05) and on day 50 and 90, there was a corresponding significant increase in ESC/testis. FSH levels were elevated 1.6-fold on day 5 (p<0.05) and 3-fold on days 10 and 15 (p<0.01). FSH levels remained elevated by <50% from day 20 to 90. Inhibin levels decreased by 32% on day 5, were not significantly different from control at days 10-20 but at days 50 and 90 were significantly decreased (p<0.01). After administration of hRI on day 10, there was a 44% reduction in serum FSH which approached control values. The inhibin levels remained unchanged.

This data confirms the hemicastration induced testicular hypertrophy and concomitant increase in FSH in prepubertal animals. Interestingly, there was not a consistent decrease in serum inhibin associated with this rise in FSH except in very young animals. Administration of hRI resulted in a decrease in serum FSH levels in prepubertal animals to near control values thus partially reversing the effects of hemicastration. Whilst it appears that inhibin is involved in regulating FSH in the newborn, the question of whether inhibin is the major factor remains open. The near normal levels of immunoactive inhibin observed at 10, 15 and 20 days in these animals may reflect the presence of pro-α-C which cross-reacts in the assay.


EFFECT OF STAGE OF BREEDING SEASON ON THE PITUITARY ACTIONS OF INHIBIN AND TESTOSTERONE TO CONTROL FSH SECRETION IN RAMS

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Both inhibin and testosterone have negative feedback actions directly on the pituitary to suppress the plasma concentrations of follicle stimulating hormone (FSH) in rams during the non-breeding season (NBS; 1). It also appears that the suppressive actions of inhibin may be enhanced by testosterone (1). These effects have not been studied during the breeding season (BS), therefore this experiment tested the hypothesis that the pituitary actions of inhibin and testosterone change with season.

The following procedure was conducted during the BS and NBS using 2 groups (n=5) of castrated rams (wethers) that had undergone hypothalamo-pituitary disconnection 2 weeks before use in the experiment and were pulsed with 125ng of GnRH every 2h. Different wethers were used in each season. Jugular samples were collected over 4h then 2x5000 i.v. injections of human recombinant inhibin A (hr-inhibin) or vehicle were given 6h apart commencing 6h after the start of sampling. Jugular samples were then collected for 6h commencing 6h after the second injection. Each wether was then treated for 7 days with 8mg/day of testosterone propionate (TP) given as 4mg i.m. injections every 12h and the sampling and treatment regimen was repeated. The treatment and sampling regimen was then repeated after 7 days of treatment of each wether with 16mg TP/day given as 8mg i.m. injections every 12h then 32mg TP/day given as 16mg i.m. injections every 12h.

Treatment with hr-inhibin and TP significantly (P<0.01) suppressed plasma concentrations of FSH while vehicle had no effect. The stage of the breeding season did not influence the direct effects of either hormone. During the BS the suppressive effects of hr-inhibin were not affected by treatment with TP (47% suppression before TP and 43% suppression with all doses of TP). In contrast, during the NBS, the degree of suppression of plasma FSH due to treatment with hr-inhibin was significantly (P<0.01) greater when the wethers were treated with 16 or 32mg TP/day (75.4% and 84.7% suppression respectively) than when not treated with TP or when receiving 8mg TP/day (51.8% and 55.9% suppression respectively). Overall, the suppression in plasma concentrations of FSH due to treatment with TP alone was 27%, 46% and 51% for 8, 16 and 32mg TP/day respectively.

Our data confirm that both inhibin and testosterone have negative feedback actions on the pituitary to influence FSH secretion in rams. While the stage of the BS did not influence the direct effects of either hormone, the suppressive actions of inhibin were enhanced by testosterone during the NBS but not the BS.

This study examined if accelerated testis growth in bulls treated with Deslorelin (D-Trp\(^6\)-Pro\(^6\)-des-Gly\(^15\)-GnRH ethylamide) (1) is associated with changes in testis morphology and sperm production. Twenty-month-old bulls received 0, 4 or 8 implants formulated to release 50 μg Deslorelin/implant/day (n = 6/group). After 120 days, bulls were castrated and the right testis was perfused with heparinised saline, followed by 5% glutaraldehyde. Testis tissue was postfixed in osmium tetroxide/potassium ferrocyanide and processed into Epon-Araldite. Sections were stained with 1% toluidine blue in borate buffer and mounted under DPX. Stereological estimates were made using an Olympus BH2 microscope fitted with a Panasonic F15 camera; the video image was genlocked to suitable stereological probes generated by the GRID 1.2 package (Graffitidata, Silkeborg, Denmark). Volume fraction of seminiferous epithelium, lumen and interstitium were assessed by point counting at a magnification of 169x. Areal density of round spermatids was assessed at a magnification of 1000x using an unbiased counting frame superimposed on a video image. Numerical density of round spermatids was estimated from the areal density and diameter data using the Rhines de Hoff equation. There were no differences between bulls treated with 4 or 8 implants so the data for these two groups were pooled for ANOVA. Data are reported as means ± SEM. Bulls treated with Deslorelin showed a greater increase in testis diameter compared with control bulls (6.3 ± 0.8 vs 2.6 ± 0.5 mm, P < 0.01) and at the end of treatment the weight of the right testis was heavier in the former bulls (238 ± 18 vs 172 ± 7 g, P < 0.01). Seminiferous tubule diameter was not affected by treatment but the total length of tubules was greater in treated bulls (5054 ± 337 vs 4099 ± 276 μm, P < 0.01). The volume fractions of epithelium, lumen and interstitium were not affected by treatment nor were the absolute volumes of the tubular lumen and interstitium. However, absolute volume of the seminiferous epithelium was greater in treated bulls (168 ± 43 vs 120 ± 14, cm\(^3\), P < 0.01). Numerical density of round spermatids in the testis was not altered but the absolute number of round spermatids per testis was increased in treated bulls (23.5 ± 2.5 x 10\(^6\) vs 16.2 ± 1.4 x 10\(^6\), P < 0.05). These findings indicated that testis growth and sperm production are increased in bulls treated with Deslorelin. The apparent absence of changes in testis morphology would suggest that the response to Deslorelin involves normal growth and maturation of the testes, albeit at an accelerated rate.


**Testis Morphology and Sperm Production in Bulls Receiving the Gonadotrophin Releasing Hormone Agonist Deslorelin**

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We have studied the effects of active immunization against oestradiol-17β (E\(_2\)) on testicular development, testosterone (T) production, testicular blood plasma flow (TBPF) and daily sperm production (DSP). Eight Merino ram lambs received a primary injection of 17β-oestradiol-6-(O-carboxy methyl) oxime-bovine serum albumin conjugate (E\(_2\)-6-BSA) in Freund's complete adjuvant (FCA) at 14 weeks of age (E\(_2\)-immunized). Nine control ram lambs were given bovine serum albumin (BSA) in FCA. Each lamb received a booster injection four weeks later with Freund's incomplete adjuvant (FIA) instead of FCA. Testicular biopsies were taken from 4 controls (CB) and 4 E\(_2\)-immunized (EB) ram lambs at 22 wks of age (left testis) and at 26 wks of age (right testis). The other lambs (5 CN and 4 EN) were not biopsied. The lambs were kept in a room with controlled light (12 hours light: 12 hours dark). All ram lambs were castrated at 30 wks of age (except for the two lightest lambs, 1 CN and 1 EN, which were castrated at 34 wks of age). E\(_2\) binding in diluted (1:5000) plasma from the E\(_2\)-immunized lambs varied between 40 and 60% after the booster injection whereas binding of E\(_2\) remained negligible (less than 4%) in the controls during the whole experiment. Testicular volumes (TV) were slightly increased between 22 and 26 wks of age in the E\(_2\)-immunized lambs however the differences between groups were not statistically significant at any age. We have noticed that TV was decreasing towards the end of the experiment in two lambs in each group. Interestingly, one lamb (#15, EN) exhibited a very important increase in TV between 23 and 25 wks of age followed by a very steep decline. Immunization treatment did not significantly affect testis-epididymis weight at castration but tended to increase TV (p < 0.05). TV was significantly smaller (p < 0.05) in CB lambs compared with CN and EN lambs (TV cm\(^3\) ± SEM: CN: 163.50 ± 18.39; CB: 110.13 ± 14.49; EN: 188.08 ± 23.37; EB: 153.07 ± 11.58). Immunization or biopsy treatment did not significantly affect DSP. The minimum DSP value (0.7 x 10\(^4\) sperm per gram testis) was found in the lamb #15 (EN) which had exhibited the most pronounced decline in TV. However, TBPF per unit weight of testis was significantly higher (p < 0.05) in controls than in E\(_2\)-immunized lambs. Before hCG injection, mean T production per testis was 188.08 ± 23.37, EB: 153.07 ± 11.58. Immunization or biopsy treatment did not significantly affect DSP. The minimum DSP value (0.7 x 10\(^4\) sperm per gram testis) was found in the lamb #15 (EN) which had exhibited the most pronounced decline in TV. However, TBPF per unit weight of testis was significantly higher (p < 0.05) in controls than in E\(_2\)-immunized lambs. Before hCG injection, mean T production per testis was significantly higher (p < 0.05) in CN lambs compared with EN lambs. Before hCG injection, mean T production per testis was significantly higher (p < 0.05) in EN lambs compared with CN lambs (CN: 5.06 ± 2.09, CN: 13.14 ± 5.27 μg/min/testis). At 22 and 26 wks of age, large vacuoles were observed in the epithelium of the seminiferous tubules of one E\(_2\)-immunized lamb (#3, EB). At 30 wks of age, nearly complete absence of germ cells was observed in E\(_2\)-immunized lamb #15 with other apparent signs of degeneration in the seminiferous tubules such as those observed in lamb #3 at 22, 26 and 30 wks of age. We conclude that the presence of oestrogen antibodies in circulation does not result in an acceleration in testicular maturation.

**Active Immunization Against Oestradiol-17β in Developing Ram Lambs Can Have Marked Effects on the Structures and Functions of the Testis.**

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THE EFFECT OF SEXUAL STIMULATION ON TESTOSTERONE IN BOARS IN TWO DIFFERENT MATING SYSTEMS.

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A new mating system for pigs has been developed at Werribee to improve the physical and sexual environment during oestrus detection and mating (1). In this system six boars are housed in stalls surrounding an oestrus detection and mating arena (DMA), in which females are detected for oestrus with the back pressure test and adjacent boars are introduced for mating. In the traditional system, females are introduced to the boar's accommodation pen for oestrus detection and mating.

Six boars in the DMA and six boars in the traditional system were fitted with permanent cannulas to study the levels of sexual stimulation provided to boars in the two systems. To avoid disturbance to the animals during sampling, these cannulas were extended during blood sampling so that the boars could be bled from outside the accommodation shed. Concentrations of testosterone from 0900 to 1700 h, and after sexual stimulation and mating were compared in the two systems. Sexual stimulation was provided by allowing boars to observe a mating from their pen or stall for 15 minutes.

Although base concentrations of testosterone tended to be higher for boars in the DMA, this difference was not statistically significant (P > 0.05). In contrast to boars in the traditional system, boars in the DMA experienced a distinct rise in testosterone concentrations following sexual stimulation (figure 1). This testosterone response of boars in the DMA may be due to increased sexual stimulation. All boars responded to mating with a rise in testosterone concentrations, although this response appears to be less than that following sexual stimulation. As with sexual stimulation, the increase in testosterone concentrations following mating was greater for boars in the DMA than those in the traditional system (figure 2).

The magnitude of the testosterone response of boars in the DMA indicates that this mating system may provide boars with high levels of sexual stimulation, which may have consequence for their reproductive performance. Blood samples are currently being analysed for LH and these data may provide further information on the level of sexual stimulation in the two mating systems.

REFERENCES


Figure: The secretion of oestradiol following infusion of ovine Growth Hormone (hatched band is mean ± sem).

4 and 8 hours after the start of the infusions. Oestradiol and androstenedione levels were assayed in ovarian venous plasma and LH, FSH and GH levels in jugular venous plasma.

Growth hormone (GH) had no effect on androstenedione secretion. Oestradiol secretion in response to a GnRH-induced LH-pulse was increased following the infusion of GH (Figure), an effect that was associated with a reduced LH pulse amplitude.

These data show that short term GH infusions can increase oestradiol secretion following a GnRH induced LH-pulse and therefore suggest a role for GH in mediating steroid secretion from the follicle. Unequivocal effects of GH on androstenedione secretion were not observed and further work is in progress using higher infusions rates of oGH.

EVALUATION OF TRANSOVARIAN UPTAKE OF METABOLITES AND PROGESTERONE SECRETION

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Nutrition influences hypothalamic-pituitary-gonadal function. However, little information is available on the effects of nutrition on steroid hormone production except that undernutrition tends to produce increased progesterone concentration (1). Our study was conducted to determine the uptake of metabolites, and progesterone secretion by ovaries of anaesthetised ewes. The ovarian vein in five crossbred anaesthetised ewes was cannulated, and a blood flow transducer (4R Transonic) placed around the ovarian artery for measuring ovarian blood flow (OBF). Sheep 1 and 2 had dominant follicular structures (P), sheep 3 and 4 had dominant luteal structures (L), and sheep 5 had no significant structures (O). Data (mean and 95% confidence interval for the mean) for OBF, glucose, acetate, cholesterol and progesterone are presented in the table. Blood flow to the L ovaries appears to be higher than F or Q ovaries. Ovarian uptake of cholesterol was significant only in one sheep. Glucose uptake was significant in 3 sheep with different ovarian structures. Only one sheep (O) had significant uptake of acetate. Progesterone secretion was significant in 3 sheep (L and F ovaries).

Table: Uptake per minute of metabolites for ovaries with different dominant structures

<table>
<thead>
<tr>
<th>Structure</th>
<th>OBF (ml/min)</th>
<th>CHOL (mmol/L)</th>
<th>GLUC (mmol/L)</th>
<th>ACET (mmol/L)</th>
<th>PROG (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>2.0 ± 0.2</td>
<td>0</td>
<td>0.6</td>
<td>0.1</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>Q</td>
<td>2.8 ± 0.5</td>
<td>-0.1 ± 0.5</td>
<td>0.9</td>
<td>0.1</td>
<td>-12.3</td>
</tr>
<tr>
<td>L</td>
<td>3.3 ± 0.3</td>
<td>0.5</td>
<td>0.9</td>
<td>0.1</td>
<td>20 ± 0.3</td>
</tr>
<tr>
<td>O</td>
<td>3.2 ± 0.2</td>
<td>0.2</td>
<td>0.6</td>
<td>0.1</td>
<td>20 ± 0.3</td>
</tr>
</tbody>
</table>

* Mean ± 95% Confidence Interval

Higher blood flow to the L ovaries is related to the large luteal vascular bed (2) and is consistent with previous studies (3). Despite previous studies showing that luteal tissue primarily utilises cholesterol for progesterone synthesis (4), our study found that uptake of cholesterol was not significant in most sheep. It appears that acetate was not significantly utilised as a substrate for ovarian steroidogenesis by most sheep. This is consistent with previous studies which found that de novo synthesis of cholesterol from acetate was unlikely (4). The data show considerable variation in glucose uptake. Glucose supplies energy used in hormone synthesis. The reasons for progesterone uptake in F and Q ovaries are unclear. Progesterone secretion in sheep 2 may be due to residual luteal tissue, luteinization of the follicle or normal follicular function. Our findings indicate the need for further research using these methods.


INFLUENCE OF PROLACTIN AND LH ON OVARIAN PROGESTERONE SECRETION DURING LATE PREGNANCY IN THE RAT

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Ovarian secretion of progesterone (P4) falls gradually during late pregnancy in the rat, and there is a subsequent, rapid increase in the secretion of 20α-hydroxyprog-4-en-3-one (20α-OHP) during day 22 (term = day 23). The pre-partum fall in P4 is generally thought to reflect withdrawal of placental luteotrophic support, but the stimulus for the increase in 20α-OHP secretion is not known. It is likely to involve the pituitary, since the rise in 20α-OHP does not occur in rats hypophysectomised at midpregnancy. In the present study, therefore, we tested the possibility that either prolactin or LH, both of which increase rapidly in peripheral blood during day 22, is responsible for the rise in ovarian 20α-OHP secretion.

Rats were anaesthetized with halothane/nitrous oxide on day 17 of pregnancy, and a cannula positioned in the dorsal aorta for subsequent collection of blood samples. At 16.00 h on days 21, 22 and 23, rats were treated with either LH antiserum (LHAS; 0.5 ml ip; n = 5), normal rabbit serum (NRS; 0.5 ml ip; n = 5), bromocriptine (BROMO; 4 mg/kg, 0.4 ml sc; n = 5) or saline vehicle (SAL; 0.4 ml sc; n = 5). Blood samples were obtained from all rats just prior to the initial treatment, and then at 2-h intervals from 8.00 am to 20.00 h on day 22, the period during which the major increase in 20α-OHP secretion occurs. The time of parturition was recorded for each rat (day 23), and ovaries were collected on the morning of day 24. Concentrations of P4 and 20α-OHP were measured by RIA in all samples, and ovaries were examined histologically to determine whether a post-partum ovulation had occurred.

Concentration profiles for both P4 and 20α-OHP were similar in the two control groups (NRS and SAL) from day 21 to 23, and so these groups were combined for subsequent analyses. As shown in Fig 1, 20α-OHP levels increased rapidly in control rats during day 22 (P < 0.01, two-way ANOVA), but in rats treated with BROMO this increase was attenuated. Thus, concentrations of 20α-OHP in BROMO-treated rats were lower (P < 0.05) than those in controls at 16.00, 18.00 and 20.00 h on day 22. BROMO had no apparent effect on P4 secretion. In contrast, LH did not prevent the rise 20α-OHP; indeed, the initial increase in 20α-OHP was evident earlier in this group (by 8.00 h on day 22), and this was associated with a slight fall (P < 0.05) in P4. In rats treated with LH, ovarian weight was reduced by more than 30% (P < 0.05; ANOVA) and there was no evidence of a post-partum ovulation, which occurred in 4/7 control rats and 3/5 BROMO rats. The timing of parturition was advanced (P < 0.05; Mann-Whitney test) in the LHAS group, but not in BROMO group.

In conclusion, these data indicate that prolactin stimulates ovarian 20α-OHP secretion during late pregnancy in the rat, apparently independent of changes in P4. LH also influences progesterone secretion at this time, but does not appear to directly stimulate 20α-OHP secretion.


Supported by an ARC Small Grant (UWA 031/259).
CORPORA LUTEA OF PREVIOUS CYCLES ARE A SOURCE OF PROGESTINS IN THE RAT

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Corpora lutea (CL) of most mammals regress functionally and structurally near the end of a non-fertile oestrous cycle. CL of the rat, however, survive for two or three cycles but are reported to have minimal steroidogenic activity unless 'rescued' by prolactin surges induced by mating at the start of the cycle (1). We have found, however, that during pregnancy CL of previous cycles grow, have a classic steroidogenic morphology, a high rate of blood flow (2) and accumulate and convert cholesterol into precursors of progestins (3).

This study focuses on the role of CL of previous cycles on progestin secretion during the cycle and early pregnancy (Day 3). Vaginal smears were taken from 19 rats to assess stage of the cycle. To assess potential secretion rates, mass of tissue and progestin concentrations (progesterone and 20α-hydroxyprog-4-en-3-one) were determined from one ovary which was dissected into components of new CL (NCL) formed since the latest ovulation, old CL of the previous generation (OCL1), old CL of generations earlier than that (OCL2) and remaining stroma which also contained luteal remnants (STRO). Rats were injected with Evans blue dye (1 ml, 1% solution) before the latest ovulation to distinguish NCL (red) from OCL (blue): OCL1 were distinguished from OCL2 by their approximately 50% lighter mass. The remaining ovary was examined histologically for steroidogenic characteristics.

NCL were not observable at oestrus and were grouped with STRO at post-oestrus. The OCL1, OCL2 and STRO collectively contained around 50% of the available progesterone in the ovary (see below) and a similar fraction of 20α-hydroxyprog-4-en-3-one, at all stages of the cycle and on Day 3 of pregnancy. All CL examined were highly vascular and, at a light microscope level, retained an active steroidogenic appearance. Pregnancy was reported to have minimal steroidogenic activity unless 'rescued' by prolactin surges induced to undergo in vitro maturation, after exposure to 4°C (2). Antifreeze proteins from all species of fish gave comparable results (3).

The effects of the antifreeze peptide from Winter Flounder (AFP) and an antifreeze glycoprotein from Antarctic Cod (AFGP) were assessed on the motility of ram spermatozoa (Dorset and Dorset x Romney) after chilling (5°C) and after freeze-thaw. Concentrations of 0, 0.1, 1 and 10 μg/ml were used for both AFP and AFGP. Semen was diluted and cooled to 5°C, then loaded into 0.25 ml straws and frozen in a liquid N2 plunge freezer.

After chilling and after freeze-thawing, the motility of the diluted semen was assessed on a scale of 0 to 5 and percent motile (2). After chilling, spermatozoal motility decreased significantly at an AFP or AFGP concentration of 0.1 μg/ml and at concentrations above 10 μg/ml (P<0.05). These proteins can have a mild cytotoxic effect at certain concentrations at 5°C. However, addition of AFP or AFGP to the freezing medium at concentration of 0.1 to 10 μg/ml significantly reduced the loss of spermatozoal motility that occurs due to the freeze-thaw process (P<0.001). The effect was not concentration dependant, nor did it depend on which antifreeze protein was added. However, due to the cytotoxicity during the chilling stage, only AFP at a concentration of 10 μg/ml increased the percentage of motile spermatozoa significantly following freezing and thawing over that of the control (P<0.05).

We propose that prevention of re-crystallisation of ice by antifreeze proteins during the brief thawing stage may have a protective effect on ram spermatozoa, particularly reducing damage to cellular structures such as membranes.

EFFECTS OF OXYGEN AND AMINO ACIDS ON CATTLE OOCYTE MATURATION, FERTILIZATION & EMBRYO DEVELOPMENT IN VITRO

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Reduced oxygen tension significantly increases the development of ruminant embryos in vitro (1). The supplementation of culture media with amino acids significantly reduces the percentage of sheep embryos which arrest in culture, provided ammonium produced from the breakdown of amino acids is avoided by renewing the media every 48 hours (1). In the present study we have examined these factors on the in vitro maturation (IVM) of cumulus enclosed cattle oocytes (Experiment 1), in vitro fertilization (IVF) (Experiment 2) and on subsequent in vitro culture (IVC) (Experiment 3). Immature oocyte-cumulus complexes were aspirated from the follicles of ovaries collected from a local abattoir. Oocytes surrounded by compact corona radiata were cultured in medium 199 + 10% fetal calf serum and 7.5IU FSH, 7.5IU LH, 50IU hCG and 1µg/ml oestradiol 17β at 39°C for 24h. In Experiment 1, 422 oocytes were matured in a gas phase of either 7%CO₂, 88%N₂ or 20%O₂, 5%CO₂, 75%N₂. Oocytes were then inseminated with frozen-thawed bull semen at 2x10⁶/ml in Fert Talp medium (3) (modified to contain 0.25mM 6-mercaptopurine and no glucose) and cultured in SOF medium + amino acids (2) + BSA (8mg/ml). In Experiment 2, 489 oocytes were matured in 20%O₂, 5%CO₂, 75%N₂ and inseminated in Fert Talp with or without amino acids. Subsequent embryo culture was in 7%CO₂ in SOF + amino acids + BSA. In Experiment 3, 486 oocytes matured in 20%O₂ + amino acids were cultured in the absence of amino acids and then cultured in SOF + amino acids + BSA, for 6 days or placed in fresh medium every 48h.

Experiment No. Cleavage(%) Blastocyst(%) EB Cell B Cell ExB Cell
1. IVM
20% O₂ 72 34 104±4 127±4 15±4 ±
7% O₂ 60** 14** 98±4 128±3 - ±
2. IVF
-aa 79 37 87±5 125±3 150±2 ±
+a +aa 53** 17** 79±6 112±6 127 ±
3. IVC
Same drop 76 15 99±7 123±3 132±10 ±
Moved @ 48h 80 39** 96±3 123±2 141±3 ±

EB, early blastocyst; B, blastocyst; ExB, expanded blastocyst; Cell, cell number ± sem; -aa, without amino acids; +aa, with amino acids; ** significant P<0.01.

The beneficial effect of high oxygen on oocyte maturation, as determined by increases in blastocyst formation is similar to the findings of DeAzambuja et al (3). We have further demonstrated a stimulatory effect of high oxygen on cleavage. In contrast to their ability to enhance embryo development, amino acids in the fertilisation medium significantly reduced the number of resultant embryos. This may be attributed to their action on either sperm and/or oocytes and warrants further investigation. Similar to the data on sheep embryos in vitro (2), the build up of ammonium in the culture medium reduced the number of cattle embryos reaching the blastocyst stage, although there was no apparent effect on resultant blastocyst cell number.

RAPID FREEZING OF MOUSE EXPANDED AND HATCHED BLASTOCYSTS

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In 1991 we rapidly froze expanded and hatched mouse blastocysts after a 3 min equilibration period at room temperature (RT) in dimethyl sulfoxide and sucrose (1). Of the 20% of embryos that reexpanded only half "plated" ie attached to the petri dish. More recently Fujikawa et al (2) obtained 95% survival of mouse blastocysts frozen after 30 seconds exposure to a solution containing 20% ethylene glycol (EG), 20% dimethyl sulfoxide (DMSO) & 10% 1,3 butanediol. We are reluctant to use butanediol as our toxicity tests indicate that both 1,3 and 2,3 butanediol are very toxic to mouse embryos (unpublished data). Solutions without butanediol are known to be highly effective for the cryopreservation of mouse morula (3). This paper therefore investigates whether late blastocyst stage embryos can be frozen in solutions without butanediol.

Mature, superovulated, C57BL x CBA F1 mice were mated with C57BL x CBA F1 males. Embryos were collected early on D 4 at the morula early blastocyst stage and cultured in DMEM with 10% FCS. Embryos were frozen on D 5 at the expanded and hatched blastocyst stage. The cryoprotectant solution developed by Kasai et al (3) (10 ml= 4 ml ethylene glycol, 1.8 g Ficoll 70 000, 0.3 M sucrose, PBS) gave variable survival of late blastocysts. Modifications to Kasai’s formulation resulted in higher survival rates. Here we present the results for solutions in which the sucrose was replaced by glucose, and the PBS with water. The cryoprotectant was either DMSO or EG. In 3 replicate experiments, embryos for freezing were randomly divided into 6 groups (9-10/group). Group 1 embryos were inserted directly into straws containing the cryoprotectant solution (CP). The other groups were pre-equilibrated in M2 containing dilute CP. Group 2 were in 10% CP for 5 min. Group 3 were in 10% CP for 2.5 min followed by 20% CP for 2.5 min. After 1 minute in the concentrated CP at RT all straws were plunged into liquid nitrogen. Straws were warmed in a 35 to 37°C waterbath for 3 to 6 sec, and the contents expelled into M2 with 0.3 M sucrose at RT. After 10 min the embryos were washed and placed in culture. The proportion of embryos which plated was significantly (* P<0.01) reduced compared to controls in only 1 of the 6 frozen-thawed groups (below).

<table>
<thead>
<tr>
<th>Cryoprotectant</th>
<th>Group 1 %</th>
<th>STD</th>
<th>Group 2 %</th>
<th>STD</th>
<th>Group 3 %</th>
<th>STD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethylsulfoxide</td>
<td>86</td>
<td>4.4</td>
<td>93</td>
<td>9.4</td>
<td>85</td>
<td>13.9</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>72 *</td>
<td>18.0</td>
<td>90</td>
<td>4.7</td>
<td>96</td>
<td>5.2</td>
</tr>
</tbody>
</table>

There were no significant differences between solutions containing DMSO and EG, or between groups pre-equilibrated in dilute CP. We conclude that the use of butanediol is not necessary to obtain high survival rates of late blastocyst stage mouse embryos.

References:

ISOLATION OF ACTIVIN A FROM Ovine ANMiotic FLUID AND PERSISTENCE OF INHIBIN IN AMNIOTIC FLUID AFTER FETAL ORCHIDECTOMY


Our previous studies have demonstrated that immunoactive inhibin was detectable in foetal gonads, foetal plasma and amniotic fluid. The addition of foetal testis extract and foetal plasma, to rat pituitary cell cultures resulted in inhibition of FSH content. Addition of amniotic fluid however caused a stimulation of FSH content. We have demonstrated by two specific immunoassays1 that this FSH stimulating activity was likely to be due to activin. To characterize the nature of activin present in amniotic fluid, pools of amniotic fluid (2-8 litres) were collected from ovine foetuses between 110-113 days gestation, and purified by dye-affinity chromatography, hydrophobic interaction chromatography, gel filtration using Sephadex-G100 in 4M acetic acid and a series of reverse-phase HPLC steps. The purification of activin was monitored by immunoassay. A progressive increase in activin specific immunoactivity was obtained at each step. Polyacrylamide gel electrophoresis of the active fractions after the final step revealed a single band with a molecular weight of 24.3 kDa which on reduction produced a single band of 16.4 kDa. NH2-terminal amino acid sequences of several fractions in the immunoactive activin peak were identical and were GLEXDGKNIXXXKQFYVSSGDI (X indicates unknown). This sequence is identical to the known sequence of ovine activin A.

In attempts to determine the source of inhibin and activin in amniotic fluid, gonadectomy was performed in 4 male foetal sheep between 113 and 119 days of gestation. Foetal and maternal carotid artery and jugular vein catheters and amniotic fluid catheters were inserted into experimental and control (n = 5) pregnant ewes and their foetuses. Post castration plasma and amniotic fluid samples were obtained for inhibin and activin measurements by radioimmunoassay. Gonadectomy caused a precipitous decrease in fotal plasma inhibin concentrations from 6.3 ± 1.3 ng/ml (± 3.4) to basal concentrations of 0.16 ± 0.01 ng/ml within 48 hrs. Amniotic fluid inhibin concentrations did not alter after gonadectomy, and were not different from those observed in amniotic fluid from control foetuses throughout the remainder of gestation (5.15 ± 0.19 ng/ml vs. 7.63 ± 0.59 ng/ml: gonadectomy vs control).

In conclusion, the results of these studies demonstrate that activin A represents the major component of the immunoactive activin in ovine amniotic fluid. Furthermore, the foetal gonads do not appear to be the major source of inhibin in amniotic fluid. Further studies will determine whether the changes in activin levels in amniotic fluid parallel those of inhibin post-gonadectomy. The possibility that activin and inhibin are locally produced by the ovine foetal membranes is currently being explored.

ACTIVIN A REGULATES PRODUCTION OF FOLLISTATIN (FS) BY OVINE ANTERIOR PITUITARY CELLS IN VITRO.

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Rat pituitary cells express mRNA for the activin-binding protein, FS, and bovine pituitary folliculo-stellate cell cultures secrete FS into the medium. In the present study, we have employed an heterologous RIA for FS to determine whether ovine anterior pituitary cells synthesize and secrete FS in a regulated manner in vitro.

Primary cultures of ovine pituitary cells were prepared by standard procedures from tissue obtained from a local abattoir. Cells were plated at 75,000 cells/0.3 ml per well in DMEM:F12 medium containing 10% fetal bovine serum, and treatments were usually added 2 days later to washed cultures in the absence of serum but presence of insulin (1 µg/ml), transferrin (10 µg/ml), and bovine serum albumin (6 mg/ml). The medium and cell lysate samples from each well were saved for subsequent RIA for FS and FSH.

Medium conditioned for several days by ovine pituitary cells contained FS-like immunoreactivity which diluted in parallel with a bovine FS standard in the RIA. The immunoreactive material co-elluted with authentic bovine FS through a routine chromatographic isolation of FS, and the purified material selectively suppressed FSH cell content in the rat pituitary cell culture bioassay for inhibin/FS.

Under serum-free conditions, dispersed ovine pituitary cells secreted immunoreactive FS at 2–5 ng/10^6 cells per day. Secretion of FS was lower in cultures of closely packed cells. Dispersed cells that had been cultured for 2 to 13 days in the presence of serum secreted up to 3-fold more FS during the subsequent 2 days, whereas serum-free conditions than did cells cultured in the absence of serum prior to the test period.

Continuous exposure of cultures to recombinant human activin A (1–10 nM) for 3 days increased FS levels in both the medium and cell lysate fractions by up to 3-fold, and concomitantly enhanced FSH secretion and cell content. Under similar circumstances, purified bovine inhibin (0.003–3.2 nM), dihydrotestosterone (1–320 nM), retinoic acid (0.03–30 µM), and the synthetic glucocorticoid, RU28362 (1–100 nM), suppressed FSH secretion and total FSH, but did not affect FS synthesis or secretion, which were likewise unaffected by GnRH, GRF, somatostatin, CRF or AVP.

From these results, we conclude that 1) ovine anterior pituitary cells synthesize and secrete FS for many days in vitro, and 2) FS production is enhanced by prolonged exposure of the cells to activin, or undefined serum factors. Activin may regulate the production of its own binding protein in the pituitary.

Supported by the NHMRC of Australia.

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EFFECT OF THE FECB GENE AND AGE ON PLASMA INHIBIN CONCENTRATIONS IN MERINO RAMS

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Utilisation of the Booroola high fecundity gene (FecB) in sheep populations is limited by an inability to identify gene carriers easily. A recent study indicated that the concentration of immunoreactive inhibin was lower in a group of prepubertal rams of high FecB gene frequency than in a group of low gene frequency (1). The present study determined plasma inhibin concentrations in Merino rams, either heterozygous or non-carriers of the gene, between 2 and 18 months of age.

Ten ram progeny from SA Merino rams and heterozygous (FecB+Fec+) SA Merino ewes were bled at 2, 3, 4, 6, 9, 12 and 18 months of age, and plasma inhibin concentrations were determined using RIA (2). FecB gene status of the rams was assessed by a progeny test.

Table 1. Least-squares means (SEM) of plasma inhibin concentrations (ng/l)

<table>
<thead>
<tr>
<th>Ram genotype</th>
<th>n</th>
<th>Age (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>FecB+Fec+</td>
<td>5</td>
<td>1694</td>
</tr>
<tr>
<td></td>
<td>(163)</td>
<td>(146)</td>
</tr>
<tr>
<td>FecB+Fec+</td>
<td>5</td>
<td>1698</td>
</tr>
<tr>
<td></td>
<td>(133)</td>
<td>(119)</td>
</tr>
</tbody>
</table>

Overall, inhibin concentrations did not vary significantly with FecB status. This is in contrast to an earlier report (1) that indicated a transient change in inhibin concentrations due to genotype at 3 and 4 months of age. A decline in inhibin concentrations with increasing age (P<0.001) confirmed previous results (1, 3). The apparent increase at 18 months may have been due to a seasonal effect (4). We conclude that plasma inhibin concentration is unlikely to be of predictive value for the selection of FecB rams.

THE DIRECT EFFECT OF INSULIN ON OVARIAN STEROID SECRETION IN EWES WITH AN AUTOTRANSPLANTED OVARY

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Sheep vary their reproduction in response to nutrition by mechanisms that are not well understood; the role of insulin in mediating direct ovarian responses to nutrition is also not clear. We report the results of an experiment in which the direct effect of insulin on the secretion of follicular steroids was determined.

Twenty three sheep with ovarian autotransplants (Goding et al., 1967) were fed, ad lib, a diet of oat straw supplemented with minerals. Following oestrus synchronisation, they were allocated to four treatments; glucose (n=6), insulin (n=6), insulin + glucose (n=5) and untreated (n=6). Treatments were administered by direct infusion into the ovarian artery for 13.5 hours on day 11 (luteal phase) of the oestrous cycle (Murray et al., 1993). Nominal rates of infusion were 1.25 g/hour and 20 μg/hour for glucose and ovine insulin respectively. All ewes were given GnRH (150 ng i/v) at -2.5h, +12h and +24h relative to the start of treatment. Ovarian venous and jugular venous blood was collected every 15 min from -30 min to +150 min relative to the GnRH injection. Androstenedione and oestradiol were assayed in ovarian venous plasma and LH, insulin and glucose in jugular venous plasma.

The infusion of insulin or glucose did not alter the secretion of androstenedione or oestradiol when compared to untreated ewes or when compared to pre-treatment responses for each ewe. However, treatment with a combination of insulin and glucose resulted in a markedly suppressed secretion of androstenedione (Figure) and oestradiol (not illustrated).

These data suggest that insulin increases the availability of glucose to follicles and that this is associated with decreased steroidogenic responses to LH. We propose that nutritional effects on ovulation may be mediated by the insulin-regulated uptake of glucose.


Figure: The secretion of androstenedione following infusion of insulin and glucose

The increased levels of the 24 and 30 kDa IGF BP s in declining bovine follicles suggest an intranovarian role for one or both of these IGF BPs in preventing the development of granulosa cell aromatase activity. Very low or negligible levels of these IGF BPs occur in bovine follicles with high (>100 ng/ml) oestradiol concentrations. The factor(s) which regulate the concentration of IGF BPs in the follicle remain to be elucidated.

INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS IN THE OVARY OF THE DOMESTIC HEN (GALLUS DOMESTICUS)

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Insulin-like growth factor-I (IGF-I) is produced by granulosa and thecal cells from pre-ovulatory ovarian follicles in the domestic laying hen and has been shown to be mitogenic for both cell types in vitro (1). Binding proteins for IGF-I have been demonstrated in mammals; so far, six of these have been identified and shown to have various effects on the actions of IGF-I in different tissues (2). Some of the proteins are known to be involved in granulosa cell activity in mammals; however, the existence of similar proteins in avian ovaries has not been previously investigated.

Granulosa and thecal cells were obtained from the largest pre-ovulatory follicles of domestic laying hens and cultured in vitro. Analysis of the resulting granulosa cell-conditioned medium (GCM) by Western ligand blotting revealed the presence of five IGF binding proteins of approximate molecular weights 32.5, 31.5, 30.5, 29.5 and 24.5 kDa. Thecal conditioned medium contained four proteins with approximate molecular weights of 32.5, 31.5, 29.5 and 24.5 kDa. Treatment of granulosa cell cultures with IGF-I (25 ng/ml) caused an increase in the concentrations of all five proteins present in GCM with the greatest increase in the 29.5 kDa protein. Similar treatments of thecal cells had no detectable effect on the protein concentrations. Treatment of granulosa cells with luteinizing hormone (LH) had no effect on protein concentrations and treatment with LH and IGF-I produced results similar to treatment with IGF-I alone. Thus there are differences in the IGF binding proteins produced by chicken granulosa and thecal cells in vitro and in the regulation of this production by IGF-I.

These results are consistent with the existence of a complex IGF-I intra-ovarian system in the domestic hen. In this system IGF-I modulates the production of binding proteins from granulosa cells, these proteins may themselves modulate the established actions of IGF-I on both granulosa and thecal cells. Further investigation is required to elucidate the nature of these proteins and thus determine whether they are similar to those already characterised in mammals. Investigations of both the in vitro and in vivo actions of these proteins may be of use in determining the intra-ovarian regulatory mechanisms controlling normal follicular growth in domestic hens.


FACTORS IN FOLLICULAR FLUID THAT DELAY OR HASTEN OESTRUS

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We reported that inhibin-depleted bovine follicular fluid (bFF) suppressed follicular development and delayed onset of oestrus when injected into heifers (1). The present study compared the effects of inhibin-depleted and inhibin-enriched follicular fluid fractions on oestrus onset in sheep. Sequential chromatography was used to commence isolation of the active fraction(s).

Charcoal treated bFF was separated into inhibin-depleted (Pool 1) and inhibin-enriched (Pool 2) fractions with a monoclonal antibody to the alpha subunit of bovine inhibin (2). Some of Pool 1 was divided into pools 3 (not-retained) and 4 (retained) with blue sepharose CL4B (Pharmacia). Sephacryl-200 (Pharmacia) was used to isolate material of molecular weights > 110 KDa (Pool 5) and 56-110 KDa (Pool 6) from Pool 3. Inhibin concentrations (ng/ml by RIA) were: Pool 1, 50 ng; Pool 2, 558 ng; Pool 4, 34 ng; Pool 5, 4 ng; Pool 6, 2 ng.

In January 1992, 100 adult ewes were treated with intravaginal prostegagen sponges. At sponge removal ewes were injected i.m. at 8 hr intervals for 2 days with pool volumes equivalent to 3 ml initial bFF starting material (additional groups injected with 1 ml of Pools 1 or 2). Animals were observed for oestrous activity, daily blood samples were taken until 1 week after oestrus onset, and laparoscopy was carried out 1 week after oestrus.

Onset of oestrus and growth of corpora lutea were delayed by Pool 1 and Pool 4 (Table 1). The differences between the data for the Pool 5' and saline groups approached significance.

Table 1. Onset of oestrus and time to reach 0.4 ng progesterone/ml plasma (Prog) in ewes injected with bFF fractions. Values are means ± se. n = 19 for the saline group and 8 - 10 for the treatment groups.

<table>
<thead>
<tr>
<th>Saline</th>
<th>Pool 1</th>
<th>Pool 2</th>
<th>Albumin</th>
<th>Pool 4</th>
<th>Pool 5</th>
<th>Pool 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 ml</td>
<td>3 ml</td>
<td>1 ml</td>
<td>3 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oestrus (hr)</td>
<td>± 5</td>
<td>±16</td>
<td>±15</td>
<td>±6</td>
<td>±8</td>
<td>±13</td>
</tr>
<tr>
<td>Prog (days)</td>
<td>±0.9</td>
<td>±0.4</td>
<td>±0.7</td>
<td>±0.5</td>
<td>±0.5</td>
<td>±0.9</td>
</tr>
</tbody>
</table>

In conclusion the main factor in bFF that delays onset of oestrus in both cattle and sheep is a factor other than inhibin. It chromatographs with albumin on blue sepharose. A second factor in follicular fluid has opposing effects.

THE RAT OVARY PRODUCES CYTOKINES DURING OVULATION

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The infiltration of leukocytes into the ovary at ovulation and the concomitant release of inflammatory mediators including eicosanoids, collagen-degrading enzymes and vasoactive substances has prompted the comparison of mammalian ovulation to an inflammatory process(1). To examine the production of cytokines by the ovary during ovulation, ovaries were obtained from immature rats and from eCG-hCG primed immature rats at different stages of the ovulatory process (prior to hCG injection, 10h after hCG, and 20h after hCG) and perfused in vitro for 5 hours(2). The steroid and cytokine contents of the recirculating perfusates were measured by immuno- and specific bio-assays, respectively(3).

Large quantities of interleukin (IL)-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) bioactivity (Fig 1 and 2) and smaller amounts of tumor necrosis factor (TNFα) and IL-1 bioactivity were found in the perfusate. Interleukin 2 and IL-3 were not detectable in the perfusion media. The GM-CSF content was significantly higher in the perfusate of ovulating ovaries (obtained 10h after hCG) compared to the earlier stages. Studies on preovulatory ovaries (prior to hCG injection) revealed that GM-CSF release was not influenced by LH, but was markedly increased when recombinant human IL-1β (4ng/ml) was added to the perfusion medium. IL-6 was released in similar amounts from ovaries at all stages. The identity of bioactive GM-CSF was confirmed by neutralisation with a specific polyclonal antibody against murine GM-CSF. Size-exclusion chromatography of perfusion medium revealed peaks of GM-CSF and IL-6 bioactivity at approximate molecular weights of 21-23kD and 24-25kD, respectively.

Cytokines have important roles in ovulatory events, including recruitment of leukocytes, activation of prostaglandins, effects on steroid biosynthesis and regulation of collagenases. These results indicate that the ovary makes cytokines in detectable quantities.

References

MESOTOCIN AND ARGinine-VASOPRESSIN IN THE CORPUS LUTEUM OF THE BRUSHTAIL POSSUM (Trichosurus vulpecula)

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The neuropeptide hormones arginine-vasopressin (AVP) and oxytocin (OT) have been found in the ovarian follicles and corpora lutea (CL) of many eutherian mammals. In ruminants, there is persuasive evidence that luteal OT is involved in luteolysis via stimulation of uterine prostaglandins. However, based on scant evidence, the marsupial ovary has been viewed as being devoid of OT-like and AVP-like peptides. In this study, corpora lutea from the brushtail possum were examined for AVP, OT and mesotocin (MT) by a combination of reverse phase HPLC, radioimmunoassay (RIA) and immunohistochemistry (IHC). Peptides extracted from each of 5 CL were separated by HPLC (C-18 RP-HPLC column; 250 x 4.6 mm) and each fraction was assayed for AVP, MT and OT. Two peaks were found, corresponding to AVP and MT standards. The amount of each peptide was 8.79±2.25ng MT/g (mean±sem) and 6.17±1.08ng AVP/g respectively. The mean AVP/MT ratio was 0.69 compared to 4.2 for the pituitary. IHC (streptavidin-peroxidase method) of Bouins-fixed CL showed staining for MT in the cytoplasm of luteal cells which was absent in stromal tissue and non-luteal ovarian tissue. Not all luteal cells were immunopositive and no topographical distribution of stained cells was observed. IHC localization of AVP was not attempted. It was concluded that the CL of the brushtail possum contains low quantities of MT and AVP, probably synthesized by the immunohistochemically staining cells of the CL.
LAPAROSCOPIC RECOVERY OF OVARIAN OOCYTES FROM SLAUGHTERED OR LIVING SHEEP

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Repeated collection of oocytes from living animals followed by in vitro maturation (IVM), fertilisation (IVF) and culture (IVC) has the potential to produce more offspring than traditional MOET procedures. We report here further results of our technique development in sheep. Forty ewes were synchronised and superovulated with our standard double CIDR® (Carter Holt Harvey, NZ) PMSG (Intervet, Holland) plus FSH (Ovagen, ICP, NZ) regimen (1). Immediately after CIDR removal the ewes were starved overnight and on the following morning were either slaughtered or subjected to laparoscopy (2). Laparoscopic oocyte recovery was attempted from the small (S; 3-6 mm) and large (L; > 6 mm) ovarian follicles of both the living and slaughtered ewe ovaries by 2 operators using 2 types of aspiration needles and modified human IVF equipment (Stortz, West Germany). Recovered oocytes were IVM, IVF and IVC in our standard system (3) and development to blastocysts recorded. As an IVM/IVF/IVC control, oocytes were recovered from our usual source, the ovaries of commercially slaughtered untreated ewes.

There were no effects of needle type on any of the parameters. The mean number of L follicles aspirated in slaughtered ewes was 8±2.1±1.7 (sem) and 3.6±1.3 in a laparoscoped ewes (P<0.01). Similar numbers of S follicles were aspirated from both ewe types (4.7±1.6, 4.1±1.3; respectively). Overall oocyte recovery rate from all follicles was 49.5%. Recovery from L follicles was 55.3% and was affected by operator (1, 69.1%; 2, 44.6%; P<0.05) and recovery date (July 6, 46.2%; July 8, 61.8%; P<0.01). Recovery from S follicles was 45.2% and was affected by operator (1, 50.0%; 2, 37.1%; P<0.05). Overall, a mean of 0.2±0.6 oocytes were recovered from each ewe. Oocyte yield was affected by operator (1, 5.5±0.8; 2, 4.6±0.8; P<0.05), follicle size (L, 3.4±0.6; S, 1.8±0.3; P<0.05) and ewe-type (laparoscopic, 3.9±1.1; slaughter, 6.6±1.1; P<0.05).

Oocyte quality did not differ between slaughtered and laparoscoped ewes and between recovery dates. However, a higher proportion of better quality oocytes (grade 1+2) were recovered from L than S follicles (73.3% v 48.6%; P<0.001). Overall development of oocytes to blastocysts from all 3 ewe types was 16.5% and was affected by follicle size (L, 20.6%; S, 2.8%; P<0.01), recovery date (July 6, 10.0%; July 8, 20.6%; P<0.01) and oocyte quality (grade 1, 21.9%; 2, 22.7%; 3, 3.4%; P<0.001). There was an inexplicable interaction between oocyte quality and ewe type (P<0.01).

The results described are an improvement on our previous work (2). Further research is still needed to increase the numbers of large follicles available for aspiration in laparoscoped ewes and to improve oocyte recovery rates.

(1) Thompson, J.G.E. et al. (1990) Theriogenology 33: 1297-1304

OOCTYE RECOVERY IS INFLUENCED BY NEEDLE TYPE AND ASPIRATION PRESSURE.

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Oocyte pick-ups in humans and livestock use a variety of needles (16G-20G single and double lumen) and aspirate at a vacuum pressure (VP) of about 100 mmHg (1). Before beginning a research program on the repeated collection of immature oocytes from heifers, we evaluated the effect of various needle types and VP's on the in vitro recovery of oocytes from bovine ovaries. To mimic the aspiration of follicles in vivo, the needle was fixed loosely in two clamps, to be held at around 10° from horizontal, the ovary was bought up to the tip of the needle, the candidate surface follicle was measured and its contents aspirated into a 50 ml Falcon tube containing 1 ml ova maintenance media. For each parameter setting, 3 operators conducted a number of runs, each run consisting of the aspiration the all surface follicles 22 mm diameter from 10 ovaries. At the completion of each run the media was searched for oocytes and their number and quality recorded.

We conducted two experiments. The first compared 17G and 20G single lumen needles at VP's of 25, 50, 75 and 100 mmHg. The second compared 17G single and 17G double lumen needles at the optimum VP of 50 mmHg. When using the double lumen needle, the follicle was subjected to continuous aspiration and flushed twice for 2 seconds with Dulbecco's PBS at a rate of 0.75 mlsec. Figure 1 demonstrates the effect of needle type and VP on the total recovery rate of oocytes (dotted lines) and the proportion that were viable (A,B,C class) for IVM/IVF procedures (solid lines).

A total of 7,300 follicles were aspirated from 968 ovaries, giving a mean of 7.54 follicles aspirated per ovary. Of these follicles, 92% were 2-4 mm, 6% 5-10 mm and 2% >10 mm in diameter. The total recovery rate of all oocytes was 50.2%.

These in vivo studies demonstrate that both the needle type and VP effect the recovery rate and quality of oocytes aspirated from bovine follicles 2 mm in diameter and greater. We recommend using either a 17G single or 17G double lumen needle at a VP of 50 mmHg to produce the greatest number of viable oocytes for in vivo follicle aspiration.

The probability that a sperm will fertilise an ovum depends upon at least one sperm surviving at the site of fertilisation until the arrival of the ovum. This obviously depends on several factors and primary among them are: the pattern of survival of sperm in the female tract, survival time of viable sperm in the tract and the probability that an inseminated sperm will reach the site of fertilisation (1). Differences between bulls or treatments that affect survival pattern of sperm will be reflected in a difference in conception rates. To a certain extent these effects can be masked by high sperm numbers. 

If fertility is arranged so that it is well below optimum levels, by manipulating sperm concentrations, maximum differences between bulls and treatments can be observed (1). In this study, two semen dilution techniques have been compared using this method. The two techniques are, rediluted deep freeze (RDF) (2) and fresh semen diluted in Caprogen (3). Ejaculates from eleven bulls were frozen at a concentration of 400 million sperm/ml by the RDF method (2) subsequently thawed and rediluted in Caprogen (3) to a concentration of 40 or 10 million sperm/ml. For fresh semen, ejaculates from the same bulls were split and diluted to contain either 5 or 1 million sperm/ml in Caprogen. A 0.5ml dose was used per insemination so that sperm numbers were 20 and 5 million for RDF and 2.5 and 0.5 million for fresh semen. All diluted semen was stored at ambient temperature (18-21°C) and used on the day after dilution. The results from the field trial are shown in Table 1.

### Table 1. Effect of optimum and sub optimum dilution rate on fertility of RDF and fresh semen.

<table>
<thead>
<tr>
<th>Dilution Technique</th>
<th>Optimum dilution techniques</th>
<th>Sperm concentration million/dose</th>
<th>% NRR</th>
<th>Sub optimum dilution techniques</th>
<th>Sperm concentration million/dose</th>
<th>% NRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDF</td>
<td>6004</td>
<td>20</td>
<td>67.6</td>
<td>3710</td>
<td>5</td>
<td>59.7</td>
</tr>
<tr>
<td>Fresh</td>
<td>14792</td>
<td>2.5</td>
<td>68.1</td>
<td>9034</td>
<td>0.5</td>
<td>61.1</td>
</tr>
</tbody>
</table>

The difference between optimum and sub optimum dilution rates for the two techniques was 7.9% for RDF and 7% for fresh. The techniques did not differ significantly at optimum dose rates (difference, 0.5%, t=0.7, p>0.1). An analysis of variance of the dilution rate results showed up some interesting effects. For both the techniques, differences between bulls and dose rates were highly significant, however, bull x dose rate interaction whilst significant for RDF, was not significant for fresh semen. The features of interest in this trial are that at the optimum dose, freezing and redilution procedures did not affect the absolute time of survival in the female tract, albeit many more sperm were required to achieve comparable fertility with fresh semen. The estimated modal time of sperm at the two dose rates was at least 8 and 2 million for RDF and 5 and 0.4 million for fresh. The significant interaction between sires for RDF indicates that the process of freezing had not altered the mean survival rate of sperm for all bulls but that individual bulls had been affected differently. It is therefore reasonable to conclude that the freezing procedure alters the probability of a sperm reaching the site of fertilisation or the pattern of survival in the female reproductive tract. Mean survival rates in in vitro trials need to be conducted at different dose rates to establish if there is any link with field fertility results.


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**Effect of Levels of Egg Yolk and Compatible Solutes on the Post-Thaw Motility of Ram Spermatzoa**

L.G. Sánchez-Partida1, J.L. Zupp1, W.M.C. Maxwell2 & B.P. Setchell1

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The presence of egg yolk in diluents for short and long term storage of ram spermatzoa is necessary to minimize the adverse effects of cold shock (1). The addition of compatible solutes are reported to enhance post-thaw motility of ram spermatzoa in the presence of egg yolk (2). In this study only the absence and presence of egg yolk were assessed, and in order to investigate the effect of different levels of egg yolk in the presence or absence of compatible solutes the following study was conducted.

A 5 x 2 x 2 experiment was designed using tris-citrate diluents with four levels of egg yolk (0.5, 1.0, 15, 20 % v/v), two levels of proline (0 and 54mM) and two levels of glycine betaine (0 and 56mM) as factors. All the diluents contained 5% glycerol (v/v) at pH 7.0. Semen was collected from 3 Merino rams by artificial vagina, consecutive ejaculates from each ram were pooled, divided into 20 parts and diluted five-fold with the respective diluents at 30°C, pellet-frozen on dry ice and stored in liquid nitrogen. Three pellets per diluent per ram were individually thawed and assessed using a Hamilton-Thorn motility analyser after incubation in a water bath (37°C) for 0, 5 or 10 h.

The presence of compatible solutes in the diluents improved post-thaw motility in the presence of egg yolk (table 1.). When egg yolk was not present in the diluent motility was lower (P<0.001) and there was no significant effect of the compatible solutes. (table 2).

### Table 2. Post-thaw motility of diluents in the absence of egg yolk and in the presence or absence of compatible solutes.

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>GB absent</th>
<th>GB present</th>
<th>GB absent</th>
<th>GB present</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>45.8</td>
<td>62.4**</td>
<td>57.8**</td>
<td>56.6**</td>
</tr>
<tr>
<td>5</td>
<td>25.5</td>
<td>36.2**</td>
<td>33.2</td>
<td>32.1</td>
</tr>
<tr>
<td>10</td>
<td>11.16</td>
<td>17.3</td>
<td>18.8</td>
<td>13.7</td>
</tr>
</tbody>
</table>

Each value is the mean of 27 observations, means different by more than 8.8 within the same incubation time would be significantly different (**: P <0.01)

### Table 1. Post-thaw motility (%) of ram spermatzoa pellet-frozen in diluents with 10, 15 or 20% (v/v) egg yolk and in the presence or absence of compatible solutes.

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>GB absent</th>
<th>GB present</th>
<th>GB absent</th>
<th>GB present</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>33.2</td>
<td>34.0</td>
<td>43.0</td>
<td>29.6</td>
</tr>
<tr>
<td>5</td>
<td>12.3</td>
<td>14.7</td>
<td>10.9</td>
<td>11.5</td>
</tr>
<tr>
<td>10</td>
<td>5.6</td>
<td>3.8</td>
<td>3.7</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Each value is the mean of 9 observations, means different by more than 15 within the same incubation time would be significantly different (P <0.01)

EFFECT OF CONCENTRATION OF TAURINE AND LEVELS OF GLYCEROL ON THE POST-THAW MOTILITY OF RAM SPERMATOZOA

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Taurine is a sulfonic amino acid compound which has been found in high concentrations in reproductive tissues and fluids in the male (1) and it may be important for sperm motility and fertilization. The addition of taurine to a high K+ phosphate buffer protected rabbit spermatozoa from loss of motility when incubated under aerobic conditions (2). The aim of this experiment, was to assess the effect of different levels of taurine and glycerol on the post-thaw motility of ram spermatozoa.

A 6 x 3 experiment was conducted using six concentrations of taurine (0 mM, 50 mM, 100 mM, 150 mM, 200 mM and 250 mM) and three levels of glycerol (0%, 3% and 5%). All the diluents were 15% egg yolk (v/v) at pH 7.0. Semen was collected from 3 Merino rams by artificial vagina, consecutive ejaculates from each ram were pooled, divided into 18 parts and diluted five-fold with the respective diluents at 30°C, pellet-frozen on dry ice and stored in liquid nitrogen. Three pellets per diluent per ram were individually thawed and assessed using a Hamilton-Thorn motility analyser after incubation in a water bath (37°C) for 0, 3 or 6 h.

The addition of taurine to the diluents containing glycerol increased post-thaw motility (table 1). When glycerol was absent post-thaw motility was lower (P < 0.001) (table 2), but taurine still had a beneficial effect on motility; no difference was observed between the 3% and 5% levels of glycerol.

**Table 1. Post-thaw motility of ram spermatozoa pellet-frozen in diluents with different concentrations of taurine in the presence of 3% or 5% glycerol.**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Taurine (mM)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>51.0</td>
<td>64.0**</td>
<td>49.6</td>
<td>30.9</td>
<td>20.7</td>
<td>14.7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>37.2</td>
<td>56.6**</td>
<td>36.7</td>
<td>20.3</td>
<td>12.3</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>21.7</td>
<td>35.8**</td>
<td>16.9</td>
<td>11.0</td>
<td>7.3</td>
<td>4.2</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean of 27 observations, means different by more than 6.9 within the same incubation time are significantly different (**: P < 0.01 higher than controls). Table 2. Post-thaw motility of ram spermatozoa pellet-frozen in diluents with different concentrations of taurine in the absence of glycerol.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Taurine (mM)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16.3</td>
<td>31.0**</td>
<td>25.0**</td>
<td>12.6</td>
<td>7.0</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9.5</td>
<td>18.6**</td>
<td>12.8</td>
<td>5.2</td>
<td>2.5</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4.9</td>
<td>8.1</td>
<td>8.2</td>
<td>3.0</td>
<td>3.3</td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean of 54 observations, means different by more than 6.9 within the same incubation time are significantly different (**: P < 0.01 higher than controls). The addition of the lowest concentration of taurine (50mM) improved post-thaw motility (P < 0.01), but motility was still lower than controls. Furthermore these results indicate that the percentage of glycerol in diluents for the freezing of ram spermatozoa can be reduced to as little as 3% without compromising post-thaw motility and this may help to counteract the negative effects of glycerol reported on the fertilization capacity of frozen thawed sperm (3).


CHANGES IN SACCHARIDE-BINDING SITES ON THE SURFACE OF RAM SPERMATOZOA FOLLOWING FREEZE-THAWING

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Artificial insemination (AI) in sheep, using semen from genetically superior rams, has the potential to hasten genetic progress in the wool producing flocks. This potential is not being realized since frozen semen has poor fertility when used with vaginal or cervical AI. This is due to a diminished longevity in, and decreased transport through, the cervix (1). Proper interaction between the cervix and spermatozoa must exist if disruption of spermatozoan transport and consequent lower fertility is to be averted. Glycoproteins on the spermatozoa surface change as the spermatozoa transit both the male and female reproductive tracts, including the addition of glycoproteins and saccharide residues in the epididymis and removal of glycoproteins in the uterus. These changes might be expected have some function, possibly related to longevity and transport in the cervix.

Preliminary studies were undertaken to define the terminal glycosyl residues on the sperm plasma membranes of fresh and frozen-thawed spermatozoa. Sperm was obtained from rams by ejaculation into an artificial vagina. Sperm was frozen, stored and thawed using techniques described (2) using a triglycerol-egg yolk medium as diluent, and frozen using the fast pellet method. Fresh or frozen-thawed spermatozoa were washed three times in PBS+ + 0.4% BSA (pH 7.2) at 500 g for 10 min. Plasma membranes were extracted and isolated by the method described (3); dilution in ice-cold TN buffer (minus protease inhibitors), disruption by vortexing at high speed for 2 min, centrifugation at 500 g for 15 min and centrifugation of the supernatant onto a 15%50% sucrose gradient at 10000 g for 90 min. The plasma membrane band at the 15%50% interface was diluted with an equal volume of TN and spun at 10000 g for 60 min. The precipitate was taken up in TN buffer, protein concentration measured, extract subject to PAGE (7.5%, 12% and 15%) and then either stained for total protein or Western blotted. Biocytinylated lectins, proteins with a high saccharide-binding affinity and specificity, were used to probe the blots, which were subsequently reprobed with streptavidin-biotin-horseradish peroxidase and colour developed.

Coomassie blue staining revealed several differences between fresh and frozen spermatozoa, including both the appearance of additional bands at >200 kDa, 150 kDa, 45-50 kDa and 25 kDa, and apparent changes in amounts of proteins between 35-45 kDa. Lectin binding studies showed that for both fresh and frozen membrane extracts, Peanut agglutinin (PNA, β-D-Gal) and Ricinus communis agglutinin (RCA, β-D-GalNAc) had high affinity, Wheat germ agglutinin (WGA, β-D-GlNAc) and Ulex europaeus agglutinin (UEA, β-D-Gal) had moderate affinity, and Bandeiraea simplicifolia lectin (BSL, α-D-Gal) and Soybean agglutinin (SBA, α-D-GalNAc) had weak affinity, results consistent with other studies (4). SBA and UEA bound more intensely, and WGA less intensely, to frozen semen compared to fresh semen. Additional or more intense lectin binding occurred consistently at around 21 kDa, 43 kDa and 66 kDa with the exception of WGA which had less binding at 66 kDa. The addition of taurine to the diluents containing glycerol increased post-thaw motility and these results indicate that the percentage of glycerol in diluents for the freezing of ram spermatozoa can be reduced to as little as 3% without compromising post-thaw motility and this may help to counteract the negative effects of glycerol reported on the fertilization capacity of frozen thawed sperm (3).

In vitro matured (IVM) and fertilised (IVF) cattle embryos produced in our laboratory have, until recently, invariably been cultured (IVC) in a non-co-culture system involving SOF medium enriched with human serum (HS) under humidified 5% CO2/5% O2/90% N2 (1). We report here the viability of embryos frozen in this and 2 systems involving co-culture.

Oocytes from slaughtered cattle were IVM and IVF as previously reported (1). They were then IVC for 24 h in SOF+HS and cleaved embryos then cultured for a further 4-6 d in either: ligated oviducts of D2 (oestrus = D0) ewes; co-cultured with bovine oviduct epithelial cells in TCM 199 + 10% FCS under 5% CO2/air; or SOF+HS. All fair to excellent quality late morula to hatched blastocysts were deep frozen in straws with glycerol (G, 10%) as cryoprotectant. Thawing was accomplished on 2 consecutive days and involved G removal via a sucrose gradient. Blastocoele development and visual quality after 24 h culture in SOF+HS was used to score embryos for survival (good, fair or poor chance of establishing a pregnancy).

Seventy percent of the embryos transferred to sheep oviducts were recovered and 10% of those recovered were freezeable. The proportion of co-cultured and SOF+HS embryos freezeable was 19 and 18%, respectively (P<0.01). Embryo survival was affected by culture system (P<0.001) as follows:

<table>
<thead>
<tr>
<th>No. embryos</th>
<th>Proportion of embryos scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
</tr>
<tr>
<td>Oviduct</td>
<td>49</td>
</tr>
<tr>
<td>Co-culture</td>
<td>42</td>
</tr>
<tr>
<td>SOF + HS</td>
<td>16</td>
</tr>
</tbody>
</table>

Survival was also affected by thawing day (P<0.01) with more embryos categorised as good or fair, and fewer as poor, on the first compared to the second thaw day (day 1: 34, 23, 43%; day 2: 21, 11, 68%). Survival was not affected by any other factor and embryo cell number at the end of post-thaw culture was not affected by culture system.

The results show that IVM/IVF cattle embryos cultured in SOF + HS have very poor survival after freezing and that culture with oviduct cells, either during co-culture or in the sheep oviduct, improves freezeability. Recent alterations to SOF (2) have, however, enabled us to produce a more freezeable product from a no co-culture system (3). The observation that thawing day affected survival is intriguing as the same media were used each day. It suggests that small and unintentional changes in protocol can effect results.

An increase in deer farming and captive propagation of endangered species has stimulated interest in applying artificial breeding techniques to deer. However, there is no basic information on gamete/embryonic physiology for any deer species. This study determined timing of oocyte maturation, fertilisation, sperm transport and motility events in red deer (Cervus elaphus).

Time to peak LH (PLH) from CIDR device withdrawal was determined in synchronised (12 d CIDR + 200 i.u. PMSG) mature red hinds (N=26). Time to oestrous onset (00) from device withdrawal was recorded. Reproductive tracts were surgically exteriorised at various times after estimated PLH (range 0-36 h). Preovulatory follicles were aspirated, and ampullary (A) and isthmic (I) regions of oviducts were flushed separately. Recovered follicular and ovulated ova were fixed and stained with 1% lactadex. Sperm motility was recorded on video.

Mean±s.e.m for 00 and actual PLH following device withdrawal were 39±1 h and 37±1 h, respectively. Ovarian morphology, ovum recovery and sperm recovery data are presented below:

<table>
<thead>
<tr>
<th>PLH (h)</th>
<th>No.</th>
<th>Ovarian morphology (n)</th>
<th>Ovum recovery (n)</th>
<th>Sperm recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6</td>
<td>3</td>
<td>7-9mm F</td>
<td>GV (2)</td>
<td>I (non-motile)</td>
</tr>
<tr>
<td>7-12</td>
<td>3</td>
<td>5-7mm F</td>
<td>M1 (1)</td>
<td>I (non-motile)</td>
</tr>
<tr>
<td>13-18</td>
<td>7</td>
<td>10mm F</td>
<td>GV (1), M1 (1), M2 (1)</td>
<td>I (non-motile, FP, CM), A (FP)</td>
</tr>
<tr>
<td>19-24</td>
<td>5</td>
<td>7-10mm F (2)</td>
<td>M2 (3)</td>
<td>I (FP, CM)</td>
</tr>
<tr>
<td>25-30</td>
<td>5</td>
<td>7mm F</td>
<td>A (1)</td>
<td>A (FP)</td>
</tr>
<tr>
<td>31-36</td>
<td>3</td>
<td>7mm F</td>
<td>OV (3)</td>
<td>OV (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A (1) I (2)</td>
<td>I (FP, CM, HA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A (1) I (1)</td>
<td>A (CM, HA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The majority of oocytes had meiotically matured (M2) by 24 h post-PLH (range 16.5-25.5 h). Mean time from PLH to ovulation was 20±1.5 h, with the earliest ovulation at 18.5 h. Fertilisation was first observed in an ovum recovered from the ampulla at 27.5 h post-PLH. All ovulated ova had lost their cumulus oophorus by the time of recovery. Sperm had reached the oviduct as early as 6 h after 00, but were all non-motile. Forward progressive motility was first observed in the isthmus 8.5 h after 00. Two other types of sperm motility were also observed: circular motion (CM) and hyperactivation (HA, "figure-8" motility pattern). One hind exhibited all three types of sperm motility at 28.5 h post-00. After 31 h post-00 (and post-PLH) no FP sperm motility pattern was observed in any hind.

These data demonstrate that, in general, gamete maturation and transport in female red deer reproductive tract follows similar patterns to those of other ruminant species, in that: meiotic maturation is complete by 24 h after PLH; ovulated oocytes rapidly lose their cellular vestments; fertilisation appears to occur shortly after ovulation; a rapid transport phase of non-viable sperm is followed by establishment of a population in the isthmus; hyperactivated sperm move from an isthmic reservoir to the ampulla.
EFFECT OF DILUENT TYPE ON MOTILITY OF RAM SPERMATOZOA STORED FOR VARYING PERIODS AT 15°C

G.C. Upreti, J.E. Oliver, R. Munday, J.F. Smith

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Successful use of fresh semen in sheep AI systems is dependant on the use of diluents that can maintain sperm viability for extended periods. We investigated the effect of two diluents on viability of semen stored at 15°C and during subsequent incubation at 38°C.

Ram semen, collected by artificial vagina, was diluted to 800 x 10⁶ spermatozoa per ml in either a defined diluent (RSD-1) or a milk diluent (1). Diluted semen was cooled to 15°C (2) and stored at this temperature for up to 8 days. The total lipids (TL) from some sources were also fractionated into neutral (NL) and phospholipids (PL). The TLs from sheep liver and cattle RBC completely inhibited motility for stored samples only. The corresponding values for milk were 75%, 45%, and 10%.

There was a more rapid decline in motility during incubation with the milk compared to the RSD-1 diluent and this was more pronounced with increasing time of storage. No motility was found after 4 h of incubation in the milk diluent even on day 0 of storage whereas motilities of 65% on day 0 to 25% on day 8 were recorded after 24 h incubation with RSD-1. The pattern of changes in velocity score was similar to that of % motile sperm. Measurements on progressive motility (at 1 hr of incubation) after storage in RSD-1 for up to 4 days did not show any changes in % progressively motile sperm or in the velocity.

RSD-1 is thus a better diluent than milk for maintaining spermatozoal survival as assessed by in vitro motility. A recent insemination trial (4) has shown, however, that semen diluted and stored in RSD-1 gave no better conception rates than semen stored in milk diluent. Thus the motility parameters we currently use for in vitro assessment of semen are inadequate for predicting fertilizing ability in vivo.


MODIFICATION OF RAM SEMEN DILUENT (RSD-1): EFFECTS OF LIPIDS ON RAM SPERMATOZOA MOTILITY

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Semen diluents based on egg yolk and milk contain considerably more lipid than RSD-1. Krebs-Ringer salts, organic nutrients and bovine serum albumin are the major components of RSD-1 (1). This study examined the effects of addition of lipids to RSD-1 on spermatozoal motility.

Qualitative variations in lipids were obtained by using lipids extracted from various biological sources such as red blood cells (RBC) and livers of sheep and cattle, egg yolk, milk and foetal-calve and calf sera. The total lipids (TL) from some sources were also fractionated into neutral (NL) and phospholipids (PL). Lipids (total or fractionated) were suspended in RSD-1 (1 mg/ml) by heating to 50°C and sonicating for 10 min, under nitrogen atmosphere. Semen was collected and diluted in RSD-1 as previously described (1). Percentage motility and velocity score were assessed for freshly diluted spermatozoal suspensions and those stored at 15°C for 2 days after 1 and 24 h incubation at 38°C (2).

Spermatozoal motility was markedly inhibited by TL from liver and RBC after 24 h incubation for both freshly diluted and stored semen. The motility was completely lost in the presence of TL from sheep liver and cattle RBC for both freshly diluted and stored samples. Similarly TL from sheep RBC completely inhibited motility for stored samples only. The percentage motility and velocity score (indicated in brackets) declined for sheep RBC - 40 (0.06), cattle liver - 10.5 (0.42) and for cattle liver TL treatment for stored samples - 5 (0.33), compared to RSD-1 freshly diluted - 50 (2.2) and stored - 40 (2.08). TL from milk or egg yolk did not significantly influence motility parameters.

The PLs from milk and sera were toxic to sperm but those from egg yolk were not toxic. NLs from egg yolk and milk were less toxic than NL from serum. After 24 h incubation, % motility and velocity score values of 30 (1.5), 38 (1.5) 21 (1.1) and 13 (0.8) were recorded for NL from egg yolk, milk, foetal-calve and foetal-calve serum respectively compared to 47 (1.9) for RSD-1. Serum NLs were less toxic in samples stored for 2 days. The corresponding % motile and velocity score values for foetal-calve serum NL and calf-serum NL were 45 (1.8) and 42 (1.6) respectively compared to 45 (2) for RSD-1 after 24 incubation of stored samples.

Incubations in RSD-1 under anaerobic conditions improved the velocity score to 3.5 after 24 h at 38°C, but did not effect the % motile. These beneficial effects of anaerobic incubations were maintained in samples stored for 2 days at 15°C. However the toxicity of fractionated PLs was retained even under anaerobic conditions.

72.

QUAIL YOLK AND COCONUT EXTRACT IN DILUENTS FOR STORAGE OF RAM SEMEN AT 30 AND 5°C.

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Cryopreservation of spermatozoa is common in the sheep and cattle industries in developed countries. However, the ingredients required for cryoprotective diluents, and the technology for cryopreservation are not readily available in many developing countries, such as Indonesia and there is little information on the requirement for maintaining viable spermatozoa at ambient or cool, rather than frozen, temperatures. The present study investigated the suitability of two diluent components that are readily available in Indonesia, and examined the motility of spermatozoa maintained in this novel diluent at ambient (30°C) and cool (5°C) temperatures. Chicken egg yolk is used widely as an important cryoprotective agent during chilling of spermatozoa. However quail eggs are more readily available in Indonesia, and have a greater yolk proportion than chicken eggs (1). Coconut water, found within the endosperm cavity, has previously been found to be a viable "natural" diluent for sheep semen (2). However, coconut extract (extracted from the coconut endosperm) has a higher protein content (3), and since protein is also important in semen diluents we have used this extract in the present studies.

Single ejaculates were collected from each of three Merino rams with an artificial vagina, twice a week, for a period of two months. Each ejaculate was split into 4 aliquots, each of which was diluted five fold at 30°C. Diluents used were a standard citrate-glucose medium containing 20% hen yolk (HYCG), the same medium with 20% quail yolk (QYCG) and 15% coconut extract plus 5% quail yolk in citrate buffer (CEQY). For each experiment four repetitions were conducted. Assessment of semen was performed with a Hamilton-Thorn Motility Analyser. Samples were stored at 30°C were maintained at this temperature in a water bath, while those to be stored at 5°C were cooled down from 30 to 5°C over 2 hours and maintained at this temperature in a water bath until assessed.

The effect of quail yolk and coconut extract on % motile sperm during storage.

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Diluent</th>
<th>Time of storage (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30°C</td>
<td>HYCG</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>QYCG</td>
<td>12.9*</td>
</tr>
<tr>
<td></td>
<td>CEQY</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>HYCG</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>QYCG</td>
<td>12.9*</td>
</tr>
<tr>
<td></td>
<td>CEQY</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>HYCG</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>QYCG</td>
<td>83.5</td>
</tr>
<tr>
<td></td>
<td>CEQY</td>
<td>83.5</td>
</tr>
<tr>
<td></td>
<td>HYCG</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>QYCG</td>
<td>84.4</td>
</tr>
<tr>
<td></td>
<td>CEQY</td>
<td>85.3</td>
</tr>
<tr>
<td></td>
<td>HYCG</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>QYCG</td>
<td>66.6</td>
</tr>
<tr>
<td></td>
<td>CEQY</td>
<td>80.8</td>
</tr>
<tr>
<td></td>
<td>HYCG</td>
<td>192</td>
</tr>
<tr>
<td></td>
<td>QYCG</td>
<td>25.4</td>
</tr>
<tr>
<td></td>
<td>CEQY</td>
<td>78.8*</td>
</tr>
<tr>
<td></td>
<td>HYCG</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>QYCG</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>CEQY</td>
<td>71.5*</td>
</tr>
</tbody>
</table>

The motility of sperm stored in quail yolk diluent was comparable to those stored in hen yolk diluent; the inclusion of coconut extract significantly (*: P<0.05, by Tukey's test) improved motility during storage at both temperatures. Incorporation of coconut extract was so effective that the use of this component may prove to be of practical importance.


73.

ABSENCE OF BINDING OF AN OVINE OESTRUS-ASSOCIATED OVODUCAL GLYCOPROTEIN TO RAM SPERMATOZOA

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An oestrus-associated glycoprotein (EGP) is known to be produced by the ovine oviduct for 3-6 days around oestrus (1). This coincides with the time at which gamete transport, fertilization and early embryo development occur in the oviduct. Preliminary work suggested that this glycoprotein, which binds to oocytes and embryos, may also bind to spermatozoa, thereby implying a role in fertilization (2). The present set of experiments were designed to reassess the ability of EGP to bind to spermatozoa.

Oviducal fluid (OF) was collected daily from catheters positioned in the fimbrial end of oviducts of cyclic ewes. Fractionation of OF was carried out using a Superose 12 FPLC column (Pharmacia), F1 being the EGP-enriched fraction. Spermatozoa were washed 3 times with PBS before being incubated in duplicate with the appropriate protein (OF, F1 or F2) at 39°C. In experiment 1, semen was collected from 2 rams and pooled. The spermatozoa were divided into 12 treatment groups, each incubated with either OF, F1 or F2 for 10, 20, 40 or 60 min. Experiment 2 involved a comparison of the abilities of capacitated and uncapacitated spermatozoa to bind EGP over periods of 60 min. or overnight. Experiment 3 tested whether EGP bound to spermatozoa in vivo, and used 2 groups of 3 ewes killed 2 h and 6 h after intrauterine A.I. Tracts were removed and divided into uterus, ampulla and isthmus which were flushed with PBS to recover spermatozoa. Control spermatozoa were incubated with oviduct proteins as described in experiment 2. In all experiments, spermatozoa were subsequently washed twice in PBS before being fixed onto slides using methanol. Specimens were then assessed for the presence of EGP; binding sites were blocked with BSA before slides were incubated overnight with a monoclonal antibody against EGP (MAC264) at a dilution of 1:1000. Slides were then washed twice in PBS before being incubated with an FITC second antibody (1:64), washed in PBS and examined under an epifluorescent microscope. All experiments included a slide of oviduct epithelial cells collected from oestrous ewes as a positive control. This was treated as sperm. A non-reactive primary antibody was used for negative controls.

In all of the experiments described, no fluorescence was associated with spermatozoa at levels higher than that obtained with negative controls. The oviducal epithelial cells gave a consistently strong signal. These results imply that EGP does not bind to spermatozoa at any stage of capacitation and/or fertilization. However, this does not necessarily mean that EGP plays no role in fertilization. It is widely documented that EGP and similar glycoproteins in a variety of species become associated with the zona pellucida and plasma membranes of the oocyte and embryo. Although we have evidence to suggest that this protein plays a role in regulating early embryo development (3), it may also influence fertilization of the oocyte.

TRANSCEVICAL INSEMINATION OF MERINO EWES

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There have been a number of reports of successful transcervical artificial insemination in the ewe, but the techniques described have not been widely adopted in Australia. The aim of this study was to compare a recently described method (1) to laparoscopic and cervical artificial insemination (AI) for use with frozen semen in Merino ewes.

Ewes were treated for 12-14 days with 30mg FGA sponges (Ovakron, ESP [exp 1] or Chronogest, Intervet [exp 2]). PMSG (350iu; Pregnecol, Horizon) was administered at sponge withdrawal. AI (51-56 hr post-sponge withdrawal) with semen frozen commercially as pellets was by either 1. laparoscopic (LAI; 2), 2. cervical (CAI) or 3. transcervical (TAI; 1) methods. Thawed semen doses were: LAI: 2x30pL; CAI: 200 (exp 1) or 100 (exp 2) pL; TAI: 80pL (diluted 6x in Dulbecco's PBS prior to AI).

Depth of TAI by experienced (exp 1) or inexperienced (exp 2) operators was scored as: 1: intravaginal; 2: deep intracervical; 3: shallow intracervical; 4: at the cervical os.

Pregnancy was determined by ultrasound 65-75 days post-insemination, and data evaluated by logit analyses. Pregnancy rates (% Preg) and the proportion (%AI) of TAI ewes falling into each depth of insemination category are shown in the table.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Datum</th>
<th>LAI</th>
<th>CAI</th>
<th>Depth of TAI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>n=251</td>
<td>% AI</td>
<td>-</td>
<td>76%</td>
</tr>
<tr>
<td>2</td>
<td>n=182</td>
<td>% AI</td>
<td>-</td>
<td>43%</td>
</tr>
</tbody>
</table>

LAI produced higher pregnancy rates than the overall TAI results, which were in turn superior to CAI, in both experiments (p<0.05). LAI produced higher pregnancy rates than uterine TAI in exp 1 (p<0.05), but not exp 2. TAI pregnancy rates were limited by low rates of cervical penetration in exps 1 and 2, and reduced fertility in exp 1. The benefit of increased depth of intracervical AI (by TAI) was less apparent in exp 2 than exp 1. It is concluded that TAI is unlikely to displace LAI in commercial practice. The results of exp 2 indicate, however, that TAI may have practical application if cervical penetration rates can be increased by operator experience or technical improvements.

The uterine endometrial surface is receptive to attachment of the trophoblast for only a short period in early pregnancy, with attachment in the sheep occurring after 15 - 16 days of pregnancy. To investigate the biochemical changes associated with this window of receptivity, frozen sections of uterus from pregnant or non-pregnant ewes on Days 11, 13, 15, 16, 17 and 18 after mating (Day 0) were cut and stained with the biotinylated lectins Dolichos biflorus (DBA), Glycine max., Arachis hypogaea, Ricinus communis-I, Concanavalin A, Ulex europaeus-I (UEA) and Wheat germ agglutinin. Only DBA and UEA reacted differentially with the endometrial epithelium of pregnant and non-pregnant ewes around the time of attachment. DBA stained strongly in the luminal and glandular epithelium of pregnant ewes up to Day 16, but showed no staining in non-pregnant ewes. UEA stained strongly both luminal and glandular epithelium on all days examined in both pregnant and non-pregnant ewes, except for a complete absence of staining on Days 15 - 16 of pregnancy. There was no difference in staining between caruncular and intercaruncular areas. The UEA receptor has been purified to apparent homogeneity by lectin-affinity chromatography and preparative gel electrophoresis. SDS-gel electrophoresis of the affinity column fractions revealed a diffuse lectin-stained band, suggestive of a heavily glycosylated glycoprotein. The purified product from the preparative gel was identified after electrotransfer by lectin staining as a discrete band of approximately 100,000. Silver-staining did not detect any protein bands. Characterisation of the protein is in progress and its association with stage-specific lectin staining of the epithelium remains to be established.

Immunoblotting revealed distinctive and identifiable a bands of collagen types I (a1, a2), III (a1 and V (a1, a2). Densitometric evaluation of the bands in relation to total collagen showed a significant reduction (by ANOVA) of type I concentration at each day in both uterine regions. A similar decline was found also for type III but this only reached significance by day 8. Type V was found to be reduced by day 8 but only in the implantation sites. Immunoblotting revealed distinctive and identifiable a bands of collagen types I (a1, a2), III (a1 and V (a1, a2). Densitometric evaluation of the bands in relation to total collagen showed a significant reduction (by ANOVA) of type I concentration at each day in both uterine regions. A similar decline was found also for type III but this only reached significance by day 8. Type V was found to be reduced by day 8 but only in the implantation sites.

<table>
<thead>
<tr>
<th>Day</th>
<th>Type I</th>
<th>Type III</th>
<th>Type V</th>
<th>Type I</th>
<th>Type III</th>
<th>Type V</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1.85 ± 0.13</td>
<td>0.29 ± 0.13</td>
<td>0.17 ± 0.04</td>
<td>3.05 ± 0.22</td>
<td>0.37 ± 0.15</td>
<td>0.45 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>1.48 ± 0.12</td>
<td>0.16 ± 0.07</td>
<td>0.11 ± 0.07</td>
<td>2.40 ± 0.25</td>
<td>0.63 ± 0.15</td>
<td>0.40 ± 0.13</td>
</tr>
<tr>
<td>8</td>
<td>0.96 ± 0.03</td>
<td>0.09 ± 0.04</td>
<td>0.05 ± 0.02</td>
<td>2.05 ± 0.08</td>
<td>0.51 ± 0.09</td>
<td>0.33 ± 0.05</td>
</tr>
</tbody>
</table>

These results indicate that in the implantation regions (and to a lesser extent in the uninvolved regions) there is a decline in all the fibrillar collagens studied. The rate of decline was less for types III and V than for type I. This may indicate slightly different catabolic mechanisms involved in remodelling of the extracellular matrix proteins as important aspects of the uterine response to early pregnancy. Further studies designed to determine the histological distribution of type III and V would ascertain if, as is the case for type I, these collagens are greatly reduced within decidualising tissues.

DECIDUALISATION AND THE REMODELLING OF LAMININ DURING NATURAL EMBRYO IMPLANTATION IN THE RAT.

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Embryo implantation in most mammalian species is associated with the differentiation of uterine stromal cells into the maternal decidual cells of pregnancy. The embryo which is located within the antimesometrial aspect of the lumen is surrounded by the large closely adhering primary decidual cells. Secondary decidual tissue is located more peripherally and contains less tightly packed polygonal cells. By day 8 of pregnancy there is a functioning choriovitelline (yolk sac) placenta on the mesometrial side of the implantation sites. Pericellular decidual laminin has been shown during an artificially stimulated decidual response (1). Expression of laminin in the early stages of natural implantation has not however been examined. This study used immunohistochemical techniques to investigate the spatio-temporal distribution of laminin over days 5 to 8 of natural pregnancy in the rat.

Rats were mated and the morning on which spermatozoa were found in the vaginal smear was called day 1 of pregnancy. A rabbit anti-mouse laminin antibody (Gibco) was used and its specificity tested by western blotting, slot blotting and immunoprecipitation. Laminin was detected using immunofluorescence on transverse frozen sections which passed through the embryo.

The presence of punctate laminin around the primary decidual cells was detected on day 6 of pregnancy but not days 5 and 7. Transient, punctate laminin expression around mesenchymal cells has also been observed at the stromal/epithelial interface during development and differentiation of both the intestine and kidney (2,3). Expression of laminin may be associated with the differentiation program and suggests that developmental mechanisms may be activated during deciduallisation. The adhesive properties of laminin suggest that it may be involved in the adhesion of the primary decidual cells. Other than the localised punctate expression the decidual cells did not have pericellular laminin and thus are unlikely to have a basement membrane.

By day 8 the mesometrial vessels of the choriovitelline placenta have formed and laminin is found in association with the arterial vessels but only faintly within the venous sinusoids. Antimesometrially immunohistochemistry also revealed a network of small vessels. It is hypothesised here that this network of vessels is essential for the transport of the endocrine factors produced in this region. These results lay the foundation for further investigations into the role of punctate laminin in the differentiation program of the uterine stromal cells and laminin expression during angiogenesis and placentaion.


HUMAN ENDOMETRIAL ENDOTHELIAL CELL PROLIFERATION DURING THE MENSTRUAL CYCLE

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Angiogenesis (new blood vessel growth) occurs rarely in normal adult tissues, however the female reproductive tract provides several exceptions. An early step in this process is endothelial cell (EC) proliferation. Previous studies indicate that there may be three separate episodes of angiogenesis in the endometrium during the primate menstrual cycle. The aims were to quantify EC proliferation in human endometrium during the menstrual cycle using immunohistochemistry, and to compare the pattern of cellular proliferation seen with two different markers: PCNA (proliferating cell nuclear antigen - a nuclear protein whose expression increases during the late G phase of cycling cells, and peaks during the S phase), and BrdU (bromodeoxyuridine - a thymidine analogue which is incorporated into DNA as it is synthesised).

Endometrial biopsies were collected from women undergoing investigation for infertility, and were used only if the infertility was unrelated to uterine or endocrine factors. Biopsies were formalin fixed and wax embedded for routine histological dating, and immunohistochemistry. Portions of biopsies for the secondary study were collected into medium with 10% FCS and incubated with 2mM BrdU for 3hr at 37°C before fixation. For the first part of the study, one section from each biopsy was stained immunohistochemically using a streptavidin-biotin method, and incorporated a double staining technique for proliferating EC's. Proliferating cells were stained to give red nuclei using mouse-anti-rat-PCNA (clone PC10; Novocastra) and AP-Blue. An index of EC proliferation was derived from the number of proliferating EC's / the total number of EC's, expressed as %. For the second part of the study, consecutive sections were stained using the PCNA antibody or mouse anti-BrdU (clone Bu20a; Dako).

Results (table) show that there was great variability in the EC proliferative indices between biopsies throughout the menstrual cycle, and from the same stage of the cycle, and that there were no significant peaks of EC proliferation.

<table>
<thead>
<tr>
<th>Menstrual group</th>
<th>1</th>
<th>2</th>
<th>Proliferative</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Secretory</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>14.02</td>
<td>2.47</td>
<td>2.56</td>
<td>17.43</td>
<td>5.79</td>
<td>18.72</td>
<td>5.34</td>
<td>8.45</td>
<td>12.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sem</td>
<td>1.43</td>
<td>1.23</td>
<td>1.23</td>
<td>6.78</td>
<td>4.87</td>
<td>9.54</td>
<td>2.28</td>
<td>3.72</td>
<td>5.57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

An unexpected finding was the pattern of cellular proliferation seen with the PCNA antibody. In particular, evidence of glandular proliferation was apparent in 11/15 biopsies in the mid- and late-secretory phases of the cycle. This is not consistent with the commonly reported pattern of mitoses seen in human endometrium through the cycle; hence the comparision between PCNA and BrdU staining. To date, the results from the second part of the study have shown that the pattern of cellular proliferation seen with PCNA, and BrdU staining are similar.

Overall the results do not support the concept of three discrete peaks of angiogenic activity through the cycle corresponding to such events as post-menstrual repair, endometrial proliferation, or spiral arteriole growth.
ENDOTHELIN SECRETION IN VITRO BY CULTURED HUMAN ENDOMETRIAL EPITHELIAL CELLS: EFFECT OF TRANSFORMING GROWTH FACTOR-β1 AND INTERLEUKIN-1α.

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Endothelin (ET) is a potent vasoconstrictor (1), which has diverse biological actions. Immunohistochemically ET localizes predominantly to endometrial glands with increasing intensity in the late secretory phase (2). ET secretion by human endometrial epithelial cells (3) and passed stromal cells (4) in vitro have been described. ET has been shown to interact with a wide variety of cytokines and growth factors in different cell types.

In this study we examined ET production by isolated human endometrial epithelial cells in culture and the secretory response to transforming growth factor-β (TGF) and interleukin-1α (IL-1). Cells known to produce both TGF and IL-1 are present in the endometrium and these cytokines are potentially involved in the regulation of endometrial ET production.

Endometrial tissue from women without evidence of endometrial dysfunction was obtained at curettage. Epithelial cells were prepared from individual or pooled specimens. Cultures were established in Medium 199 with antibiotics (M199) and 10% charcoal-treated (CT) fetal calf serum (FCS) for 48 hours, cells washed and further incubated in M199 and a serum free mixture: insulin, transferrin, selenite, hydrocortisone and 0.01% BSA (SF). TGF (2, 5, 10 ng/ml) or IL-1 (1, 10, 100 IU/ml) in triplicate wells, was added for a further 48 hours. SF alone and 10% FCS were negative and positive controls respectively. Media was assayed for ET and cells for DNA content and ET secretion was calculated as pg/mcg DNA. Mean ET secretion by unstimulated individual cell preparations is variable (133-353 pg/mcg DNA), however, is increased 140%-192% by FCS. Comparative analysis was performed by expressing ET secretion as a percentage of the negative control.

TGF at all doses stimulated ET production (P<0.05) (N=3). Mean ET% control +/- SE ranged from 122% +/- 22 to 191% +/- 36. This effect was not dose responsive and in general less than that induced by 10% FCS (Mean ET% control +/- SE: 144% +/- 38 to 192% +/- 13). Preliminary data suggests that IL-1 also stimulates ET secretion (P=0.053). Mean ET% control +/- SE: 110% +/-3 to 164% +/-10.

These results demonstrate in vitro stimulation of ET by TGF and IL-1. This is potentially important in the regulation of ET production and in understanding the role of ET in human endometrium.

Supported by the NH & MRC and WHO.

NUTRIENT UPTAKE DURING EMBRYONIC DIAPAUSE AND SUBSEQUENT REACTIVATION IN MOUSE BLASTOCYSTS

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Many oocytes, including the mouse (Mus musculus), carry a diapausing blastocyst in the uterus while suckling young. This delayed implantation is readily induced and terminated experimentally by administering hormones to ovariectomised pregnant mice (1). It has been determined that within 6h of oestradiol-17β (E2) administration, there are significant changes in enzyme activities in the reactivating mouse blastocyst (2). Previous studies on nutrient utilisation of blastocysts during delay and reactivation have employed radiolabel techniques, requiring extended incubation periods to assess metabolism (1,2). In contrast, microfluorimetry, employed in this study, allows the accurate determination of nutrient uptakes within minutes as opposed to hours.

CF1 mice were ovariectomised on day 4 of pregnancy before 8 a.m. and injected with progesterone on days 6 - 9 of pregnancy, which delays implantation (diapause). Blastocyst reactivation was induced by injecting E2 on day 9. In each experiment half of the mice with diapausing blastocysts were injected with E2, so that diapausing and reactivated embryos were examined simultaneously to control for interexperimental variation. Individual blastocysts were incubated at 37°C for 3h in 100ml drops of modified MTf medium (3) containing 0.5mM glutamate, pyruvate and glucose with 2mg/ml BSA. Serial nanolitre samples of medium were taken to determine substrate uptake by the blastocysts. After metabolic analysis, blastocysts were incubated in colcemid (2µg/ml) for 2h before cell number and mitoses per embryo (mitotic index) were determined.

Mitotic index and cell numbers increased significantly within 8h of E2 administration, compared to that of delayed controls (0.5 ± 0.1 vs 0.22 ± 0.02; P<0.01, and 137.9 ± 0.5 vs 134.6 ± 0.8; P<0.05, respectively). There was no difference in glutamate uptake between delayed controls and reactivating blastocysts at any timepoint studied. In contrast, pyruvate uptake by reactivating blastocysts (9.3 ± 1.1) was significantly higher than delayed controls (5.8 ± 1.6 pmol/o/h; P<0.05), within 4h of E2. However, significant differences in glucose uptake between the two groups were not evident until 20h after E2 (reactivating, 12.5 ± 1.4; control, 7.3 ± 0.8 pmol/o/h; P<0.05). In contrast, at 20th after E2, pyruvate uptake by reactivating blastocysts was significantly different to the delayed controls. Metabolic analysis of reactivated embryos 24h after E2 was not possible as embryos could not be preserved and are presumed to have implanted.

The delayed mouse blastocyst has a similar substrate preference to the early cleavage stage embryo, ie preferential utilisation of pyruvate over glucose. The ATP/ADP ratio in both diapausing and reactivated blastocysts was high (attributed to low levels of biosynthesis), inhibiting phosphofructokinase, a key regulatory enzyme of the glycolytic pathway (1,4). As biosynthesis increases during reactivation, and the ATP/ADP ratio falls as a result of ATP utilisation for cleavage and biosynthesis, glycolysis will be de-inhibited, resulting in an increase in glucose uptake. As glucose utilisation increases, pyruvate uptake decreases concomitantly. The reactivated blastocyst therefore resumes a nutrient preference of a non-delayed embryo.

DEVELOPMENT OF IN-VIVO AND IN VITRO DERIVED OVINE EMBRYOS IN MEDIA SUPPLEMENTED WITH HUMAN SERUM OR FATTY ACID-FREE BOVINE SERUM ALBUMIN.

J.M. Bowen1, P.A. Pugh & J.G. Thompson


Domestic animal embryo culture systems are largely undefined, comprising either somatic cells in co-culture or simple media supplemented with human (HS) or animal sera (1). Cell-free, defined media are advantageous in allowing direct examination of media components on development and reducing variability in culture conditions. This study compared development of ovine embryos in two serum-free media (a modified SOF, known as JAM) and CR1aa (2), both supplemented with 5 mg/ml fatty acid-free bovine serum albumin (FAF-BSA) and SOF supplemented with 20% HS. Embryos were derived either from IVF/F, or from day 1.5 superovulated ewes. Embryos were incubated for 6 days at 39°C in either humidified 5% CO2/air or 5% CO2/7% O2/88% N2 and in one of the 3 media; SOF+HS, JAM, CR1aa. In addition, some in vivo embryos were incubated in SOF+5 mg/ml FAF-BSA (SOF+BSA). Development to blastocyst (B1) or hatched blastocyst (HB1) stages were as follows:

<table>
<thead>
<tr>
<th>[O2]</th>
<th>Medium</th>
<th>In Vitro Derived No. %B1 %HB1</th>
<th>In Vitro Derived No. %B1 %HB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>7%</td>
<td>SOF+HS</td>
<td>42  33  10  13  23  46</td>
<td>SOF+BSA - - - 6  67  0</td>
</tr>
<tr>
<td>20%</td>
<td>SOF+HS</td>
<td>66  3   0   10  0   0</td>
<td>(air) JAM 66 3 0 12 25 0</td>
</tr>
<tr>
<td></td>
<td>JAM</td>
<td>78  3   0   12  0   0</td>
<td>CR1aa 40 6 0 12 0 0</td>
</tr>
<tr>
<td></td>
<td>CR1aa</td>
<td>57  9   0   11  18  0</td>
<td>SOF+BSA - - - 6  33  0</td>
</tr>
</tbody>
</table>

More embryos developed to blastocyst in 7% O2 than in air (P<0.001), thus confirming a previous report (3). Highest levels of development were observed in SOF+HS when incubated under 7% O2 (43±8% and 69±13% for in vitro and in vivo derived embryos, respectively). In vivo derived embryos performed equally well in JAM and SOF+HS media. However, at 7% O2, in vitro embryos had improved development in SOF+HS (43±8%) compared to JAM (19±6%). Development in CR1aa was poor for embryos of either source.

A striking morphological difference was observed between embryos, particularly blastocysts, incubated in media containing HS or FAF-BSA. Embryos incubated in FAF-BSA were much lighter in appearance and had fewer lipid-like inclusions that characterised embryos incubated in SOF+HS. We believe this morphology may be induced by low density lipids and lipid peroxides present in serum.

Performance of embryos in JAM, particularly in vivo derived embryos, demonstrate that this medium is suitable for investigation of factors affecting embryonic development.

EFFECT OF CO-CULTURE WITH OVIDUCT CELLS and the Addition of Glutamine on the Development of Sheep Embryos in Vitro

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The most successful methods for the in vitro culture of sheep embryos use oviduct epithelial cell support (1) or synthetic oviduct fluid (SOF; 2). We examined whether oviduct cell support will improve the viability of embryos cultured in SOF. In addition, the effects of glutamine, which was shown to be beneficial for hamster embryos (3), were investigated on the development of sheep embryos.

Day 2 sheep embryos were cultured with or without oviduct cell support in SOF + BSA supplemented with 0 or 1 mM glutamine. The embryos were collected at the 1 to 8-cell stage (2 days after insemination of donor ewes) and cultured in 96-well culture plates. The stage of embryonic development reached was evaluated after 4 days in vitro culture. Additionally, in the last two of four replications of the experiment, the viability of embryos which developed to the late morula/blastocyst stage was tested in vivo by transfer into recipient ewes (1 or 2 embryos/recipient).

The proportions of embryos which developed to or beyond the late morula (LM) stage were not affected by treatments (Table 1). However, there was an interaction between the effects of oviduct cell support and glutamine on the proportions of embryos which reached the early blastocyst (EB), blastocyst (BL) or late blastocyst (LB) stages (P<0.05) and on the proportions of recipients pregnant (P<0.02). In the absence of oviduct cells, the addition of glutamine improved development to the early to late blastocyst stages (P<0.01) and the proportion of recipients pregnant (P<0.05).

In the presence of oviduct cells, glutamine caused a non-significant reduction in the viability of embryos.

Table 1. The effect of sheep oviduct epithelial cells (SOEC) and glutamine (Gln) on the percentages of embryos developing to or beyond different developmental stages and on the percentages of recipients pregnant on Day 51

<table>
<thead>
<tr>
<th>SOEC</th>
<th>Gln (mM)</th>
<th>Development in vitro</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>LM</td>
<td>EB</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
<td>36</td>
<td>53</td>
</tr>
<tr>
<td>1</td>
<td>31</td>
<td>48</td>
<td>35</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>34</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>31</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>48</td>
<td>22</td>
</tr>
</tbody>
</table>

These results demonstrated that glutamine, used as a single amino acid, can improve the viability of sheep embryos cultured in SOF. It was also shown that the requirements for glutamine are different for embryos cultured with or without oviduct cells. This may be explained by differences in the metabolism of embryonic and somatic cells.


EFFECT OF TYPE AND CONCENTRATION OF SERUM ON THE DEVELOPMENT OF IN VITRO MATURATED AND FERTILISED SHEEP EMBRYOS


Department of Animal Science, University of Sydney, NSW 2006

Synthetic oviduct fluid (SOF) supplemented with 20% human serum (HS) has been reported to support development to blastocysts of in vitro produced sheep zygotes (1). We examined four sources of serum for their ability to support development to blastocysts of in vitro matured and in vitro fertilised sheep zygotes.

Immature oocytes, collected on 4 separate days, were cultured at 39° C under low O2 tension (5% O2, 5% CO2, 90% N2) in M199 + 10% FBS (foetal bovine serum), LH (10 μg/ml), FSH (10 μg/ml) and oestradiol (1 μg/ml). After 22 hr, 427 oocytes were inseminated with frozen-thawed sperm (90% NJ) 2 days after ovulation and FBS each ID: w23-28.

Transf.1: A.I.1: will in M199 + 10% FBS (foetal bovine serum), S.E.M.) AND THE ADDITION OF 500 μl 162-167.

<±.23.60) female), HS2 (human serum from 1 ewe), HS3 (sheep serum from 4 males and 1 female), HS4 (human serum from 2 females), SS (sheep serum from 1 ewe, 2 days after ovulation) and FBS each at 10 and 20% v/v with SOF (+ 1 mM glutamine, - glucose). Embryos were cultured for 6 days in 500 μl of appropriate medium in 4-well dishes under low O2 tension at 39° C. On the 4th day after insemination, glucose was added to the culture media to give a final concentration of 1.5 mM. The number of cleaved embryos developing to blastocysts was not affected by concentration of serum (37/158, 10% vs 27/169, 20%) but was affected by type of serum (Table 1).

Table 1. Effect of Type of Serum on Embryo Development.

<table>
<thead>
<tr>
<th>Type of serum</th>
<th>No. Blastocysts/ Cleaved zygotes (%)</th>
<th>Mean Cell No. (± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS1</td>
<td>26/88 (30)*</td>
<td>83.5 (±23.60)</td>
</tr>
<tr>
<td>HS2</td>
<td>20/85 (24)*</td>
<td>107.5 (±34.20)</td>
</tr>
<tr>
<td>SS</td>
<td>10/76 (13)*</td>
<td>80.2 (±18.88)</td>
</tr>
<tr>
<td>FBS</td>
<td>7/74 (9)*</td>
<td>73.5 (±34.94)</td>
</tr>
</tbody>
</table>

P<0.01 and P<0.05

Media containing human serum were superior to those containing SS or FBS/P<0.001). There was no differences in mean cell numbers per blastocyst but overall the cell numbers were higher than those generally reported for totally in vitro produced blastocysts. These higher cell numbers may indicate an increase in viability in vivo.

PARTITIONING OF GLUCOSE CARBON IN POST COMPACTION Ovine EMBRYOS

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Sheep and cattle embryos appear to metabolise glucose in a different manner to mouse embryos. Indeed, one study concluded that little glycogen production occurs in 8- to 16-cell sheep embryos (1) in contrast to the mouse, where glycogen production constitutes a significant proportion of glucose metabolism (2). The current study examined glucose partitioning in post-compaction sheep embryos.

Day 5 sheep embryos (late morulae and early blastocysts) were surgically recovered from superovulated ewes. Groups of embryos (4-7) were incubated for 24 h at 39°C under humidified 5% CO\(_2\), 95% N\(_2\) in 50 μL microdrops of a substrate-free SOF medium (12 mg/ml BSA, m-SOF) containing 0.9 mM cold glucose, 0.1 mM [U-\(^{14}\)C]-glucose (5.6. 10.6 GBq/mmol) and with or without 1.0 mM glutamine. Following incubation, embryos were washed in m-SOF (0.9 mM cold glucose) and stored at -70°C until fractionation.

Embryos were fractionated into acid-soluble (glycogen/non glycogen) and acid-insoluble (desmoglycogen/non glycogen) fractions as described in (3). Partitioning of glucose carbon is presented below:

<table>
<thead>
<tr>
<th>Glutamine</th>
<th>Reps</th>
<th>Total Glucose</th>
<th>Acid soluble</th>
<th>Acid insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>5</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>2.3±0.4</td>
</tr>
<tr>
<td>+</td>
<td>5</td>
<td>73.8±7.3</td>
<td>&lt;1</td>
<td>7.7±6.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.6±0.1</td>
<td>14.2±2.7</td>
<td></td>
</tr>
</tbody>
</table>

To further characterise glucose carbon partitioning in the acid-soluble (AS) fraction, 100μL samples were fractionated by HPLC (0.6 μL/min) into 5 fractions (AS1-5). Fraction AS2 corresponded to the retention time for glucose and AS4 corresponded to lactate. Proportion (%) of radioactivity in each fraction follows:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Not recov’d</th>
<th>0-9</th>
<th>9-10.5</th>
<th>10.5-13</th>
<th>13-14.5</th>
<th>14.5-20</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>16±6</td>
<td>15±5</td>
<td>44±12</td>
<td>20±4</td>
<td>5±1</td>
<td>2±1</td>
<td>89±13</td>
</tr>
<tr>
<td>+</td>
<td>15±1</td>
<td>17±4</td>
<td>36±9</td>
<td>23±4</td>
<td>8±2</td>
<td>2±1</td>
<td>75±14</td>
</tr>
</tbody>
</table>

Results demonstrate that negligible glycogen production occurs in post-compaction sheep embryos, thus extending the earlier observation in 8- to 16-cell embryos (1). The presence of glutamine tended to improve morphological development over the 24 h culture period, but had no significant effect on glucose incorporation. Finally, most glucose carbon was associated with the acid-soluble non glycogen fraction. HPLC revealed that this was predominantly glucose (approximately 40%), in addition to other metabolic intermediates. A smaller than expected portion was isolated in the lactate fraction. This does not, however, reflect total lactate production. Lactate would perfuse into the incubation medium as well as serve as an oxidisable energy substrate.

FERTILISING ABILITY OF SPERMATOZOA FROM RAMS
SUBJECTED TO INTERMITTENT SCROTAL INSULATION AND
DEVELOPMENT OF THE RESULTANT EMBRYOS IN VITRO

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In 1992, we reported (1) that insulating ram scrotas for 16h per day for 3 weeks resulted in the production of spermatozoa which were motile but less effective in fertilising eggs in vitro. In the present study, we have extended the duration but reduced the intensity of the treatment to 8h/day, and continued weekly observations through the recovery period. The techniques used were the same as described previously (1). In a room maintained at 21°C, 2 rams were kept as controls and insulating bags which raised the subcutaneous scrotal temperature by about 2°C were applied to 2 rams at 0900h and removed at 1700h each day. As in the previous experiment, the percentage of eggs fertilised by the same number of motile, capacitated spermatozoa was less during the period of insulation, and furthermore, the effect continued with the semen from one ram for 7 weeks after the end of the 5-week period of insulation. Semen from the second ram deteriorated during insulation to such an extent that after 3 weeks of insulation, it was no longer possible to obtain enough motile spermatozoa for the in vitro studies. Insulation was therefore stopped after 4 weeks but the lack of motile spermatozoa continued for a further 3 weeks; then, the semen quality returned to normal, but for the last 2 weeks of the experiment, spermatozoa from this ram also fertilised a lower proportion of the eggs than controls. Development of the embryos was assessed by classifying them as 2 or 4 cell, 8 or 16 cell, morula or blastocyst; when the proportion of embryos in the 4 classes was compared, development of the embryos produced was significantly slower than control, for one ram during the 5-week period of insulation and for 5 weeks afterwards, and for the other during the pre-insulation period (associated with appreciable numbers of abnormal sperm), and for the 3 weeks of insulation before the number of motile spermatozoa fell. During the last 2 weeks of the study, spermatozoa from both rams produced embryos which developed normally. When the same calculation was made on the data from the previous experiment, a small but significant retardation of development could again be demonstrated, and development was significantly different between the two studies, which were conducted in April and August-November respectively.

Pre-insulation Scrotum insulated 8h/24. Post-insulation weeks 1-5 Post-insulation weeks 6-7
% ova fertilized
Ram 47 58(58)w 31(71)***w 48(60)* 65(91)***w
Ram 33 54(99)w 40(56)***w - 64(91)***w
Development scores
Ram 47 2.00(2.33) 2.24(2.83)***w 2.16(2.54)* 2.94(2.89)
Ram 33 1.92(2.53)*** 2.30(2.79)*** - 3.38(2.89)##
Rams 12+16 3.57(3.48)w 2.99(3.34)***w from previous exp.

Bold figures are for the insulated rams, 36 ± 1.8 eggs/ram/week; figures in parentheses are pooled values for the two controls, for different corresponding periods as insulat;on of ram 33 began 1 week later than ram 47. 1w, 2w or 3w: no of weeks for which data are pooled. Development scores were calculated by counting 1 for 2-4 cell embryos, 2 for 8-16 cell, 3 for morula and 4 for blastocyst. **, ***: P < 0.01 or 0.001 respectively less than controls. ##: P 0.01 greater than control by 4x2 chi-square test.


TIME AND INCIDENCE OF FOETAL MORTALITY IN ALPacas

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Low reproductive performance in alpacas is a major problem in South America, with an estimated 50% of females failing to produce young each year. A high proportion of this loss is attributed to embryo and foetal mortality (1). Alpacas in New Zealand graze abundant green pasture over most of the year and this may mean lower levels of embryo and foetal mortality than reported in South America. This paper presents information on the incidence of foetal mortality in two alpaca flocks in New Zealand.

Over the years 1990-92, 46 autumn (Feb.-Apr.) and 33 spring (Oct.-Dec.) mated alpaca at Flock House, and in 1992, 34 autumn mated alpacas at Tara Hills Research Station were diagnosed as pregnant by ultrasonography. Pregnancy in spring 1990 was diagnosed using a 3.5 mHz transabdominal probe with the first scan being at 49-113 days gestation. In the subsequent years a 5 mHz transrectal probe was used with first scans of the autumn 1991 mated alpacas being at 51-60 days, while in spring 1991 and autumn 1992 most first scans were at 21-30 days of gestation. Once an alpaca was diagnosed as pregnant it was scanned every 10-14 days at Flock House and every 15-36 days at Tara Hills. The transrectal probe was used up to 80 days gestation and thereafter the transabdominal probe was used until the foetus became too large to scan.

Overall 23% of foetuses were lost between days 21-30 of gestation and parturition with the losses being 28% for autumn and 18% for spring mated alpacas at Flock House, and 23% for autumn mated alpaca at Tara Hills. The major foetal loss (6%) occurred between the scannings on days 21-30 and days 31-40 gestation. The 6% foetal loss after day 100 of gestation indicates that losses in late pregnancy can occur.

Table 1: Incidence of foetal mortality over gestation

<table>
<thead>
<tr>
<th>Days gestation</th>
<th>Foetal loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 - 40</td>
<td>5.5</td>
</tr>
<tr>
<td>41 - 70</td>
<td>6.1</td>
</tr>
<tr>
<td>71 - 100</td>
<td>5.2</td>
</tr>
<tr>
<td>&gt;100</td>
<td>6.1</td>
</tr>
</tbody>
</table>

Total embryo and foetal loss from the alpacas in this experiment was likely to be greater than the 23% recorded since only a small proportion of the losses from fertilisation to day 30 of gestation would have been identified in this experiment. Foetal loss in the alpacas was much higher than the 2-6% loss in sheep after days 30-40 (2).

CALCIUM, PHOSPHATE AND CITRATE IN SOWS' MILK
DURING LACTOGENESIS II

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Holt & Muir (1) proposed a physicochemical model to explain some of the ion interactions in milk and predicted a direct relationship between the diffusible fractions of calcium and citrate in milk. The model has been corroborated in the milk of cows collected in different seasons (1), in the milk of eight different species (2) and in human milk during lactogenesis II (3). Citrate has been called the 'harbinger of lactogenesis' (4), increasing in concentration during lactogenesis II in all species previously studied. However, Holmes & Hartmann (5) have shown that the concentration of citrate in the milk of sows decreases during lactogenesis II. Therefore, we examined the relationship between diffusible calcium (Ca\text{diff}) and diffusible citrate (Cit\text{diff}) in sow milk during the peripartum period to further test the model (1).

The concentration of lactose in the blood plasma began to increase during farrowing, and reached a peak 4-6h after farrowing, confirming the occurrence of lactogenesis II during or just before farrowing. Ca\text{total} increased from 20mM before farrowing to 42mM by day 2 after farrowing and remained above 40mM for the remainder of the study period. Ca\text{total} was 9mM before farrowing, increasing to 12mM on day 2 after farrowing, and decreasing again to 8mM on day 3. The casein concentration of sow milk increases from the time of farrowing until 2d after farrowing [calculated from (6)], and this may be responsible for binding the non-diffusible calcium. Ca\text{2+} showed a similar pattern of change, starting at 4mM before farrowing, increasing to 7mM on day 2 after farrowing, and decreasing again to 5mM on day 3. Ca\text{2+} decreased from 11mM before farrowing to 6mM by day 2 after farrowing. Ca\text{2+} was significantly related to Cit\text{diff} (r² = 0.585, p = 0.0001), and this relationship improved slightly when Ca\text{2+} was corrected for Ca\text{diff}² (r² = 0.621, p = 0.0001).

The general relationship between Ca\text{diff} and Cit\text{diff} in established lactation applies during the rapid increases in Ca\text{diff} and Cit\text{diff} in human milk during lactogenesis II. We have now shown that the predicted relationship still applies during lactogenesis II in the sow which is unique in showing a decrease in Cit\text{diff} concentration concurrent with a rapid increase in Ca\text{total}.


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FOLLICULAR DYNAMICS IN COWS FROM A HERD WITH A HISTORY OF TWIN-CALVING


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Ultrasonic monitoring studies of ovarian follicle turnover in cows have led to the development of models highlighting follicular dominance as a major mechanism by which cows remain uni-ovular. Although twin-calving is relatively rare in cattle (1-3% in most breeds) studies have confirmed that repeated twin-calving invariably arises as a result of twin ovulations. Furthermore, the incidence of twin ovulation and twin-calving can be increased if sufficient selection pressure is applied. The Ruakura twin-calving selection herd was established in 1982 to study genetic and physiological factors associated with twinning in cattle (1). In the present study cows from the Ruakura twin-calving selection herd and an unrelated control herd were used to compare ovarian and oestrous activity using ultrasound scanning. In particular we were interested in testing the hypothesis that twin-ovulations were the result of two rather than one dominant follicle developing during the latter part of the oestrous cycle, probably as a result of a diminishing of the dominance effect observed in single-ovulating cows. Oestrus was synchronised in 14 cows from the twin-calving selection herd (Selected) and 16 cows from an unrelated herd with no history of twin ovulations or twin calving (Control). Beginning on the day of oestrus, ovaries were examined once a day using a 7.5 MHz ultrasonic probe and the growth of each follicle greater than 4 mm was monitored. Ovarian monitoring ceased once cows had shown a second oestrus and ovulated. Data from 3 Control cows were excluded for being incomplete.

Table 1  Follicle data from Selected and Control cows

<table>
<thead>
<tr>
<th></th>
<th>No. Cows</th>
<th>Max. Follicle Diameter mm</th>
<th>Folicle Waves per Cycle</th>
<th>Mean Daily Total Follicles</th>
<th>Multiple Ovulations %</th>
<th>Multiple Ovulations % Cycle Length days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selected</td>
<td>14</td>
<td>14.4</td>
<td>2.7</td>
<td>15.1</td>
<td>71</td>
<td>43</td>
</tr>
<tr>
<td>Control</td>
<td>13</td>
<td>13.4</td>
<td>2.9</td>
<td>15.2</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22.8</td>
<td>22.2</td>
</tr>
</tbody>
</table>

There was no difference in the maximum diameter of the largest follicle during the cycle, the number of dominant follicle waves per cycle or the mean total number of follicles present per day (Table 1). A major difference favouring the Selected cows was the number of follicle waves with more than one dominant follicle and the incidence of multiple ovulations (Table 1). In all instances, twin ovulations were immediately preceded by the development of twin dominant follicles. Cycle length ranged from 19-28 days in Selected cows and 20-26 days in Control cows. These data support the hypothesis that twin ovulations in cows are associated with a preceding development of twin dominant follicles. A pair of dominant follicles is present for several days and can occur during the 1st, 2nd or 3rd follicle wave. It is thus likely that dominance is attenuated in cows with a history of twin-calving. As a consequence, more follicles continue to grow and the incidence of multiple follicles/wave and therefore multiple ovulations is increased in cows with a history of twin-calving. Furthermore, the twin-follicles present during the ovulatory follicle wave are more likely to twin-ovulate in the Selected group compared with Controls. Thus, a higher incidence of twin-follicle waves as well as a higher propensity to ovulate appear to have contributed to the higher incidence of twin-ovulations and therefore twin-calving in the Selected herd.

FOLLICULAR DYNAMICS, OVULATION AND OESTRUS IN BEEF COWS SUCKLING EITHER ONE OR TWO CALVES

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Recent ultrasound scanning studies on post partum activity in cows have focused on dominant follicle turnover as being the key ovarian event leading to the timing of first oestrus and ovulation. In single-suckled beef cows under Irish conditions the extended period of post-partum anoestrus, when compared with dairy cows, is due to the lack of ovulation of dominant follicles rather than a failure of dominant follicles to develop (1). Twin-suckling can further increase the interval to first oestrus activity, but dominant follicle activity has not been monitored in twin-suckled cows. The aims of the current study were to describe ovarian and oestrous activity in cows rearing either one or two calves. In particular, we wanted to test the hypothesis that the extended interval to first oestrus in twin-suckled cows was a consequence of continued dominant follicle turnover rather than the absence of dominant follicle development. The ovaries of twenty three single-suckled Hereford × Friesian cows and 12 contemporary twin-calved and twin-suckled cows were scanned daily beginning about 1 week after calving until first oestrus and follicle development described. All cows initiated a dominant follicle but 1 cow in each group did not ovulate or show oestrus (day 77 and 120 post-partum).

Table 1

<table>
<thead>
<tr>
<th></th>
<th>No. Cows</th>
<th>Interval to First Dominant Follicle (days)</th>
<th>'Silent' Ovulations (% of cows)</th>
<th>Interval to 1st Ovulation (days)</th>
<th>Interval 1st to 2nd Ovulation (days)²</th>
<th>Interval to First Oestrus (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-suckled</td>
<td>23</td>
<td>10.5</td>
<td>64</td>
<td>38</td>
<td>10</td>
<td>43</td>
</tr>
<tr>
<td>Twin-suckled</td>
<td>12</td>
<td>13.3</td>
<td>27</td>
<td>46</td>
<td>12</td>
<td>45</td>
</tr>
</tbody>
</table>

¹ P<0.05 ² P<0.1 NS NS NS

in cows with 'silent' ovulations

The first dominant follicle took longer to appear in twin- compared with single-suckled cows (Table 1). There was no relationship (r² <5%) between the interval to the appearance of the first dominant follicle and either the interval to first oestrus or the interval to first oestrus. There was a tendency for fewer twin-suckled cows to show a 'silent' ovulation before first oestrus, and for the interval from calving to first oestrus to be longer (Table 1). The interval from the first to the second ovulation as well as from calving to first oestrus was similar in both groups of cows (Table 1). Our results in single-suckled cows are very similar to those achieved in Irish studies, although interval to oestrus were about 10 days shorter in the present study (1). We could not test our hypothesis because twin-suckling did not extend the intervals to ovulation or oestrus. The lack of a relationship between the timing of the appearance of the first dominant follicle and the interval to either first oestrus or ovulation indicates that the major factors controlling the timing of these events are different. These results indicate that twin-suckling need not necessarily be associated with extended intervals from calving to first oestrus.


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FOLLICLE AND OVULATORY PATTERNS IN ROMNEY EWES TEASED BY RAMS DURING LATE ANOESTRUS

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In British sheep breeds such as the Romney, the proportion of ewes ovulating in response to teasing increases linearly over the mid-January to mid-February period in New Zealand (1). We know very little about why some ewes respond and others do not, but between-ewe differences could arise from differences anywhere along the hypothalamus-pituitary-ovary axis. The aim of this study was to describe some ovarian factors that may explain some of the differences. Eighty four Romney ewes, continuously isolated from rams for several weeks, were examined using a laparoscopy to count surface ovarian follicle numbers (>1-2 mm diameter) during a 3 week period beginning in mid-January. The ewes were then continuously teased with rams and four days later ovaries were again examined. Non responsive (ie anovular) ewes were then either re-teased with novel rams or re-isolated for 4 days and their ovaries re-examined. Total follicle numbers per ewe were 13.9 ± 0.65 (mean ± SEM, range 3-36) prior to first teasing. About 17% of these follicles were >3 mm diameter, although 77% of ewes had at least one such follicle. Twenty five percent of ewes had at least one large (>4.5 mm) follicle. Overall, 61% of ewes ovulated to first teasing and 37% of these were twin ovulators. Total follicle numbers >1-2 mm prior to first teasing were similar to cows with 'silent' ovulations.

1. Sensitivity to gonadotrophin and/or gonadotrophin secretion patterns may be more important. 2. Immediately re-teased ewes will respond to novel rams. This finding questions the need for long term isolation and highlights the urgency for a description of 'physiological' rather than physical isolation. 3. Ovarian responses to teasing can be 'carried over' for several days since more re-isolated ewes than continuously isolated ewes ovulated. Current models used to describe ram-induced ovulations need to be modified to explain these findings.

EFFECTS OF OESTRADIOL ON SYNCHRONY PATTERNS IN CATTLE TREATED WITH AN INTRAVAGINAL PROGESTERONE DEVICE

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An experiment was designed to test the hypothesis that the concurrent administration of oestradiol benzoate (ODB) at the initiation of a treatment with progesterone would alter the subsequent synchrony pattern in lactating dairy cows. Cycling animals in 12 seasonal dairy herds each had a CIDR device (EaziBreed CIDR, Inter Ag, Hamilton) containing 1.9 g progesterone inserted into the vagina for 7 or 12 days. Half of the devices had a gelatin capsule containing 10 mg ODB placed in a grooved surface before insertion. The dates of insertion were arranged so that similar numbers of cows within a herd had devices removed during the morning milking on each of Days 20 to 24 (Oestrus = Day 0). The herd owners recorded oestrous and insemination dates for the trial cows. Those not detected in oestrus within 14 days of device removal were presented for a veterinary examination.

The analysed results were from 1028 of 1091 cows (94.2%) after excluding 2.4% which lost their device, 0.5% which were anoestrus and 2.8% which ovulated without being detected in oestrus. There were 41 to 65 cows in each of the 20 sub-cells (7 vs 12 days x +ODB x 5 removal days).

The addition of ODB in a 7-day CIDR treatment reduced the percentage of cows detected in oestrus by 48 h after device removal (61% vs 29%; p<0.001) with compensatory increases by 72 h (25% vs 46%; p<0.01) and 96 h (5% vs 14%; p<0.05). These effects were most pronounced when a device was removed on Days 20 or 21 (day x treatment; p<0.01).

A contrasting effect occurred with a treatment period of 12 days. The inclusion of ODB increased the 48 h synchrony rate (65% vs 75%; p<0.02) with these increases varying from 4% (Day 24) to 14% (Day 20; p<0.10). There were 96.8% of these cows detected in oestrus by 96 h after device removal if they were treated with ODB compared to 92.3% of those which did not receive ODB (p<0.05).

The synchrony patterns were similar for animals treated with a CIDR device for 7 or 12 days without ODB. The maximum synchrony rate at 48 h was 72% with device removal on Day 23. This Day coincided with the equivalent maximums for cows also treated with ODB, but it was only 49% when associated with a 7-day CIDR compared to 84% for 12 days. Minimum synchrony rates at 48 h were 16% and 17% if the use of ODB occurred with 7 days of treatment and device removal on Days 20 or 21 (p<0.01).

Further studies are being completed to test a consequential hypothesis that oestradiol from the capsule in combination with progesterone from the CIDR device is altering follicle wave patterns in late dioestrus as well as the time of luteolysis.

OESTROUS SYNCHRONY, OVULATION RATE AND EMBRYO SURVIVAL IN RECIPIENT EWES COMMENCING SYNCHRONY TREATMENT IN EITHER MID OR LATE CYCLE

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Oestrous synchrony treatment duration approximating the luteal phase can lead to normal or extended cycle lengths depending on the stage of the oestrous cycle at the commencement of treatment. Some of the effects of extended cycle length on donor ewes have been reported (1) but little is known of the effects on ewes treated for synchrony only. The aim of this study was to descriptor and ovariatic activity as well as embryo survival in ewes treated for synchrony only. Two hundred and nine Romney ewes were each treated with a CIDR® device for 12 days. Beginning either in mid cycle (d 7-9) or late cycle (d 13-15), a second CIDR® was inserted for 12 days. Interval to onset of oestrus was recorded twice daily for 3 days. Ovulation rate was recorded in about half of the ewes and 2 fresh embryos were transferred to each. Correlations between interval from CIDR® removal to onset of oestrus were calculated.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No Ewes</th>
<th>% Oestrus over 3 days</th>
<th>Interval to Oestrus</th>
<th>Correlation between Intervals</th>
<th>Ovulation Rate</th>
<th>% Embryo Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid Cycle</td>
<td>99</td>
<td>97</td>
<td>36.5</td>
<td>0.36</td>
<td>1.65</td>
<td>71</td>
</tr>
<tr>
<td>Late Cycle</td>
<td>110</td>
<td>100</td>
<td>32.9</td>
<td>0.20</td>
<td>1.75</td>
<td>73</td>
</tr>
</tbody>
</table>

No ewes were in oestrus within 8 h of CIDR® removal and 96% of cyclic ewes had commenced oestrus by 48 h after CIDR® removal. Other results show that: 1. Oestrus can be synchronised in virtually all cyclic ewes within 48 h of CIDR® removal, 2. Ewes treated from late in the cycle have a 3-4 h shorter interval to oestrus (P<0.05, Table 1), mainly because more ewes are in oestrus between 8 and 24 h (30 vs 19%, NS), 3. Within ewes, the interval to oestrus is inconsistent from treatment to treatment (Table 1). 4. Neither ovulation rate nor embryo survival is affected by synchrony treatment (Table 1). Since none of the ewes were expected to have functional corpus luteum (CL) at CIDR® removal, differences in onset pattern may reflect differences in: clearance of exogenous progesterone (unlikely); follicle parameters (turnover and/or follicle sensitivity to gonadotrophin); or higher neural centres controlling oestrous behaviour. Whatever the explanation, the difference (3-4 h) is unlikely to be of practical significance in AI or recipient synchrony programs. The low correlations reported indicate that interval to onset in individual ewes cannot be reliably predicted from cycle to cycle. Extended periods of progesterone treatment (19-27 d) do not appear to impair corpus luteum function or the capacity of the uterus to support pregnancies following embryo transfer. However, additional studies are required to determine the implications of extended progesterone exposure on sperm transport, ovum competence for fertilisation and subsequent embryo development. We may find that synchrony systems to maximise reproductive competence in recipients differ from those that maximise reproductive competence when artificial insemination or natural mating is planned.

It was hypothesised that (a) progesterone (P4) pre-treatment would increase the proportion of ovulations accompanied by oestrus and (b) oestradiol-17β (E2) treatment following P4 pre-treatment would increase the number of ovulations in non-cycling post-partum mixed-age dairy cows. These hypotheses were tested with a 2 x 2 design. One hundred and sixty-nine cows were milk sampled 3 times weekly from calving for subsequent analysis of P4 concentration by RIA (Coat-a-count, DPC, Calif, USA). At weekly intervals, those cows that were 14-20 days postpartum were stratified on the basis of age (2,3-3 years) and palpated per rectum for the presence of a CL. Half of the cows then received either an intravaginal device containing 1.9 g of P4 (CIDR-B, InterAg, Hamilton, NZ) and the others a blank device (Blank). The device was removed after 5 days, and 2 days later (Day 0), each cow was treated with either 600 μg of E2 or 3 ml of 0.9% sodium chloride (saline) i.m.. Cows were observed twice daily for oestrus. Cows were removed from analysis if they had a reproductive tract pathology upon palpation (n=11), were late calvers or induced to calve (29), or had ovulated (ie had a milk P4 concentration >2.5 ng/ml) or were detected in oestrus on, or before, Day 0 (31). Oestrus was defined as having occurred if a cow was detected in oestrus within 14 days and ovulation if the P4 was >2.5 ng/ml within 19 days of oestrus removal and subsequently being pregnant. Fewer Group P animals were receptive over the 10 days of CIDR insertion. Mean size of the dominant follicle decreased in Group P animals following CIDR insertion, but increased in Group C animals. Follicle size increased in both groups after CIDR removal, but two more Group C alpacas ovulated following a single mating four days later. Regression of the CL subsequently formed was noted in 3/8 Group C and 1/10 Group P alpacas within twelve days of mating. Forty-six days after mating 4 Group C and 5 Group P alpacas were impregnated (Group P, n=11) controlled internal drug releasing-device (CIDR) inserted in the anterior vagina for a period of 10 days. Four days after CIDR removal all females were mated to one of four entire males. Each female was blood sampled, tested for receptivity to a male (2) and had its ovaries observed by ultrasound on the day of CIDR insertion, and every second day thereafter for twenty-eight days.

Table 1. Response to treatment with P4 and/or E2 of non-cycling cows.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group C (n=10)</th>
<th>Group P (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days from CIDR insertion</td>
<td>0 2 10 14</td>
<td>0 2 10 14</td>
</tr>
<tr>
<td>Number receptive</td>
<td>8 10 9 7</td>
<td>8 4 8</td>
</tr>
<tr>
<td>Mean follicle size (mm)</td>
<td>6.2 (0.8)</td>
<td>7.7 (0.8)</td>
</tr>
<tr>
<td>(±SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number ovulated</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

P4 pre-treatment increased the number of cows expressing behavioural oestrus at ovulation (51% vs 38% of ovulating cows had concurrent behavioural oestrus in cows pre-treated with P4 or Blank; respectively). E2 treatment did not alter the number of animals ovulating, exhibiting behavioural oestrus or both compared to saline treated cows. The hypothesis that P4 increases the proportion of cows expressing behavioural oestrus is accepted, but E2 does not increase ovulation rate either alone or following P4 pre-treatment.

GnRH - INDUCED ACCESSORY CORPORA LUTEA IN RED DEER AND SHEEP

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Red deer hinds, unlike sheep, often have an accessory corpus luteum during pregnancy, although their origin and function are unknown. They may represent incompletely regressed corpora lutea formed during an oestrous cycle prior to conception, or be formed following ovulation during pregnancy. The aims of this experiment were to determine:

1. Whether accessory corpora lutea could be induced with a single bolus of GnRH at two stages during the luteal phase of the oestrous cycle;
2. Whether accessory corpora lutea influence the timing of luteolysis of the primary corpus luteum and
3. The fate of accessory corpora lutea following luteolysis of the primary corpus luteum.

Mature hinds (n = 18) and ewes (n = 18) were synchronised with exogenous progesterone (CIDR-G, AHI Plastic Moulding Co.) and then treated with either saline or GnRH (Fertagyl, Intervet) at the rate of 250 µg (ewes) or 500 µg (hinds) 11 or 14 days after progesterone withdrawal. Ovaries were examined laparoscopically under sedation (ewes) or general anaesthesia (hinds) 48 hours prior to saline or GnRH treatment, and again 72 hours and 18 (day 11 treated animals) or 15 (day 14 treated animals) days later. Blood sampling was undertaken to monitor plasma LH after GnRH (2 h for 6 h) and progesterone (daily).

Plasma LH concentrations reached a peak 2 hours after GnRH treatment: mean 9.86 ± 2.10 (sd) ng/ml in GnRH-treated hinds compared with 0.04 ± 0.02 ng/ml in untreated hinds. Ewes averaged 57.41 ± 21.97 ng/ml 2 h after GnRH compared with 0.50 ± 0.45 ng/ml without treatment.

Accessory corpora lutea, absent in all saline-treated animals, were observed in GnRH treated animals as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Proportion of animals with induced corpora lutea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deer</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0/3</td>
</tr>
<tr>
<td>GnRH</td>
<td>4/6</td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0/3</td>
</tr>
<tr>
<td>GnRH</td>
<td>4/6</td>
</tr>
</tbody>
</table>

One hind had not ovulated prior to treatment and was removed from the experiment. The remaining hinds all had a single corpus luteum and in 8 animals GnRH treatment induced a further easily identifiable single accessory corpus luteum. The ewes had 2 or 3 corpora present at the time of the first laparoscopy, and GnRH induced the formation of a further 1 or 2 accessory corpora lutea in 8 animals. All induced or accessory corpora lutea appeared macroscopically normal, but were younger-looking, and smaller than the primary corpus luteum.

Plasma progesterone profiles indicated that luteolysis had occurred at the same time in control and GnRH-treated animals in each species (hinds: 20.9 ± 1.30 d and ewes: 18.4 ± 1.04 d after CIDR withdrawal, respectively.

At laparoscopy during the subsequent luteal phase (16 or 15 days after GnRH) 3/6 hinds but 0/6 ewes that had previously had accessory corpora lutea had an abnormal or pale and partially regressed luteal structure in addition to a new, apparently normal primary corpus luteum. These results demonstrate that accessory corpora lutea can be induced in both hinds and ewes by a single bolus of GnRH during the luteal phase. Furthermore, their formation does not appear to affect the timing of regression of the primary corpora lutea. However, in the hinds at least, the accessory corpora lutea may not completely regress at this time but they possibly persist into the luteal phase of the next oestrous cycle.