Society for Reproductive Biology
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ABSTRACTS
(Nos 1–110 in order of presentation)

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1. MODELLING CELL JUNCTIONS IN THE TESTIS

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Spermatogenesis is critically dependent on the pituitary hormones FSH and LH, and on androgens produced in the testis in response to LH. However, little is known about the molecular endpoints of hormone action in the testis. Our data from rat and human models suggests that FSH and testosterone (T) regulate the formation and maintenance of cell junctions in the testis at three key sites, these being at i) the inter-Sertoli cell junctional complex, ii) between Sertoli cells and step 8 round spermatids, and iii) between Sertoli cells and step 19 elongate spermatids. These junctions are essential for maintaining the blood–testis barrier and sperm production, and are potential targets for hormonal-based contraception in men. The inter-Sertoli cell junctional complex consists of numerous junction types. We have looked at the regulation of tight junctions (TJ), adherens junctions (AJ), and the testis-specific ectoplasmic specialisation junction (ES) in vitro and in vivo, as these junctions contribute to the formation and maintenance of the blood–testis barrier. In the absence of hormones, immature AJ and TJ form, but FSH and T are respectively required for these junctions to resemble their mature in vivo phenotypes. In contrast, ES junctions do not form in the absence of hormones, but require FSH stimulation. Another site of hormone action is at the Sertoli cell–round spermatid interface. Previous data have demonstrated that this junction is regulated primarily by T in vivo. In order to identify T-regulated genes involved at junctions between these cells, we have employed laser-capture microdissection to isolate enriched populations of round spermatids and analysed these by differential display and real-time RT-PCR. A number of genes have been shown to be T-regulated, and their role(s) in junction maintenance will be discussed. The third site of action of both FSH and T is in controlling the junctions involved in the final release of mature elongate spermatids from Sertoli cells. A reduction in circulatory FSH and T levels prevents the release of sperm, which we propose is due to a change in the adhesive properties of Sertoli cell junctions at this site. This presentation will demonstrate that a variety of in vivo and in vitro models coupled with new techniques (laser-capture microdissection) and sensitive detection systems (differential display PCR, confocal microscopy) can be successfully used to delineate the hormonal regulation of cell junctions in the testis.

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2. ADVANCING THE UTILITY OF MODELS OF ORGAN DEVELOPMENT

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The prostate gland is an organ of the reproductive system that undergoes branching morphogenesis. Beginning during fetal life and continuing through puberty, there is a complex set of spatial and temporal events leading to mature gland development. In order to understand how abnormal prostate development occurs and to identify genes controlling branching morphogenesis and growth, normal development was previously mapped in several ways. Most commonly this involved the use of ductal spreads of isolated prostates and visualisation of branching using 2D images. Usually the number of main ducts was reported and/or the number of branch tips; occasionally stereological techniques were used. One of the main limitations of this older type of methodology relates to the 3D organisation of the gland, which is not represented in 2D images. We have developed a system to detect and quantify the normal patterns of branching morphogenesis in the prostate using confocal microscopy and computer-based algorithms. Using this system we measure the gland volume, branch length and the number of branches, branch points and branch terminal tips. Analyses of transgenic mice with a prostate phenotype identify the parameters affected by the modified gene and thus identify the mechanism of action of the genes on the branching process. Using this model system we report the differential effects of the aromatase and BMP 4 genes on branching morphogenesis that results in prostatic hypertrophy and hyperplasia.

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3. *IN VIVO* ELECTROPORATION METHODOLOGIES TO STUDY MALE REPRODUCTIVE GENE EXPRESSION

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For the past several years our laboratory has been interested in the "lumicrine" regulation of epididymal gene expression by testicular luminal growth factors. To understand the *in vivo* regulation of epididymal genes via this mode of regulation we have used a modified *in vivo* electroporation technique (1). Initial studies were performed with a construct containing EGFP under the control of the CMV promoter to determine the optimal conditions for experiments in the rat epididymis. Approximately 20 μL containing 1–4 μg of construct DNA was microinjected into the lumen and/or interstitium of different epididymal regions using micropuncture techniques. Following injection, the DNA was electroporated into the cells of the epididymis using a pair of tweezer electrodes in conjunction with a BTX 830 electroporator. The electrodes were kept at a constant distance of 0.2 cm to deliver 8 pulses of 21–24 V/50 msec pulse. Following an appropriate time interval, the tissue was removed and analyzed for EGFP fluorescence. The initial segment clearly showed expression of EGFP in every epithelial cell in the region of the electrodes. The electroporation technique was used to perform an *in vivo* promoter analysis of gamma-glutamyl transpeptidase mRNA IV promoter, a gene specifically expressed in the rat initial segment, and electroporated various dominant negative and activating mutant constructs to dissect the FGF/FGFR-MAPK signal transduction pathway within the initial segment of the rat epididymis. This technique has also proved useful for testing "knockout" constructs for biological activity and gene silencing using RNAi and antisense morpholinos.

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(1) Muramatsu, T., Shibata, O., Ryoki, S., Ohmori, Y. and Okumura, J-I. (1997). *Biochem. Biophys. Res. Commun*., **233**, 45–49.

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4. PREDICTING FUNCTION FROM SEQUENCE

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The sequence of a genome contains the plans of the possible life of an organism, but implementation of genetic information depends on the functions of the proteins and nucleic acids that it encodes. Many individual proteins of known sequence and structure present challenges to understanding their function. In particular, a number of genes responsible for diseases have been identified but their specific functions are unknown. Whole-genome sequencing projects are a major source of proteins of unknown function. Annotation of a genome involves assignment of functions to gene products, in most cases on the basis of amino acid sequence alone. Three-dimensional structure can aid the assignment of function, motivating the challenge of structural genomics projects to make structural information available for novel uncharacterised proteins. Structure-based identification of homologues often succeeds where sequence-alone-based methods fail, because in many cases evolution retains the folding pattern long after sequence similarity becomes undetectable. Nevertheless, prediction of protein function from sequence and structure is a difficult problem, because homologous proteins often have different functions. Many methods of function prediction rely on identifying similarity in sequence and/or structure between a protein of unknown function and one or more well-understood proteins. Alternative methods include inferring conservation patterns in members of a functionally uncharacterised family for which many sequences and structures are known. However, these inferences are tenuous. Such methods provide reasonable guesses at function, but are far from foolproof. The development of whole-organism investigations permits other approaches to function prediction when the data are available. These include the use of protein–protein interaction patterns, and correlations between occurrences of related proteins in different organisms, as indicators of functional properties. Even if it is possible to ascribe a particular function to a gene product, the protein may have multiple functions. An underlying problem is that function is in many cases an ill-defined concept. Here we discuss the state of the art in function prediction and describe some of the underlying difficulties and successes.

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5. INVESTIGATION OF THE EFFECT OF OESTROGENIC AGENTS ON DNA DAMAGE IN THE MALE GERM LINE

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There is evidence to suggest that paternal exposures to certain environmental toxicants may result in germ line mutations and adverse reproductive outcomes. Oestrogenic agents have been linked with various types of DNA damage, particularly damage induced via oxidative mechanisms. However, at present the effect of oestrogenic agents on DNA in the male germ line is poorly characterised. In this study, we have investigated the susceptibility of GC-2 cells to DNA damage resulting from oestrogenic agent exposure. GC-2 cells were treated with diethylstilbestrol (DES; 0–1 μM) over a 1-h period. Alternatively, GC-2 cells were glutathione depleted for 12 h with 100 μM buthionine sulphoximine (BSO), or treated with vehicle alone, and subsequently treated with either 500 μM $4OHE_2$ or 1 mM H_2O_2 for 1 h. Cell vitalities were determined by trypan blue assay after treatment. DNA was purified, and damage assessed in an 8.8-kb fragment of the β-globin gene and a 10.4-kb fragment of mitochondrial DNA by the quantitative polymerase chain reaction assay (QPCR). Double-strand breaks in DNA were assessed by pulsed-field gel electrophoresis. Redox activity was also monitored in some experiments using lucigenin-dependent chemiluminescence. No treatment was found to significantly affect cell vitality. QPCR analysis did not detect DNA damage in either the nuclear or mitochondrial DNA fragments of cells treated with DES. However, in cells treated with BSO and $4OHE_2$, a significant ($P<0.05$) increase in damage in the β-globin gene was detected (0.39 lesions/10 kb). In this DNA fragment, damage was also significantly increased in H_2O_2 treated ($P<0.05$; 0.38 lesions/10 kb) and H_2O_2 + BSO treated cells ($P<0.05$; 0.48 lesions/10 kb). In the mitochondrial DNA fragment, significant increases in DNA damage were observed in cells treated with BSO ($P<0.05$; 0.20 lesions/10 kb), $4OHE_2$ ($P<0.01$; 0.30 lesions/10 kb); BSO + $4OHE_2$ ($P<0.05$; 0.29 lesions/10 kb), H_2O_2 ($P<0.01$; 1.3 lesions/10 kb), and H_2O_2 + BSO ($P<0.01$; 2.5 lesions/10 kb). Pulsed-field gel electrophoresis showed no difference in the occurrence of double strand breaks in the DNA of these samples. Lucigenin-dependent chemiluminesecence of GC-2 cell suspensions was not altered as a result of either DES or $4OHE_2$ treatment. These results indicate a susceptibility of GC-2 cells to DNA damage as a result of $4OHE_2$ exposure, particularly under conditions of oxidative stress. As DNA double-strand breakage and redox cycling were not found to be enhanced as a consequence of $4OHE_2$ exposure, it appears likely that DNA adducts are the predominant type of lesion formed.

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6. *IN VITRO* ASSESSMENT OF FUNCTION OF SEX-SORTED FROZEN–THAWED RAM SPERM

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Lambs of predetermined sex have been produced by laparoscopic AI with low numbers of frozen–thawed sex-sorted sperm. However, sperm that have undergone both sorting and freezing may have lower fertility than those that have only been frozen (1). In the present study, we attempt to evaluate *in vitro* the altered function of sex-sorted sperm in the female reproductive tract (FRT). Semen (3 rams, 3 ejaculates per ram) was collected and either a) used directly (FRESH), b) frozen by a commercial method (FT Control; 1), or c) Hoechst 33342 stained and sex-sorted using a modified high-speed cell sorter (2) then frozen (FT Sort; 1). Assessments of sperm (*i*) binding to ovine oviduct epithelial cell (OEC) monolayers (3), (*ii*) migration through artificial cervical mucus (HA; 4), (*iii*) acrosomal integrity using FITC-PNA, and (*iv*) motility were made over 4 h. FRESH sperm bound to the OEC during incubation (61.6, 74.6 and 75.9% bound at 0.5, 2 and 4 h, SEM = 5.3%) whereas FT Control and FT Sort sperm were released from the OEC with incubation (52.1, 45.6, 29.9% and 45.6, 29.0 and 18.1% bound respectively at 0.5, 2 and 4 h). More FT Sort sperm were released at 2 h than FT Control sperm ($p<0.05$). More FRESH sperm (241.6 ± 11.9 sperm; $p<0.05$) migrated 0.5 cm into the HA than either the FT Control (76.4 ± 11.9) or FT Sort (73.9 ± 11.9) samples. There was no difference between treatments in the distance migrated by the vanguard sperm. Overall, more FT Sort sperm (88.6 ± 1.5%, $p<0.05$) were acrosome-intact than FRESH (84.0 ± 1.5%) and FT Control sperm (81.8 ± 1.6%). Motility of FT Sort (51.1, 39.4 and 23.3% motile, SEM = 2.6%) decreased more rapidly during incubation ($p<0.05$) than that of both FRESH (89.4, 85.6 and 78.9% motile) and FT Control sperm (67.2, 58.3 and 35.6% motile at 0, 2 and 4 h, respectively). The rapid release of FT Sort sperm from OEC and their decreased longevity may indicate that FT Sort sperm have a shorter period of time within the FRT to encounter the oocyte than FT Control sperm.
(1) Hollinshead *et al*. (2002) *Reprod. Fertil. Dev.* **14**:505. (2) MoFlo®, DakoCytomation, Fort Collins, CO, USA. (3) Gillan *et al*. (2000) *Reprod. Fertil. Dev.* **12**:237. (4) Hollinshead *et al*. (2002) *Reprod. Fert. Dev.* Suppl. 14:53.

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7. THE AVIAN EPIDIDYMIS: A MODEL TO STUDY EPIDIDYMAL FUNCTION?

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It is proposed that the Japanese quail is a good model animal for studying the mechanism of post-testicular sperm maturation as the process is simple in the quail, only involving the development of motility during exposure for less than a day to secretions of the undifferentiated ductus epididymidis. By comparison, sperm maturation in mammals involves changes to numerous parameters during exposure, for a week or more, to secretions of the highly differentiated ductus epididymidis. We report our findings on epididymal function in the Japanese quail and compare them to reports on the Wistar rat. Although fluid output by the testis of the quail is about 4 times greater than the rat, fluid reabsorption by the efferent ducts is 8 times greater in the quail than rat so that sperm entering the ductus epididymidis are more concentrated in the quail than rat. Fluid reabsorption of the major ions by the ductuli is essentially isosmotic for both species. There is a slight deviation from this in the quail for K^+, glutamate and glutamine (which increase in luminal concentration) and Ca^{2+} and Mg^{2+} (which decrease in luminal concentration). Most of the protein entering the ductuli is reabsorbed in the quail and rat. Fluid reabsorption in the ductus epididymidis is low in the quail and rat. In the quail, it is accompanied by little change in concentration of inorganic electrolytes which account for most (74%) of the osmotic pressure in the fluid, and the ratio of Na:K in the distal ductus is 5.3. However, in the rat the concentration of inorganic electrolytes is reduced substantially to only account for 29% of the osmotic pressure in the fluid, and the ratio of Na:K in the distal ductus is 0.9. Numerous proteins are present in the luminal fluid of the ductus epididymidis of the quail. However, unlike in the rat, the testis is the source of all major proteins except one. This, QEP16, is an unknown protein with a M_r of 16,000, and its secretion is androgen dependent. We are identifying it and determining its role in sperm maturation.

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8. THE IDENTIFICATION OF MUTATIONS AFFECTING MALE FERTILITY USING DENATURING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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For ~40% of infertile men the underlying cause of their infertility remains unidentified, but it is expected that many will be genetic in origin. It is essential that these mutations are identified as with the rising popularity in vitro fertilization and intra-cytoplasmic sperm injection, the genetic alterations will be passed onto the next generation thus perpetuating the problem with potentially serious additional consequences. We are using denaturing high performance liquid chromatography (DHPLC) for the high throughput detection of base-pair changes (mutations or single nucleotide polymorphisms (SNPs)), insertions or deletions within genes thought to be involved in spermatogenesis. Two types of gDNA samples are currently being screened, human infertile males and mice from an ethylnitrosourea (ENU) mutagenesis program. Human samples chosen for screening are selected based on gene and protein expression. Genomic DNA samples are taken from the Andrology Australia DNA repository and compared to known fertile controls. Patients displaying asthenozoospermia are currently being screened for *TPX-1* changes. TPX-1 is a member of the CRISP family of proteins and a component of the sperm head and tail. To date, 7 SNPs have been found in equal proportions in normal and infertile men. Additionally, 1 mutation was identified in a patient with severe teratospermia and resulted in the change of a highly conserved cysteine to an arginine in the cysteine rich carboxyl end of *TPX-1*. A kinase anchor protein 4 (AKAP4) is the major protein of the fibrous sheath of the sperm tail. The gene for this protein is also being screened for mutations and thus far alterations have been found in both the translated and untranslated regions. Further we have refined the DHPLC technology to allow the high throughput screening of gDNA from ENU-mutagenized founder mice, showing that a single heterozygous base pair change is reliably detected within pooled gDNA of 3 mice. This technology coupled with the high mutation load of the founder mice will allow for the rapid screening and subsequent generation of mouse models of altered gene function at a greatly reduced cost compared to traditional knockout and transgenic technologies.

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9. LACK OF SEASONAL VARIATION IN MALE REPRODUCTIVE PARAMETERS MEASURED IN A CAPTIVE AND WILD POPULATION OF COMMON WOMBATS IN NEW SOUTH WALES

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Despite their abundance in the wild, common wombats (*Vombatus ursinus*) do not breed regularly in captivity, such that there is little published about their captive reproductive management. As part of the development of an artificial insemination program in this species and in an attempt to establish important baseline reproductive information, 4 captive male common wombats at Western Plains Zoo (Dubbo, NSW) were systematically examined over a 12-month period in order to assess whether male reproductive function was seasonally dependent. The reproductive parameters investigated included, peripheral plasma testosterone secretion, testis volume and quality of semen (% motility, % live and % normal) collected by electro-ejaculation. An attempt was also made to determine the extent of seasonal change in male reproductive function of common wombats in wild populations. Plasma samples and reproductive tracts were collected from 12 wild male common wombats from the Kangaroo Valley district (NSW) in June (n = 7) and November (n = 5); plasma testosterone secretion, epididymidal sperm characteristics (% motile, % live and % normal) and measurements of testis, prostate and bulbourethral glands were compared. Our results indicated that male common wombats in captivity at Western Plains Zoo did not appear to be seasonal breeders in terms of testosterone secretion or electro-ejaculate quality (% motile, % live and % normal); there was, however, a significant increase in testis size between the months of June to October (F = 4.04; P = 0.05). Lack of distinct seasonal variation in male reproduction was also evident in wild common wombats in Kangaroo Valley. There was no significant difference ($P > 0.05$) between wild wombats collected in June or November with respect to plasma testosterone concentration, epididymidal sperm quality or testis, prostate and bulbourethral gland dimensions.

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10. CRYOPATHOLOGY OF MACROPOD SPERMATOZOA

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Cryopreservation of kangaroo spermatozoa has proven a significant challenge (1). This study documents kangaroo sperm pathology at an ultrastructural level associated with glycerol cytotoxicity and cryoinjury in an attempt to better understand the causes of poor post-thaw sperm survival in this species. Spermatozoa were dissected from the cauda epididymides of eastern grey kangaroos (n = 2) and recovered into a Tris citrate buffer (pH 7.2). Sperm preparations were then exposed to one of three temperature treatments in buffer containing a final glycerol (G) concentration of either 0 or 20%. Treatment 1 involved storing sperm at 35°C for 10 mins. In treatment 2, sperm were initially cooled from 35°C to 4°C at 10°C / min before a 10 min exposure to 0 or 20% G. Sperm in treatment 3 were frozen–thawed according to a standard macropod sperm cryopreservation protocol (1). Glycerol in the freeze–thaw treatment was added after the sperm had cooled to 4°C. All sperm treatments were then subsequently fixed and processed for standard transmission electron microscopy. Sperm injury was described in detail and quantified based on ultrastructural damage and disruption of the axoneme (A), mitochondria (M), plasma membrane (P) and the presence of distinctive periaxonemal spaces along the sperm tail (S). The incidence of each pathology was determined after evaluating approximately 100 spermatozoa. Means for each pathology were compared separately using balanced ANOVAs and least significance difference (P = 0.05) post hoc tests. Results indicate the cytotoxic nature of 20% glycerol at 35°C on kangaroo sperm ultrastructure; an effect that was not as detrimental when sperm were cooled to 4°C and exposed to glycerol. While glycerol was clearly detrimental to sperm at 35°C, it did provide some cryoprotective value during the freeze–thaw procedure, reducing the incidence of mitochondrial, axonemal and periaxonemal pathology.

(1) *Reprod. Fertil. Dev.* **11,** 345–353.

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11. SPERM TRANSPORT, OVULATION AND AI IN THE TAMMAR WALLABY

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In marsupials, fertilization occurs in a narrow time window because the oocyte is transported through the oviduct in less than 24 h (1) during which it acquires a mucoid coat that entraps and inhibits sperm penetration (2,3). To improve our artificial insemination (AI) success, the distribution of sperm in the reproductive tract, and the timing of ovulation were characterized in naturally mated female tammar wallabies (*Macropus eugenii*). Females were watched for the first post-partum (p.p.) mating then isolated. Reproductive tracts were dissected at 0.5 h (n = 5), 6 h (n = 4), 18 h (n = 5), 36 h (n = 5) and 40h (n = 5) post coitum (p.c.), ligated into 13 major anatomical sections, and sperm & embryos were recovered by flushing. A further 13 females were isolated from males for AI and checked for births every 12 h. Semen ($\geq 10^6$ sperm) was deposited into the uterus via laparotomy at different times between 21.7-42.6 h p.p. In 4 females, ovaries were examined and tracts flushed for embryos at 6 h post AI; 9 others were left in order to check for births resulting from AI. First mating occurred 14.7 ± 2.5 h (n = 20) after birth was first detected and lasted 7.2 ± 0.8 min. Within 0.5 h after a single mating the tract contained 26 ± 11 × 10^6 sperm (n = 5) and 22 ± 10 g (n = 5) of seminal plug, much of which is lost within 18 h p.c. Sperm reach the uterus ipsilateral to the follicle within 0.5 h and the upper oviduct within 18 h p.c. Graafian follicles were observed in 3 females dissected at 36.0 h and embryos were seen 36 h (n = 2) and 40 h (n = 5) p.c. Thus ovulation occurred in roughly half of the animals at 36 h p.c. (50.7 h p.p.). A fertilized embryo was recovered 6.4 h post AI (49 h p.p.) and one AI offspring was born after insemination 34 h p.p. This confirms that anaesthesia and laparotomy do not prevent ovulation, successful fertilization can occur after intrauterine AI between 34.0 and 42.6 h after birth, and that embryos formed by AI can develop normally.

(1) Tyndale-Biscoe, C.H. and Rodger, J.C. (1978) *J. Reprod. Fertil.* **52**: 37-43. (2) Renfree, M.B. and Lewis, A.M. (1996) *Reprod. Fertil. Dev.* **8**: 725-42. (3) Roberts, C.T., Breed, W.G. and Mayrhofer, G. (1994) *J. Exp. Zool.* **270**: 321-31.

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12. MIMICKING THE EVENTS OF MENSTRUATION IN THE MURINE UTERUS: A MODEL FOR STUDYING ENDOMETRIAL BREAKDOWN AND REPAIR

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Menstruation and endometrial regeneration occur during every normal reproductive cycle in women and some old world primates. Many of the cellular and molecular events of menstruation have been identified by correlative or in vitro studies, but the lack of a convenient model for menstruation in a laboratory animal has restricted functional studies. In this study, a mouse model for menstruation first described by Finn in the 1980s was modified for use in a commonly used inbred strain of mouse. A decidual stimulus was applied into the uterine lumen of appropriately primed mice and leukocyte numbers and apoptosis were examined over time following progesterone withdrawal. Endometrial tissue breakdown was initiated after 12-16 h and by 24 h the entire decidual zone had been shed. Reepithelialization was nearly complete by 36 h and the endometrium was fully restored by 48 h. Leukocyte numbers increased significantly in the basal zone by 12 h after progesterone withdrawal, preceding stromal destruction. Stromal apoptosis was detected by TUNEL staining at 0 and 12 h but decreased by 16 h after progesterone withdrawal. Matrix metalloproteinases (MMP) -3, -7 and -9 were also detected during tissue breakdown and repair. This mouse model thus mimics many of the events of human menstruation and has the potential to assist in elucidation of the functional roles of a variety of factors thought to be important in both menstruation and endometrial repair.

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13. EFFECT OF GRAFT SITE ON OVARIAN TISSUE GRAFTS IN THE MOUSE

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The rate at which grafts become revascularized differs at different sites. The number of follicles which survive grafting also differs at different graft sites, it is, however, not known whether the quality of oocytes grown at different sites differ in their capacity to form fetuses and live young. To investigate this question ovaries of C57Bl×CBA F1 mice were cut in half and grafted to either of three different graft sites (ovarian bursa, kidney capsule, subcutaneous tissue) in females of the same F1 line. Oocytes were collected 3 weeks after grafting directly from the grafted ovaries and then both matured and fertilized in vitro. As controls, we investigated oocytes collected from the ovary (these mature to MII after overnight in vitro maturation) and ovulated, mature MII, oocytes. Results: graft recovery (no. recovered/no. grafted) was higher for the kidney capsule (46/48, 96%) and bursa (35/40, 88%) than for subcutaneous grafts (60/96, 63%). Subcutaneous grafts gave the lowest oocyte recovery (55 oocytes from 60 grafts). All oocytes recovered from the grafts were matured and fertilized in vitro. The fertilization of the control IVM IVF group was equivalent to the IVM IVF grafted groups, but all IVM IVF groups were below the in vivo matured, control (81–85% 2-cells). Two-cell embryos were transferred to pseudopregnant recipients and collected at day 15 of gestation. Embryos derived from grafts to the bursa were also transferred and left to go to term. Oocytes collected from grafts to the ovarian bursa gave rise to fetuses (4/14, 28%) and live young (2/8, 25%) with the same efficiency as normal IVF controls (5/20, 30% and 3/12, 25% respectively). We also obtained fetuses from the kidney capsule (2/20) and subcutaneous (1/1) grafts. Embryos derived from grafts to the kidney capsule were associated with a large number of fetal resorptions (9 of 11 implanted embryos), but the weights of the two remaining fetuses were comparable to that in the IVF control group (both were 0.26 ± 0.01 g). Further studies are being conducted to ascertain whether the observed differences in oocyte developmental potential are a result of the effect of the graft site on the developing oocyte.

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14. EFFECTS OF A HIGH MATERNAL PHYTOESTROGEN DIET ON THE REPRODUCTIVE DEVELOPMENT OF MALE OFFSPRING

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Environmental oestrogens have been implicated in the reported decline in human sperm counts [1,2]. Administration of oestrogen during the neonatal period has been shown to reduce Sertoli cell numbers [3]. This project investigated the effects of a high maternal phytoestrogen (PO) diet during pregnancy and lactation on the reproductive tract of their male offspring. Three treatment groups were used: LP, male animals born to mothers that had received low PO diet (112 μg/g isoflavanoid) and weaned on to a low PO diet; HP, male animals whose mothers were transferred to a high PO diet (465 μg/g isoflavanoid) at the time of mating and weaned on to a high PO diet; HLP, male animals whose mothers were transferred to a high PO diet at the time of mating and then weaned onto a low PO diet. Groups of male rats (n = 8) were killed at 18 days and 16 weeks (adult) postpartum. One testis from each animal was fixed in Bouin's fluid and the other frozen. Blood was collected for hormone assays. Sertoli cell and germ cell numbers were counted using the optical disector method [4]. At 18 days postpartum, the rats exposed to the high PO diet had significantly fewer Sertoli cells (P<0.001), and higher plasma FSH concentrations (P<0.005) than the LP rats. Sertoli cell (P<0.01) and total germ cell (P<0.01) numbers were significantly reduced in the HP and HLP adult rats. Returning the rats to a low PO diet (HLP) at weaning (21 days postpartum) did not increase Sertoli or germ cell number. No changes in plasma FSH, LH or testosterone or testicular levels of testosterone or dihydrotestosterone were seen between the adult rats. Within the testis each Sertoli cell can only support the development of a finite number of germ cells. These data suggest that a high PO diet during pregnancy and lactation decrease Sertoli cell number and the potential sperm production of male offspring.

(1) Carlsen E *et al*. (1992) *BMJ* **305**, 609–613. (2) Sharpe RM & Skakkebaek NE (1993) *Lancet* **341**, 1392–1395. (3) Atannossova N *et al.* (1999) *Endocrinol*. **140**, 5364–5373. (4) Wreford NG (1995) *Micros. Res. Tech.* **32**, 423–436.

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15. SEASONAL BREEDING IN A VICTORIAN POPULATION OF THE SWAMP WALLABY (*WALLABIA BICOLOR*)

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The swamp wallaby (*Wallabia bicolor*) is a common though unique macropodid marsupial, but much of its basic biology is unstudied. In Victorian populations, seasonal breeding was inferred from pouch young measurements of a small number of animals (1,2). The only other published information about seasonality is for two NSW populations, only one of which appeared to breed seasonally(3). In this study, data was obtained from 253 adult swamp wallabies collected from culled animals throughout the year in south-eastern Victoria from 2000 to 2003. The sex ratio was 1 female:1.84 males, which is comparable to the NSW populations. Both studies are based on shot specimens so it is uncertain whether the sex ratio bias reflects true population trends or a shooting bias. 75.3% of females were pregnant (66.3% blastocysts in diapause, 8.5% early embryos and 3.9% were near-term fetuses). Pregnancies other than blastocysts in diapause were found only in spring or summer. 86.5% of adult females had pouch young. Date of birth was interpolated from pouch young head length. The number of pouch young born in summer (n = 23), as well as the number of pouch young born in spring (n = 23) was significantly higher than the number born in winter (n = 11) (P<0.05 for both). Correspondingly, males had relatively larger testes and prostates in summer than winter (P<0.05). These results indicate that swamp wallabies in south-eastern Victoria have a seasonal pattern of reproduction.

(1) Moyle R (1997) *unpubl. Hons. thesis,* Department of Zoology, The University of Melbourne. (2) Edwards GP and Ealey EHM (1972) *Aust Mammol.* **1**: 307–317. (3) Robertshaw JD and Harden RH (1986) *Aust. Wildl. Res.* **13**: 141–63.

10.1071/SRB03Ab16

16. EARLY HORMONAL RESPONSES TO AN INCREASE IN PLANE OF NUTRITION IN MALE SHEEP

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In Merino rams, an increase in the plane of nutrition stimulates GnRH/LH pulse frequency within 3 days (1) and this effect is correlated with changes in the circulating concentrations of metabolic hormones such as insulin, leptin, IGF-1, and thyroid hormones (2). To provide information on the dynamics of these responses within the first 3 days, we studied intact rams that were fed continuously with diets that were low in energy (9 MJ ME/day) and protein (n = 6) or were changed from the Low diet to one that is high energy (21 MJ ME/day) and protein (n = 6). Jugular blood was sampled every 20 min for 96 h, including a control period of 24 h before the change of diet in the second group. No changes were observed in rams continuously fed the Low diet. In rams that were changed from the Low to the High diet, LH pulse frequency increased only 6 h after first feeding of the High diet, returned to pre-treatment values for 24 h, and then increased again (P<0.05). In the same period, insulin concentration increased (P<0.05) after 4 h and leptin concentration increased (P<0.05) after 8 h. Thereafter, concentrations of both hormones remained high. Diet did not affect the concentrations of thyroid hormones or IGF-1 over the 3 days of observation. Thus, after increase in the level of nutrition, there are fluctuations in the response in LH pulse frequency, but the effect becomes stable after 48 h. The data support roles for both insulin and leptin in the early activation of the GnRH/LH axis, but the temporary decrease in LH pulse frequency that follows the initial increase, while concentrations of both insulin and leptin were still high, suggests that other mechanisms are also involved.

(1) Martin, G.B., Tjondronegoro, S., Blackberry, M.A. (1994). *J. Reprod. Fertil.* **101**, 121–128. (2) Miller, D.W., Blache, D., Boukhliq, R., Curlewis, J.D., Martin, G.B. (1998). *J. Reprod. Fertil.* **112**, 347–356.

10.1071/SRB03Ab17

17. PROGESTERONE CONCENTRATIONS AND CORPUS LUTEUM SIZE FROM 25 DAYS AFTER INSEMINATION IN PREGNANT AND NON-PREGNANT COWS

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On average, 25% of Holstein-Friesian (HF) cows in pasture-fed herds in Victoria experience extended periods of anovulatory anoestrus (AA) (1). Their conception rates to first insemination (1st AI) are lower than those obtained in spontaneously ovulating (cycling; CYC) herdmates when AA cows have been treated with progesterone (P4) and oestradiol benzoate (ODB) to stimulate oestrus and induce ovulation (30% *v.* 45%; 1). The aim is to compare plasma P4 concentrations (PPC) and corpus luteum (CL) size in HF cows from 25 to 37 days after 1stAI associated with a spontaneous ovulation or an induced ovulation involving treatment for AA, and were pregnant to that 1stAI or had an extended luteal phase and were not pregnant. Blood was sampled from each of 127 HF cows in 3 herds at 25, 30 and 37 days after their 1stAI (day 0). These cows had not been observed in oestrus after these inseminations. Their pregnancy status was diagnosed by uterine ultrasonography on days 30 and 37 when the dimensions of each CL were also recorded. A standard treatment for AA had been used with 96 of the 127 cows and meant that ovulation had been induced with injected ODB (1 mg). Eighteen of the 96 cows (18.75%) treated for AA had elevated PPC on day 25 but were not diagnosed pregnant on days 30 or 37. The average PPC for all cows increased from 5.6 ng/mL on day 25 to 6.9 ng/ml on day 30 and to 7.4 ng/mL on day 37 ($P<0.001$). Cows treated for AA had lower PPC on each of these days (5.4 *v.* 6.3 ng/mL, $P<0.01$; 6.5 *v.* 8.1 ng/mL, $P<0.001$; 7.0 *v.* 8.7 ng/mL, $P<0.001$). Pregnancy status did not affect average PPC in either spontaneously ovulating or AA-treated cows (5.50 vs. 5.63 ng/ml; $p> 0.2$). The average diameter of the CL on day 30 was 3.12 cm compared to 3.27 cm on day 37 ($P>0.1$). These averages were not influenced by pregnancy status or whether the animal had either ovulated spontaneously or been treated for AA. The average PPC in cows treated for AA was lower than spontaneously ovulating contemporaries from day 25 to day 37 after 1stAI. These averages were not affected by pregnancy status and were not associated with differences in CL size. Nonetheless, the lower PPC in these cows may be reflected in their lower conception rates to 1stAI.

(1) Eagles, V.M. *et al*. (2001) *Proc. NZ Soc. Anim. Prod.* **61**: 1.

10.1071/SRB03Ab18

18. ALTERED LUTEAL FUNCTION FOLLOWING INDUCED OVULATION IN DAIRY CATTLE

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Oestradiol benzoate (ODB) and gonadotrophin-releasing hormone (GnRH) are two drugs commonly used to induce ovulation in cattle. Evidence suggests that luteal function may be compromised following induced ovulation (1). When Segwagwe (2) used GnRH or ODB to induce ovulation a decreased plasma progesterone concentration occurred as compared to spontaneously ovulating animals. The aim of the present experiment was to examine CL size and concentrations of progesterone in plasma and luteal tissue in cows that were induced to ovulate or ovulated spontaneously. Oestrus was synchronised in 21 non-lactating dairy cows and ovulations were either spontaneous (control), or induced with GnRH (GnRH) or oestradiol benzoate (ODB). The protocol was repeated three times in a cross-over design so that every cow received each of the three treatments. Luteal cross sectional area was measured daily after behavioural oestrus (day 0) and blood samples were collected every second day. On day 12, a luteal biopsy sample was collected to measure luteal progesterone concentration. Higher concentrations of progesterone in plasma were measured on days 4 ($P<0.05$) and 10 ($P<0.01$) when ODB was used to induce ovulation as compared with GnRH or no ovulatory treatment (0.9 ± 0.02, 0.6 ± 0.02 and 0.5 ± 0.02 ng/mL; and 5.3 ± 0.31, 4.0 ± 0.31 and 3.6 ± 0.31 ng/mL for ODB, GnRH and control respectively). The increase from day 4 to day 10 was also larger (4.5 ± 0.29, 3.4 ± 0.29 and 3.1 ± 0.29 ng/mL, for ODB, GnRH and control respectively) ($P<0.01$). Luteal cross-sectional area was not affected by inducing ovulation since CL size did not differ between treatments at any time point ($P>0.4$). The related trends in progesterone concentrations in luteal tissue (56.8 ± 6.01, 49.6 ± 6.03 and 44.6 ± 6.01 ng/mg for ODB, GnRH and control respectively) were not statistically significant ($P=0.5$). Induction of ovulation with ODB was associated with increased plasma progesterone concentrations that could not be related to luteal progesterone concentrations or CL size.

(1) Lucy and Stevenson (1986) *Biol. Reprod.* **35**: 300–311. (2) Segwagwe (2001) MVSc Thesis, University of Melbourne.

10.1071/SRB03Ab19

19. EFFICACY OF OESTRUS SYNCHRONIZATION REGIMENS WITH $PGF_{2\alpha}$ AND PROGESTERONE IN DAIRY HEIFERS

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Luteal status can affect oestrus response rate and conception rate to prostaglandin-based ($PGF_{2\alpha}$) synchrony treatments (1). To evaluate the efficacy of an oestrus synchrony protocol with 2 injections of prostaglandin with or without progesterone supplementation in dairy heifers. This study included 840 dairy heifers from 4 spring-calving dairy herds. All heifers were treated with 2 i.m. injections of $PGF_{2\alpha}$ (Lutalyse; Pharmacia Australia) 11 days apart. The heifers were randomly assigned to a progesterone (P4; n = 424) or untreated group (control; n = 416). Heifers in the P4 group were treated for 5 days prior to the second $PGF_{2\alpha}$ injection with an intravaginal progesterone-releasing device containing 1.38 g of progesterone (CIDR, Genetics Australia). Blood samples were collected via puncture of a coccygeal vessel at 7 days (Day –7) and 2 days (Day –2) prior to the start of breeding. There were no differences (P>0.05) in plasma progesterone concentrations on Day –7 [(P4, 3.44 ± 0.27, ng/mL) *v.* (control, 3.46 ± 0.26, ng/ml)]. P4-treated heifers had higher (P<0.001) progesterone concentrations on Day -2 [(P4, 7.77 ± 0.39, ng/mL) *v.* (control, 6.23 ± 0.39, ng/mL)]. A greater proportion (P<0.001) of P4-treated heifers (375/424 (88%)) were submitted for artificial insemination than controls (332/416 (80%)). Conception rate [P4, 178/375 (47%) *v.* control, 151/332 (45%)] or pregnancy rate [P4, 178/424 (42%) *v.* control, 151/416 (36%)] did not differ (P>0.05) between treatments. The greater proportion of heifers submitted for AI in the P4-treated group was associated with increased plasma progesterone concentrations 2 days prior to the start of breeding.

(1) Xu *et al*. (1997) *Theriogenology* **57**: 687–701.

10.1071/SRB03Ab20

20. DAILY VARIATION IN PLASMA GLUCOSE LEVELS OF DAIRY COWS

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The insulin-IGF-1 axis has been implicated in the control of reproductive function in dairy cattle, especially in the control of ovarian function. These results describe differences in plasma glucose concentrations in lactating dairy cows. Holstein-Friesian (HF) cows, of New Zealand (NZ) (>87.5% NZ genetics) and North American (100% NA genetics; NA) ancestry, were fed either high quality pasture (pasture) or a total mixed ration (TMR) throughout lactation (1). All animals were synchronised and allowed to have an uninterrupted oestrous cycle. Daily transrectal ultrasound identified the timing of luteal regression. When luteum size had decreased in size by 5 mm from one day to the next, a jugular catheter was inserted and 4-hourly blood sampling initiated, continuing until ovulation. Plasma glucose concentrations were analysed during a 76-hour period around the time of the pre-ovulatory surge in luteinising hormone. Cows calved between 29 June and 11 September 2001, with intensive blood sampling occurring between September and November 2001. The average interval from calving to the start of the sampling period was 81.6 ± 1.2 days for the 29 animals enrolled. Data were analysed using REML with ARI covariance structure for repeated measurements and with diet, genotype, time and their interactions as fixed effects. Plasma glucose showed time (P<0.001), diet (P<0.001), genotype (P=0.037), and time by diet effects (P=0.05). Cows fed pasture had lower and more variable plasma glucose concentrations when compared to cows fed TMR (3.04 mmol/L *v.* 3.54 mmol/L, s.e.d. = 0.048). NAHF had lower plasma glucose compared to NZHF (3.34 mmol/L *v.* 3.25 mmol/L, s.e.d. = 0.048). Other genotype and diet differences in the insulin-IGF-1 axis are now being investigated with data for plasma IGF-1 and insulin being analysed. This will provide important insights into differences between cows of differing genotype fed widely differing diets.

(1) Kolver *et al.* (2002). *Proc.NZ Soc. Anim. Prod.* **62**: 246–251.

10.1071/SRB03Ab21

21. PLASMA CONCENTRATIONS OF INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND RESUMPTION OF CYCLICITY IN PASTURE-FED DAIRY COWS

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Low plasma concentrations of IGF-I in early lactation have been associated with extended periods of calving to first ovulation in dairy cows (1). The objectives of this study were to compare plasma concentrations of IGF-I in cycling and anoestrous Holstein-Friesian (HF) cows and to establish any relationship between these concentrations and intervals from calving to first ovulation. Experiments 1 and 2 were conducted in 2001 and 2002. Before the AI program commenced in 2001, 23 of the 72 cows had >1 oestrous cycle, 26 had only 1 cycle, 10 cows ovulated but did not display signs of oestrus and 13 cows remained anovulatory. Year 2 included 30 cows from Year 1 that cycled early (12), late (12) or very late (6). Plasma concentrations of IGF-I were measured at calving, and at 1, 5 and 10 weeks into lactation with an ELISA. Experiment 3 involved 40 cycling and 30 anoestrus HF cows. Plasma concentrations of IGF-I were measured on Days 0 (day of insemination), 6, 12 and 18. In Experiment 1, cycling cows had higher (P<0.01). mean plasma concentrations of IGF-I than anoestrous cows; concentrations ranged from 48.8 ± 5.9 to 68.5 ± 4.4ng/mL for the anoestrous and cycling cows respectively. Cows that were anovulatory had a longer interval from calving to first ovulation than the other three groups (76.5 ± 2.5 *v.* 39.9 ± 2.9 days, P<0.001). Early cycling cows in Experiment 2, had higher plasma concentrations of IGF-I compared to the very late cycling cows (96.5 ± 10.9 *v.* 51.4 ± 15.4 ng/mL, P<0.05). In Experiment 3, plasma concentrations of IGF-I in cycling cows were higher compared to anoestrous cows (76.50 ± 3.85 *v.* 60.30 ± 4.4ng/mL, P<0.01). Cows with low IGF-I concentrations in early lactation are at greater risk of experiencing extended intervals from calving to first ovulation and of not showing signs of oestrus at that ovulation.

(1) Gong J.G. (2002) *Domest. Anim. Endocrinol.* **23**: 229–241.

10.1071/SRB03Ab22

22. DIFFERENCES IN PLASMA CONCENTRATION OF INSULIN-LIKE GROWTH FACTOR-1 BETWEEN PREGNANT AND NON-PREGNANT DAIRY COWS

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Insulin-like growth factor-1 (IGF-1) is associated with the reproductive performance of the dairy cow. The association between IGF-1 concentrations in early lactation and interval to first oestrus and conception are widely reported (1). Changes in plasma IGF-1 in mid and late lactation, which may also be associated with advancing pregnancy, have not been reported in the dairy cow. Given the relationship between bovine somatotropin and IGF-1 (2), it would be expected that plasma IGF-1 concentrations would increase as the pregnancy proceeds. The aim of this study is to determine if pregnancy would alter the pattern of changes in plasma IGF-1 concentrations in mid and late lactation. The study was conducted at the Department of Primary Industries, Kyabram, Victoria. All cows were high-producing multiparous Holstein-Friesians, receiving a pasture-based diet. Plasma samples were collected frequently throughout lactation and analysed using the DSL non-extraction IGF-1 ELISA kit. Data were analysed using univariate analysis of variance, with body condition score as a covariate. Data for pregnant cows (n = 24) were analysed relative to actual conception dates for each cow. This was compared to that from non-pregnant cows (n = 10) relative to day 86 of lactation (average conception day of pregnant cows). Average plasma IGF-1 of both pregnant and non-pregnant cows peaked at 103 ng/mL at around week 12 of lactation. It decreased dramatically over the next 5 weeks to 67 ng/mL, and remained relatively constant over the next 10 weeks. Plasma IGF-1 of pregnant cows was numerically higher than that of non-pregnant cows throughout the data collection period. This difference increased after conception and became statistically significant 15 weeks post-conception (P<0.05). At that time, plasma IGF-1 concentrations were 75 ng/mL for pregnant cows and 53 ng/mL for non-pregnant cows. This difference is expected to increase as the pregnancy proceeds.

(1) Thatcher *et al.* (1996) *Reprod. Fertil. Dev.* **8**: 203–217. (2) Lucy (2000) *J. Dairy Science* **83**: 1635–1647.

10.1071/SRB03Ab23

23. CELL-SPECIFIC EXPRESSION OF βC-ACTIVIN IN THE RAT OVARY

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Previous northern blot analysis of adult mouse tissues revealed significant amounts of βC-activin mRNA only in liver (1). We have assessed βC-activin mRNA and peptide levels and activin C dimer formation in the rat ovary, using real-time reverse transcription and real-time polymerase chain reaction (RT-PCR), non-reducing Western blotting and immunohistochemistry with a specific monoclonal antibody (2). Although βC-activin mRNA was predominately expressed in liver, real-time RT-PCR revealed small, but detectable amounts of mRNA in 3 of 5 extracts of rat ovary. Western blots of ovarian extracts contained immunoreactive bands at sizes suggestive of an inhibin C dimer (32 kDa) and a βAβC dimer (23 kDa), but there was no evidence for a βCβC (21 kDa) dimer. Specific βC-activin immunoreactivity was demonstrated in the granulosa cells of primordial and primary follicles and possibly in the oocyte cytoplasm of some primary follicles, but not in the granulosa cells of antral follicles. The nucleus of the oocyte was stained in some antral follicles. The theca interna was positive in all healthy antral follicles. Within the corpora lutea (CL) there was some cytoplasmic and nuclear staining of large luteal cells. Not all nuclei were stained and fewer cells were stained in regressed or degenerating CL. There was little or no βC-activin subunit immunoreactivity in the ovarian surface epithelium, but the mesothelium at the hilum of the ovary was frequently immunoreactive. The epithelium lining the rete ovarii was also strongly stained for βC-activin. The observation of stage-specific expression in gonadal cells suggests this activin subunit has specific roles, different from those of other activin subunits. Small amounts of mRNA in the presence of significant βC-activin peptide may indicate a rapid turnover of a labile mRNA.

(1) Lau AL *et al.* (1996) Structural analysis of the mouse activin beta C gene. *BBA-Gene Struct. Express.* **1307**: 145–148. (2) Gold EJ *et al*. (2003) Changes in activin and activin receptor subunit expression in rat liver during the development of CCl4-induced cirrhosis. *Mol. Cell. Endocrinol.* **201**: 143–153.

10.1071/SRB03Ab24

24. BASIC FIBROBLAST GROWTH FACTOR EXPRESSION IN HUMAN OVARIAN FOLLICLES

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Basic fibroblast growth factor (bFGF) is a growth factor that is involved in cell proliferation, differentiation, and angiogenesis (1). It has long been known that bFGF acts as a powerful mitogen for various mammalian granulosa cells in culture (2). To investigate the possible involvement of bFGF expression in follicle initiation and growth, quantitative PCR on isolated human follicle populations was performed. Human ovarian biopsies were obtained from healthy fertile women undergoing tubal ligation. Oocytes and granulosa cells of follicles at different stages of growth were isolated with laser capture microdissection in RNase-free conditions. Follicles were characterized as primordial, primary, small secondary, large secondary or antral, using morphological criteria. Owing to the very small amounts of tissue retrieved for primordial and primary follicles, these samples were pooled for each patient. RNA was extracted from samples, reverse transcribed, and relative quantitation determined with TaqMan real-time PCR, using 18S rRNA as the endogenous control. The probe and primers for human bFGF were commercially available in a pre-developed assay mix (Assays-on-Demand: ABI). Preliminary results suggest an upregulation of bFGF mRNA expression with increasing follicle growth. This study demonstrates that a possible relationship exists between bFGF mRNA expression and follicle development.

(1) Gospodarowicz, D. *et al*. (1986) *Mol. Cell. Endocrinol.* **46**: 187–204. (2) Neufeld, G., *et al*. (1987) *Endocrinology* **121**: 597–603.

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25. CHARACTERIZATION OF THE RAT OOLEMMA

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In vitro manipulation of the murine embryo has advanced over the last 50 years with the introduction of ICSI, knockout and cloning technologies. Yet the same technologies have not developed in the rat despite high similarities between proteins with a function in fertilization and fundamental differences seem apparent. A robust *in vitro* culture medium, mR1ECM capable of supporting pre-implantation development through to the blastocyst stage has only recently emerged. *In vitro* culture (IVC) of *in vivo* fertilized embryos in mR1ECM with PVA showed 28.6 % of zygotes and 79.3 % of 2-cell embryos could develop to blastocyst. The major problem was in the initial division after collection. Almost half the failure to develop occurred at this point. KSOM proved to be significantly worse than mR1ECM+PVA for IVC with arrest prominent at the 2-cell stage (89.8%). mR1ECM even supported limited hatching of blastocysts from the zona pellucida (ZP). *In* vitro fertilization (IVF) with mR1ECM has not been successful. This may be due to failure of sperm to undergo capacitation. We unsuccessfully attempted to induce capacitation over a range (0.5–6.0 h) of preincubation times. A major difficulty is that a characterised marker for capacitation in the rat has not been identified. Another problem for successful IVF is that the majority of oocytes collected underwent spontaneous activation resulting in arrest and an oocyte incapable of fertilization (78.6%). This was characterised by cortical granule exocytosis and an alteration to the ZP molecular structure, which precludes sperm binding to the ZP. These significant limitations need to be overcome if we are to be able to study interaction between rat sperm and egg at the molecular level. The overall goal of this project is to be able to identify and characterise oolemma proteins that interact with sperm counterparts as the two gametes undergo fusion.

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26. BONE MORPHOGENIC PROTEIN RECEPTOR-II IS A KEY RECEPTOR FOR TRASMITTING THE ACTIONS OF OOCYTE-SECRETED FACTORS AND GROWTH DIFFERENTIATION FACTOR-9 IN GRANULOSA CELLS

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Oocytes regulate ovarian follicle growth and development by secreting paracrine growth factors that act on granulosa cells. Little is known about the identity of these oocyte factors or the receptor system(s) they use. We have recently determined that growth differentiation factor-9 (GDF-9) accounts for ~1/2 of the total mitogenic activity of oocytes [1]. The present study was conducted to examine the receptor and intra-cellular signalling system utilised by oocytes to promote granulosa cell proliferation. We used an established oocyte-secreted mitogen bioassay, where denuded oocytes co-cultured with primed-mouse mural granulosa cells (MGC) promote cell proliferation in a dose-dependent manner [2]. At sub-maximal doses of mGDF-9, additional co-culture with oocytes had an additive effect on MGC ^{3}H-thymidine incorporation. However, at a saturating dose of 80 ng/mL mGDF-9, GDF-9 + oocyte additivity was lost, suggesting total oocyte mitogenic activity may function through the GDF-9 signalling system. Consistent with oocyte secretion of bioactive GDF-9, oocytes led to phosphorylation of granulosa cell Smad2 intracellular signalling molecules as detected by Western blot. Because it is known that the type-II receptor for GDF-9 is the bone-morphogenic protein receptor-II (BMPR-II), we tested the capacity of the receptor ectodomain (BMPR-II ECD; R&D Systems) to neutralise oocyte mitogenic activity. The BMPR-II ECD antagonised both oocyte and mGDF-9 bioactivity in a dose-dependent manner, completely abolishing activity of both mitogens at 1 μg/mL. The antagonistic actions of the BMPR-II ECD were specific, having no effect on bioactivity of the closely related TGF-β1 and partially antagonising activin-A. This study provides evidence that BMPR-II is a key receptor in transmitting oocyte-secreted factors in granulosa cells, and that the bioactivity of oocytes not accounted for by GDF-9 is likely to be due to a closely related molecule utilising this receptor.

[1] Gilchrist RB *et al.* (2003) *Reprod. Fertil. Dev.* Suppl. **15**, Abs. 93. [2] Gilchrist RB *et al.* (2001) *Dev. Biol.* **240**, 289–298.

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27. ANALYSIS OF A SAGE (SERIAL ANALYSIS OF GENE EXPRESSION) CATALOGUE OF HUMAN GRANULOSA CELLS

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Mammalian ovarian follicles are composed of an oocyte surrounded by granulosa (or follicle) cells. Granulosa cells are essential for successful oocyte maturation and ovulation. A gene expression profile, or transcriptome, provides a catalogue of the genes expressed by a cell or tissue and the levels of individual transcript expression. In the case of the granulosa cell, its transcriptome should allow insight into granulosa cell function and potentially lead to markers of follicle quality for use in human assisted reproduction such as *in vitro* fertilization (IVF). We used SAGE (serial analysis of gene expression) as a technique to determine the transcriptome of granulosa cells. SAGE relies on the assumption that a 14-base sequence whose position is defined in relation to the 3'-end of any transcript uniquely identifies the gene from which the transcript originated (1). Human granulosa cells were obtained from Otago Fertility Services, Dunedin, New Zealand from 4 adult females, at the time of IVF treatment. After purification, total RNA (5.96 μg) was extracted from these cells using the Qiagen RNeasy kit. The SAGE library was constructed with the Invitrogen I-SAGE kit. Briefly, SAGEtags were extracted from concatemerized ditag sequences using SAGE2000 software (Invitrogen, version B). In total 59 clones were sequenced, yielding 1339 SAGEtags. Selected human SAGE libraries (normal ovary, heart, liver, lung, pancreas and white blood cell) were downloaded from the NIH Sagemap website (ftp.ncbi.nih.gov/pub/sage/seq/) and their relative SAGEtag abundances were compared to that of the human granulosa SAGE library. It was found that human granulosa cells had a unique pattern of gene expression and a number of tags were found that had high abundance in the human granulosa SAGE library, but were absent from other libraries (for example, hydroxysteroid (11-beta) dehydrogenase I and scavenger receptor class B member 1). Those genes that were common to many cell and tissue libraries tended to be structural and house-keeping genes (for example gamma actin). PCR techniques were used to independently validate the SAGE catalogue.

(1) Velculescu, V. E., Zhang, L., Vogelstein, B., Kinzler, K. W. (1995). Serial analysis of gene expression. *Science* **270**, 484–487.

10.1071/SRB03Ab28

28. LOCALIZATION OF ADAMTS-1 AND PROTEOLYTIC CLEAVAGE OF VERSICAN DURING CUMULUS MATRIX EXPANSION AND OVULATION

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Prior to ovulation a specialized matrix is assembled around the oocyte and accompanying cumulus cells. Hyaluronan (HA) and a group of HA binding proteins are major components of this matrix and we recently found that the hyalectan versican is selectively incorporated, most likely acting as a crosslinking matrix organizer. The protease ADAMTS-1 (a disintegrin and metalloprotease with thrombospondin motifs-1) is a member of the ADAM family of metalloproteases that cleave members of the hyalectan family of proteoglycans including versican. ADAMTS-1 is rapidly induced in the periovulatory follicle and female progesterone receptor knockout (PRKO) mice have impaired periovulatory induction of ADAMTS-1 mRNA. PRKO as well as ADAMTS-1 null mice display anovulatory infertility due primarily to impaired ovulation. We therefore investigated the protein localization and function of ADAMTS-1 in ovulating ovaries. Specific antibodies against the pro-domain of ADAMTS-1 identified the 110 kDa pro-protein in mural granulosa cells that appeared localized to cytoplasmic secretory vesicles. An antibody against the metalloprotease domain detected the 85 kDa mature (pro-domain truncated) form secreted from cells and selectively bound to the extracellular matrix of the ovulating cumulus oocyte complex (COC). Versican in the ovulating COC matrix was found to be cleaved yielding a 70 kDa N-terminal fragment immunopositive for the neoepitope DPEAAE generated by hyalectanase mediated cleavage. This extracellular processing of versican was reduced in ADAMTS-1 deficient PRKO mouse ovaries, suggesting that ADAMTS-1 is at least partially responsible for this process. Cleavage of versican may alter the matrix structure, adhesive or viscoelastic properties of ovulating COC. These observations indicate that one function of ADAMTS-1 in ovulation is to cleave versican in the expanded COC matrix and suggest that the anovulatory phenotype of PRKO mice is at least partially due to loss of this function.

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29. CHARACTERIZATION OF THE EXTRACELLULAR MATRIX OF THE HUMAN CORPUS LUTEUM

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The ovarian follicle is composed of an epithelium (membrana granulosa) surrounded by a specialized stromal layer (theca). Like other epithelia the granulosa cells are compartmentalized and derive polarity from a basal lamina. On ovulation the follicular basal lamina is degraded and the granulosa cells develop into mesenchymal luteal cells during luteinization (1). Unlike follicles the matrix composition of the corpus luteum has not been determined or characterized. We therefore undertook an immunohistochemical and electron microscopy study of the extracellular matrix of human corpora lutea. Collagen type IV alpha 1 and laminin chains (alpha 1 to 4, beta 1 and 2, gamma 1) and the proteoglycan versican were immunolocalized to frozen sections of human corpora lutea staged as early (0–4 days following ovulation, $n = 3$), mid (5–9 days, $n = 5$) or late (10–14 days, $n = 6$) or regressing (excised during the follicular phase, $n = 1$). Collagen type IV alpha 1 chains were present in all corpora lutea with minimal staining in early corpora lutea and maximal staining in the mid to regressing corpora lutea. Staining was localized to the subendothelial basal laminas and within the luteal parenchyma. Laminin chains alpha 4, gamma1, and beta 2 were localized to the blood vessels, and both laminin beta2 and alpha2 were present within the luteal parenchyma. Versican was present in the connective tissue septae (considered to be largely derived from theca) at all stages of luteal development, and in connective tissue sheaths surrounding large blood vessels. Laminin alpha1 and alpha3 were not detected. One early, 3 mid and 3 regressing corpora lutea were processed for electron microscopic examination. At the electron microscope level subendothelial basal laminas were present. Whilst electron dense extracellular material was deposited intermittently adjacent to the luteal cells, no continuous classic basal lamina structure was observed. This material probably contains laminin beta2 and alpha2 and collagen type IV alpha1. Thus luteal cells appear not to have a continuous basal lamina surrounding them and the matrix environment of the human corpus luteum contains laminin chains, collagen IV and versican.

(1) Rodgers RJ, Irving-Rodgers HF (2002) *Mol. Cell. Endocrinol*. **191**, 57–64.

10.1071/SRB03Ab30

30. UTERINE EXTRACELLULAR MATRIX COMPONENTS ARE ALTERED DURING DEFECTIVE DECIDUALISATION IN INTERLEUKIN-11 RECEPTOR α DEFICIENT MICE

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Implantation is dependent on the differentiation of endometrial stromal cells into decidual cells, and is facilitated by dramatic remodelling of the uterine extracellular matrix. Female interleukin-11 receptor α (IL-11Rα) deficient mice are infertile due to disrupted decidualisation, suggesting a critical role for IL-11 and its downstream target genes in implantation. The molecular targets of IL-11 in the uterus are unknown, but it is likely that IL-11 signalling modifies the expression of other genes important in decidualisation. This study aimed to identify genes regulated by IL-11 during decidualisation in mouse uterus and to examine their expression and localisation as an indication of functional significance during early pregnancy. Decidualisation was induced in pseudopregnant (plug = day 0) wildtype (*IL-11Ra*+/+) and IL-11Rα deficient (*IL-11Ra*-/-) littermates by oil injection into the uterine lumen on day 3. Test RNA extracted from whole uterus at 48 h after induction of decidualisation ($n = 2$/genotype) and reference RNA from wildtype unstimulated uterus ($n = 16$) were used to generate target cDNA for hybridisation of NIA 15K microarrays. Among 15,247 DNA probes, 14 showed increased and 4 decreased expression in *IL-11Ra*-/- uterus. These included 4 genes encoding extracellular matrix proteins – collagen III α1 (2.9-fold increase), secreted acidic cysteine-rich glycoprotein (SPARC, 2.3-fold increase), biglycan (1.8-fold increase) and nidogen 1/entactin (1.8-fold increase). Immunohistochemistry confirmed increased collagen III and biglycan protein expression in *IL-11Ra*-/- uterus at this time. In both *IL-11Ra*-/- and wildtype uterus, collagen III and biglycan were primarily localised to the outer connective tissue and smooth muscle cells of the myometrium, with diffuse staining in the cytoplasm of decidualised stromal cells. Interstitial compartments underlying luminal and glandular epithelium and surrounding blood vessels also showed strong immunoreactivity for both proteins. In the absence of IL-11Rα, stronger staining for collagen III was particularly evident underlying luminal epithelium and in the extracellular matrix supporting antimesometrial decidual cells. These data suggest that IL-11 may regulate changes in the uterine extracellular matrix that are necessary for decidualisation. By elucidating the role of IL-11 regulated genes in murine decidualisation, this study may identify novel targets for the manipulation of human fertility.

10.1071/SRB03Ab31

31. IMMUNOLOCALISATION OF INTERLEUKIN-11 AND ITS RECEPTOR IN ENDOMETRIUM OF INFERTILE WOMEN DURING THE IMPLANTATION WINDOW

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The human endometrium is normally a hostile environment to embryo implantation except for a limited phase of the menstrual cycle known as the "window of receptivity". Mice with a null mutation in the gene encoding for the interleukin (IL)-11 receptor alpha (Rα) are infertile due to a failure of embryo implantation[1]. In the human endometrium IL-11 localises in a pattern suggesting a role in human fertility[2]. The aim of this study was to examine the temporal and spatial location of IL-11 and IL-11Rα in endometrium from infertile women (infertile) and women with normal menstrual cycles and no known endometrial dysfunction (normal). Immunohistochemistry was performed on tissues collected between 5 and 10 days after ovulation. IL-11 and IL-11Rα immunoreactivity was absent in a sub-population of tissues from infertile women. In the infertile tissues that exhibited staining, IL-11 stained minimally in glandular and luminal epithelial cells, stromal cells, and vascular smooth muscle and endothelial cells. Similarly, IL-11Rα immunoreactivity was minimal in all major cellular compartments, with the luminal epithelial cells and vascular smooth muscle cells showing the lowest staining. By contrast, IL-11 and IL-11Rα immunostaining was found in all normal tissues. Furthermore, staining for IL-11 and IL-11Rα was high in the glandular epithelial cells of the normal tissues. Moderate to low staining for IL-11 and IL-11Rα was seen in stromal and vascular endothelial cells, while low staining was apparent in the vascular smooth muscle and luminal epithelial cells. Staining for IL-11 and IL-11Rα was overall markedly higher in normal compared to infertile tissues. These data suggest a role for IL-11 in the preparation of a receptive endometrium, which is critical in the establishment of pregnancy.

(1) Robb *et al*. (1998) *Nat. Med.* **3**: 303–308. (2) Dimitriadis *et al*. (*2000*) *Mol. Hum. Reprod.* **6**: 907–914.

10.1071/SRB03Ab32

32. THE EXPRESSION OF OVULATORY MEDIATORS BY MACROPHAGES ISOLATED FROM THE GONADOTROPHIN-STIMULATED MOUSE OVARY

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IL-1beta (IL-1β), TNF alpha (TNFα), nitric oxide (NO) and macrophages have been shown to be important in stimulating the ovulatory event [1-4]. Since macrophages produce these mediators elsewhere, it was our aim to determine if ovarian macrophages are a source of these known ovulatory mediators. Ovaries from gonadotrophin-primed immature mice were removed at various times and enzymatically digested. Macrophages were tagged with the specific antibodies F480 or anti-MHC II (anti- Ia) and isolated using antibody panning. mRNA was isolated from some macrophages and analysed by quantitative RT-PCR; other macrophages were cultured and secreted protein levels measured. mRNA levels, expressed as fold changes from the first isolation time point and normalised to the housekeeper gene HPRT, show that ovarian macrophages exhibit regulated cytokine mRNA profiles in response to hCG. Both IL-1β and TNFα mRNA increased significantly (*) post hCG injection (IL-1β:Ia. 7.3 ± 2*, F480 5.1 ± 0.8*, TNFα:Ia 2.39 ± 0.5, F480 2.47 ± 0.21*). Secreted TNFα protein increased 6 h post hCG and increased further 24 h post ovulation and then returned to pre-hCG levels 48 h after ovulation. Prior to hCG administration IL-1β was not detectable in conditioned media, but could be detected in media from Ia cells following hCG administration. No changes in total NO activity were detected across the stimulated cycle. Therefore, ovarian macrophages exhibit regulated cytokine expression, particularly of TNFα, in response to hCG. Since TNFα is known to modulate follicular rupture, its production by ovarian macrophages may contribute to the ovulatory process.

(1) Brannstrom M *et al*. (1993) *Endocrinology* **132**: 399-404. (2) Bonello N *et al*. (1996) *Biol. Reprod*. **54**: 436 445. (3) Brannstrom M *et al*. (1995) *Reprod. Fertil. Dev*. **7**: 67-73. (4) Van der Hoek KH *et al*. (2000) *Biol. Reprod.* **62**: 1059-1066.

10.1071/SRB03Ab33

33. IDENTIFICATION OF CHEMOKINES IMPORTANT FOR LEUKOCYTE RECRUITMENT TO THE HUMAN ENDOMETRIUM DURING EMBRYO IMPLANTATION

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At the time of implantation the endometrium becomes a specialised immune environment to allow implantation of the semi-allogenic embryo. A large population of leukocytes, predominantly uterine NK cells and macrophages, infiltrate the endometrium and are believed to modulate trophoblast invasion and facilitate decidualisation. Despite their importance, the factors responsible for recruiting these cells to the endometrium and inducing their activation/differentiation to the 'uterine phenotype' are unknown. In this study, a gene array approach was utilised to identify which chemokines (family of chemotactic factors for leukocytes) are expressed by the endometrium at the time of embryo implantation. We identified eight chemokines that were expressed at this time: monocyte chemoattractant protein (MCP)-3, eotaxin, macrophage inflammatory protein (MIP)-1β, fractalkine, 6Ckine, macrophage-derived chemokine (MDC), hemofiltrate CC chemokine (HCC)-1, and HCC-4. Real time RT-PCR was utilised to analyse their expression across the menstrual cycle and in early pregnancy. mRNA expression of MCP-3, 6Ckine, HCC-1, HCC-4, MDC and MIP-1β was upregulated in the mid-secretory phase, with high expression maintained by MCP-3, 6Ckine, HCC-4 and MDC in early pregnancy. Immunohistochemistry was conducted to examine protein production and cellular source. All chemokines were predominantly localised to the glandular and luminal epithelial cells and decidualised stromal cells. HCC-1, MDC and 6Ckine were upregulated in the secretory phase and early pregnancy whilst MCP-3 and MIP-1β were high throughout the cycle and early pregnancy. Immunostaining was also detected in infiltrating leukocytes for all chemokines, with maximal numbers of MDC-, HCC-1-, and MCP-3-positive leukocytes in early pregnancy. Furthermore staining for HCC-1, 6Ckine and MDC was prominent in vascular endothelium. All of the identified chemokines possess chemotactic activity for monocytes/macrophages (HCC-1 and -4, MCP-3, MIP-1β) or NK cells (MCP-3, MDC, MIP-1β), with the exception of 6Ckine which is a potent T cell chemoattractant. Chemokines expressed by the endothelium are likely to be involved in the initial recruitment of leukocytes from vessels, whilst chemokines produced by epithelial and stromal cells could be more important for the positioning and activation of leukocytes within the endometrium. This data supports a role for these chemokines in the establishment of pregnancy through the recruitment of pregnancy-associated leukocytes.

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34. RECURRENT SPONTANEOUS ABORTION (RSA) IS ASSOCIATED WITH REDUCED ENDOMETRIAL EXPRESSION OF IL-6 mRNA DURING THE SECRETORY PHASE OF THE MENSTRUAL CYCLE

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The success of embryo implantation and ongoing pregnancy is facilitated by the generation of an appropriate maternal immune response. Specific cytokines, principally those associated with type 2 immunity, are implicated in assisting this response. The aim of this study was to quantify mRNA expression for a panel of type 1 and 2 cytokines in the endometrium of fertile and non-fertile women during the secretory phase of the menstrual cycle utilising real-time RT-PCR. Groups of women were classified as; proven fertile (control, $n = 12$), recurrent spontaneous abortion (RSA, $n = 9$), and repeated IVF-failure (IVF-F, $n = 10$). During the 3rd week of the menstrual cycle, biopsy tissue was collected using a Pipelle endometrial sampler and placed in RNA Later (Ambion). Total cellular RNA was extracted (Tel-Test), reverse transcribed (Invitrogen), and subjected to PCR amplification in the presence of SYBR Green (Applied Biosystems) in a 5700 Sequence Detection System (Applied Biosystems). Cytokine mRNA data was normalised to β-actin and analysed by Kruskal-Wallis H and Mann-Whitney U tests. Expression of mRNA encoding IL-6 was significantly reduced in RSA (mean ± SEM, 29 ± 6) compared with control (100 ± 38), but not IVF-failure (55 ± 9). The relative abundance of other cytokines examined including IFNγ, IL-2, TNFα, IL-4, IL-5 and IL-10 was not affected by fertility status. These results shows that IL-6 mRNA is differentially expressed in the endometrium of fertile and RSA patients during the menstrual cycle. The result confirms observations showing diminished IL-6 in endometrial tissue of RSA women (1,2) and suggests this type 2 cytokine is a key mediator of implantation and potentially of maternal immune tolerance towards the conceptus during early pregnancy.

(1) Lim K *et al*. (2000). *Fertil. Steril.* **73**: 136–142. (2) von Wolff M *et al*. (2000). *Mol. Hum. Reprod.* **6**: 627–634.

10.1071/SRB03Ab35

35. OVARIAN MACROPHAGE REGULATION OF INFLAMMATORY RESPONSES AT OVULATION IN MURINE OVARIES

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Ovulation has been likened to an inflammatory reaction [1]. We have observed transient increases in inflammatory cytokine mRNA production in ovarian macrophages accompanied by similar increases in secreted proteins. To produce an inflammatory profile the production of anti-inflammatory agents must occur following ovulation, and it was our aim to examine the production of the anti-inflammatory agent IL-10 to determine if this cytokine plays a role. Ovaries from gonadotrophin-primed immature mice were removed at various times and enzymatically digested. Macrophages were tagged with the specific antibodies F480 or anti-MHC II (anti-Ia) and isolated using antibody panning. mRNA was isolated from some macrophages and analysed by quantitative RT-PCR, other macrophages were cultured and secreted IL-10 levels measured. Analysis of IL-10 mRNA and protein levels in ovarian macrophages showed that although it is produced by these cells, there were no changes in cytokine mRNA or protein across the stimulated cycle, suggesting that this cytokine does not influence the inflammatory cytokine profile. Alternative means of controlling inflammation associated with ovulation have therefore been examined. The nuclear receptor peroxisome proliferator-activated receptor (PPAR) gamma has anti-inflammatory activity, including downregulation of macrophage secreted factors such as iNOS, gelatinase-B, IL-1β, IL-6 and TNFα in splenic, peritoneal and alveolar macrophages [2]. We have demonstrated for the first time that PPARs are expressed in ovarian macrophages, and further analysis of their expression profile during the gonadotropin-stimulated cycle indicates that the regulated expression of these receptors may modulate the inflammatory reaction that occurs at ovulation.

(1) Espey L. (1980) *Biol. Reprod.* **22**: 73–106. (2) Ricote M. *et al* (1999) *J. Leukoc. Biol.* **66**: 733–9.

10.1071/SRB03Ab36

36. IFN-GAMMA AND UTERINE EPITHELIAL RESPONSIVENESS TO TGF-BETA

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Positive pregnancy outcomes are critically dependant on an immunologically receptive environment in the female reproductive tract. Seminal fluid is important in generating maternal immune tolerance to paternal transplantation antigens, and TGF-beta 1 has been identified as the key factor in seminal fluid, which initiates this process (1). IFN-gamma is a potent immune modulator known to interfere with TGF-beta 1 signalling in other cell systems, and IFN-gamma is often present in high levels in the semen of partners of women suffering recurrent miscarriage (2). Modulation of uterine epithelial cell responsiveness to TGF-beta 1 by IFN-gamma was investigated using an in vitro cell culture model. Uterine epithelial cells were harvested from estrous female mice and exposed to a range of doses of TGF-beta 1 or beta 3 and IFN-gamma either individually or in combination. GM-CSF and IL-6 were measured as an indication of responsiveness to TGF-beta, using specific bioassays. Addition of TGF-beta 1, beta 2 or beta 3 alone resulted in a 4-fold and 2-fold increase in GM-CSF and IL-6 production, respectively, and the response was dose-dependent. IFN-gamma elicited a dose-dependent inhibition of up to 75% in GM-CSF production but did not affect IL-6 production. When cytokines were added in combination, both TGF-beta 1 and beta 3 overcame the inhibitory effect of IFN-gamma. Similarly, when penicillin - a known IFN-gamma binding molecule - was added to the culture system, the inhibitory effects of IFN-gamma were neutralised. We conclude that there is a mutually antagonistic relationship between TGF-beta and IFN-gamma in semen. High concentrations of IFN-gamma in semen may act to inhibit the immune tolerance-inducing properties of TGF-beta during early pregnancy. The findings support the use of exogenous TGF-beta as a therapeutic strategy in treating miscarriage in women.

(1) Robertson SA, Ingman WV, O'Leary S, Sharkey D and Tremellen KT (2002) *J. Reprod. Immunol.* **57**, 109–128. (2) DJ Sharkey, KP Tremellen, GA Dekker, SA Robertson (2002) *Reprod. Fertil. Dev.* Suppl. **14**, 81.

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37. ANALYSIS OF CELLS EXPRESSING NATURAL KILLER (NK) CELL MARKERS IN THE RAT TESTIS

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Natural killer (NK) cells are large lymphocytes, which are able to recognize abnormal or transformed cells without prior immunization, thereby contributing to innate immunity. NK T cells are a subset of T cells that exhibit both T cell and NK cell surface phenotypes and regulate immune responses by producing regulatory cytokines (IFN-γ or IL-4) in addition to supporting immune surveillance. In mice and humans, NK T cells are identified by binding of the MHC class I-like tetramer, CD1d/αGC. Cells expressing NK cell specific markers are found in the interstitial tissue of the rat testis, but the proportions of these cells that are classical T cells, NK cells, or NK T cells is not known. Using flow cytometry, we established that 40% of non-adherent leukocytes in the adult rat testis interstitium express CD3 (a T cell receptor), 25% express the NK receptor (NKR), and 19% express both CD3 and NKR (i.e. are NK T cells). However, the CD1d/αGC tetramer did not bind to the NK T cells in either testis or blood, suggesting the rat NK T cells may not bind the tetramer. After stimulation *in vitro* by phorbol myristate acetate (PMA) and ionomycin, IFN-γ was expressed by 61% of T cells and 17% of NK T cells in the testis, but IL-4 was not detected. Cells expressing NK cell markers only exhibited little expression of either IL-4 or IFN-γ. The majority of T cell and NK T cells also expressed the CD8 co-receptor. This provides the first evidence for the existence of a substantial population of both NK and NK T cells in rat testes. Since IFN-γ-producing $CD8^+$ NK T cells have been found to play an essential role in the prevention of graft rejection, a similar role in the testis may be expected. Continuing studies on the NK and NK T cell populations of the testis may reveal the mechanisms behind extended graft survival in this organ, and protection of the male reproductive tract from viruses and tumors.

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38. FUNCTIONAL CHARACTERISATION OF A LYMPHOCYTE-SUPPRESSING ACTIVITY IN GONADAL FLUID

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Protection of the developing gametes from attack by the immune system is essential for reproductive success, and autoimmune infertility represents a failure of this protection. The mechanisms are poorly understood, but there is evidence that local suppression of immune cell (i.e. T cell or antigen presenting cell) function by gonad-specific regulatory molecules is involved. Extracts of testicular and ovarian follicular fluid contain a potent inhibitor of T cell activation and proliferation as measured using a standard phytohemaglutinin (PHA) activated thymidine-incorporation assay. This activity has been partially purified from bovine follicular fluid, and initial characterisation indicates that it is a novel molecule. The inhibitor suppresses both basal and PHA-activated T cell proliferation *in vitro* within six hours in a dose-dependent manner. The suppression of proliferation does not appear to involve inhibition of the autocrine growth factor, interleukin-2. At sub-maximal inhibitory doses, the suppression of proliferation is reversible by withdrawal of the inhibitor, but at higher doses suppression is irreversible. This difference in reversibility is due to induction of T cell apoptosis at the higher doses, as indicated by Annexin V/propidium iodide dual-staining flow cytometry and DNA fragmentation analysis. In studies of the activity in several other cell types of different lineages, T cells and B lymphocytes (MPC-11 cell line) show a 10-fold or higher sensitivity to inhibition compared with non-lymphoid cells (HepG2 liver cells, NR8383 macrophages, NRK49F fibroblasts and K562 erythroid cells). These results indicate that the inhibitory mechanism involves T cell growth arrest leading to apoptosis, suggesting that the inhibitor triggers a specific lymphocyte deletion mechanism. Further characterisation of the function and immunoregulatory role of this inhibitor in the gonads is of great importance for understanding immune infertility, and more widely, has potentially significant clinical implications for improvement of transplant survival treatment of leukaemia and autoimmune diseases in general.

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39. THE MAKING OF AN EMBRYO: SHORT-TERM GOALS AND LONG-TERM IMPLICATIONS

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At first sight, the construction of the preimplantation embryo in eutherian mammals seems such a simple process: the generation of a hollow ball of cells comprising an outer trophectoderm epithelium (TE) layer and enclosing an inner cell mass (ICM) with placental and fetal fates, respectively. However, the fascination is in the detail and the subtle mechanisms utilising cell-cell interactions, differentiative cell divisions and an inherent gene expression programme to guide the formation, segregation and relative size of these two critical cell populations. The mouse embryo has become an ideal model for understanding how an epithelium forms in a step-wise manner in a 'real' tissue *in situ* engaging temporally controlled gene activity. We have focused on the mechanisms coordinating biogenesis of intercellular adhesion and multi-protein membrane junction complexes in the TE and these will be discussed. With the advent of reproductive biotechnologies, it has become apparent that these short-term goals of lineage formation and diversification prior to implantation may have more lasting consequences. The mammalian early embryo is sensitive to its environment, which may influence both early morphogenesis but most significantly later fetal and postnatal growth and physiology. Thus, in two models we have developed in rodents, (i) maternal low protein diet fed exclusively during the preimplantation period and (ii) in vitro culture followed by embryo transfer, both have been shown to alter postnatal growth, systolic blood pressure and organ allometry in a gender-specific manner. Such potential 'programming' during early development has clear healthcare implications. To unravel mechanisms of dietary influence upon embryos, analysis of maternal serum, uterus or uterine fluid composition indicates potential roles for amino acid and growth factor environments in the mediation of programming. Analysis of embryo responses to adverse conditions indicate subtle changes occur in lineage allocation and gene expression potential, in particular associated with imprinted genes. The legacy of such early changes is under current investigation. For example, in one direction, we find that post-implantation nutritional support provided by the rodent visceral yolk sac becomes compromised. Thus, we consider that embryonic programming involves a combination of interacting processes operating at metabolic, genetic, cellular and physiological levels. The fascination with simple embryos continues!

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40. RELAXIN – A REGULATOR OF OESTROGEN RECEPTORS IN THE FEMALE REPRODUCTIVE TRACT?

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The 6 kDa peptide hormone relaxin (RLX) is predominantly produced by the ovaries or placenta of pregnant females and facilitates parturition via its action on the cervix and vagina. Another important biological effect of RLX is its ability to induce uterine growth, similar to oestrogen. These uterotrophic effects of RLX are negated by treatment with an oestrogen receptor (ER) antagonist, implying that RLX is capable of ligand-independent activation of ERs (1). Interestingly, RLX treatment also causes a rapid down-regulation in uterine ERβ1 and ERβ2 gene expression in immature ovariectomized rats (2). Thus, a RLX-mediated decrease in ERβ expression may be a prerequisite for oestrogen and other ER activators to exert their effects on target tissues. To study the interactions between RLX and ERs in more detail, we conducted studies in a RLX gene knockout mouse ($Rlx^{-/-}$). The majority of $Rlx^{-/-}$ mice are fertile and produce litters of normal size (3). They also appear to have no difficulty giving birth. However, proliferation of the epithelium lining the vagina, cervix and uterus is reduced in $Rlx^{-/-}$ mice and there is abnormal accumulation of dense collagen fibre bundles in the stroma of these tissues. These phenotypes can be reversed in late pregnant $Rlx^{-/-}$ mice by infusion of 0.05 μg/h recombinant human RLX. Our recent studies demonstrate that ERα gene expression increases on day 14.5 p.c. in $Rlx^{+/+}$ mice and remains high throughout late gestation. In contrast, ERα mRNA concentrations in pregnant $Rlx^{-/-}$ mice are significantly lower compared to $Rlx^{+/+}$ mice. Preliminary data in $Rlx^{+/+}$ mice show that ERβ expression is higher in the early stages of gestation, and decreases when RLX concentrations in the plasma start to increase around day 10 p.c. This also coincides with the upregulation in ERα expression. In summary, our data show that RLX positively influences ERα expression in the uterus of pregnant mice. This is due either to a direct stimulatory effect of RLX on ERα transcriptional activity or an indirect effect involving a RLX-mediated down-regulation in ERβ expression.

(1) Pillai SB *et al*. *Endocrinology* 140, 2426–2429. (2) Pillai SB *et al*. (2002) *Biol. Reprod.* **67**, 1919–1926. (3) Zhao L *et al*. (1999) *Endocrinology* **140**, 445–453.

10.1071/SRB03Ab41

41. GNRH AND TRH RECEPTORS: MONITORING THE FORMATION OF DYNAMIC PROTEIN COMPLEXES IN LIVING CELLS

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Regulated protein-protein interactions are a key feature of many aspects of receptor activation and deactivation. Genetically encoded luminescent and fluorescent fusion proteins in conjunction with biophysical methods such as bioluminescence resonance energy transfer (BRET) have allowed us to monitor dynamic protein-protein interactions involving G-protein coupled receptors (GPCRs). GPCRs have been reported to undergo oligomerization and the formation of receptor complexes could alter both receptor pharmacology and function to provide an additional level of regulation. Using BRET we have confirmed the existence of oligomeric GPCR complexes in living cells and have been able to quantitatively assess the functional interactions of GPCRs with adaptor proteins such as the β-arrestins as well as with several other partner molecules. We show that subtypes of the TRH receptor (TRHR1 and TRHR2) undergo oligomerization and that each subtype interacts differentially with β-arrestin 1 and 2 isoforms. TRHR2 does not utilize β-arrestin 1, however this interaction does occur when TRHR1 is also present, suggesting that formation of the hetero-oligomeric unit can alter receptor trafficking. Although another GPCR, the GnRH receptor (GnRHR) is also capable of forming oligomers, its unique features make it unable to utilize β-arrestins to promote agonist-dependent internalisation rates. We have investigated interactions between the GnRHR and other proteins and demonstrate a novel interaction between GnRHR and E2F transcription factors involved in cell cycle arrest. By monitoring this interaction with BRET, we observed a rapid loss in binding between GnRHR and E2F after treatment with GnRH. This coincided with a GnRH-mediated change in E2F cellular distribution. GnRH mediates an antiproliferative effect in a range of cells expressing the GnRHR and our finding indicates the involvement of cell cycle arrest. Studies have utilized siRNA to knock-down expression of E2F transcription factors, while chimeric receptors and mutagenesis have been applied to define the sites of interaction between GnRHR and E2F factors. By monitoring dynamic interactions of engineered BRET fusion protein partners involved in GPCR-mediated events, we can more fully understand the mechanisms of ligand-induced processes like receptor trafficking, cell cycle and cellular proliferation.

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42. THE SMALL GTP BINDING PROTEIN RalA MEDIATES SIGNALLING PATHWAYS IN BRAIN AND TESTIS VESICLE TRAFFICKING

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A major vesicle traffic pathway in cells is the secretory pathway, which targets proteins destined for secretion out of the cell or for integration into the plasma membrane. Newly synthesised proteins processed by the Golgi are incorporated into different secretory vesicles, which are targeted to the membrane or to various types of endosomes. Another major type of vesicle traffic is endocytosis, where surface receptors are internalised and sorted via the endosomal system for degradation or recycling. Vesicle traffic is tightly controlled by molecular mechanisms that are directed by small GTPases. RalA is a small GTPase involved in the regulation of vesicle trafficking events. It is abundant in brain, testes and platelets. RalA is activated by growth factors and is involved in cell proliferation, oncogenic transformation, filopodia formation, and vesicle trafficking events. We find Ral on at two types of intracellular vesicles: the recycling endosome and a new ring-shaped vesicle that may be involved in the secretory pathway. RalA cycles in a controlled manner between "active" GTP-bound and "inactive" GDP-bound forms. The active form binds to two specific proteins. The first is RalBP1, which has a role in endocytosis of activated receptors. The second is the exocyst complex, which is involved in targetting secretory vesicles to sites of secretion. Ral signalling via the exocyst is involved in the regulation of both exocytosis and filopodia formation. Although Ral has a number of activator proteins, it was thought not to have any inhibitory factors. Through mass spectrometry we have discovered that the protein ERp57 binds inactive Ral. ERp57 is a redox-regulated RalGDI, a type of inhibitory regulator that keeps Ral in its GDP-bound (inactive) state. ERp57 only inhibits RalA when it is in its oxidised form, a state that is generated by oxidative stress in cells. GDI proteins for other small GTPases are important in the regulation of vesicle traffic, so ERp57 is likely to play an important role in RalA-dependent vesicle traffic. Therefore Ral is at the centre of a signalling complex regulated by ERp57 and which signals to either the exocyst or RalBP1. This complex directs intracellular vesicle traffic from recycling endosomes to the plasma membrane and for the secretory pathway.

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43. CALBINDIN-D9K AND -D28K: ARE CRITICAL FOR EMBRYO IMPLANTATION

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Calbindin-d9k (d9k) and calbindin-d28k (d28k) are highly upregulated in uterine epithelium at the time of implantation in mice[1,2]. This study aimed to investigate their functional roles in this process. Both wildtype (W/T) C57Bl6 and C57Bl6^{tmpin} mice (d28k-/-) were used, the latter strain can breed but with reduced fertility[3]. Uterine CaBP-d9k translation was disrupted using morpholino-modified anti-sense oligonucleotides (MO). Antisense d9k MO (30 nM/20µL in special delivery reagent EPEI) was administered by injection into one uterine horn day on either d2.5 or d3.5 prior to implantation (d4.5), (plug = d0). The other horn was injected with an irrelevant MO (n = 3/4 mice per treatment group). Numbers of implantation sites (N) were counted on d5.5. In preliminary studies intraluminal injection of FITC labelled MO showed their high integration into uterine luminal epithelium (the site of both d9k and d28k at implantation). Injection of specific MO into d28k-/- mice on d2.5 completely blocked implantation (Table 1), while no effect was seen following injection on d3.5. Similarly, no effect of MO injection was seen in W/T mice. The results show that endometrial expression of both d9k and d28k is necessary for implantation. The functions of these two calbindins are proposed to be overlapping as implantation can occur successfully when only one is present (i.e. in d28k-/- mice and in W/T treated with anti-d9k MO). The calbindins can now be added to the very small number of proteins shown to be critical for the process of implantation. These studies have clear implications for fertility regulation.

Table 1. Comparison of no. implantation sites in W/T and d28k-/- mice, treated with MO

Phenotype	*n*	Injection	N/Control Horn	N/Treated Horn
W/T	3	d2.5	3.5	2.5
d28k-/-	4	d3.5	3.5	3.5
W/T	4	d2.5	4.7	0
d28k-/-	3	d3.5	3.3	4.7

(1) Nie *et al. Biol. Reprod*. 2000; (2) Luu *et al.* Proc. ASRB 2001; (3) Luu *et al. Reprod. Fertil. Dev*. Suppl. **14**, 2002.

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44. INVOLVEMENT OF A VOLTAGE-DEPENDENT CALCIUM CHANNEL IN SIGNAL TRANSDUCTION IN THE 2-CELL EMBRYO

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Platelet-activating factor (PAF) is an autocrine trophic factor for the preimplantation embryo that induces an increased $[Ca^{2+}]_i$ in the 2-cell embryo. The $[Ca^{2+}]_i$ transient had an absolute requirement for influx of external calcium. The transients were inhibited by blockers of L-type calcium channel blockers but not by a variety of non-L-type channel blockers. This study used whole-cell patch clamp methodology to assess whether the early mouse embryo expressed a functional calcium channel with the properties of an L-type channel. Standard whole-cell patch clamp techniques were used to study Ca^{2+} currents in two-cell embryos. Membrane potential was held at -60mV and depolarizing voltage pulses of 1 s duration were applied between -20 and +80 mV at intervals of 5 s. Currents were low-pass filtered, sampled and digitized at 0.2 kHz. Ba^{2+} was used as the charge carrier. The currents at each voltage-step were recorded before and after treatment of embryos with different kinds of L-type Ca^{2+} channel blockers: diltiazem (75 µM), nifedipine (80 µM) and verapamil (80 µM). Inward currents were measured as the difference between the whole cell currents before and after the addition of a drug or control to the bath solution (NaCl 55 mM, KCl 4.69 mM, $MgCl_2$ 0.2 mM, Na_2EDTA 0.11 mM, glucose 5 mM, $CaCl_2$ 2.04 mM (equivalent to 1.94 mM free-Ca^{2+}), Hepes 20.4 mM, $BaCl_2$ 50 mM (equivalent to 49.99 mM free-Ba^{2+}), adjusted to pH 7.4, 300 mosM/kg. Using diltiazem, a current of 0.23 ± 0.03 nA (mean ± SEM) was detected and was maximal at a voltage of 36.94 ± 2.59 mV. A similar current was evident when either nifedipine or verapamil were used. Prior treatment of embryos with exogenous PAF resulted in a significant ($P < 0.05$) reduction in the proportion of embryos expressing the current and the size of the current compared with those pretreated with rPAF acetylhydrolase. The results show that 2-cell embryos possess a depolarisation-activated membrane channel, with the properties of an L-type calcium channel. The desensitisation of channel activity by prior PAF challenges suggest that the current was activated during PAF-induced calcium signaling.

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45. ANALYSIS OF PROTEIN EXPRESSION IN SMALL NUMBERS OF MOUSE OOCYTES AND PREIMPLANTATION EMBRYOS

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Western blotting (WB) is a widely used method for analysis of protein expression. It is generally used with many thousands of cells. The detailed study of protein expression in mouse oocytes and preimplantation embryos has been limited by the low sensitivity of WB and the high cost and logistical difficulty of collecting large numbers of oocytes or preimplantation embryos. This reports describes the development of methods that allows quantitative analysis of proteins in small numbers of cells. We used a combination of the Pharmacia PhastSystem for electrophoresis and Pierce Chemiluminescent Substrates for detection. We choose several protein targets that we expected to expressed at different levels within the embryo: Lis-1 (a structural protein), Bad (a regulatory protein that forms heterodimers) and CREB (a transcription factor). Oocytes were collected in 2 µL of PBS and proteins extracted in 2 µL lysis buffer containing (2× PBS, 2% Triton X-100, 24 mM deoxycholic acid, 0.4 mM Na vanadate, 1% NP-40, 0.2% sodium dodecyl sulfate, 20 mM NaF, 20 mM $Na_4P_2O_7$, 2 mM PMSF, 3.08 µM Aprotinin, 42 µM Leupeptin and 2.91 µM Pepstatin A). Samples were then boiled for 7 min with 1.5 µL loading buffer (50 mM Tris-HCl, 5 mM EDTA pH 8.0, 12.5% sodium dodecyl sulfate, 0.05% bromophenol blue and 25% beta-mercaptoethanol). Size separation was performed on 20% homogenous SDS polyacrylamide gels (Pharmacia) on a PhastSystem apparatus (Pharmacia, Sweden). Blotting onto PVDF membranes (Hybond-P, Amersham Pharmacia) was performed overnight by capillary action. Membranes were then incubated with primary antibody overnight at 4°C. Followed by washing and incubation with horseradish peroxidase conjugated secondary antibody for 1 h at RT. Membranes were washed and developed with either 1:2 diluted Pico SuperSignal or 1:4 diluted Femto Maximum Chemiluminescent Substrates (Pierce, Rockford, IL, USA) at room temperature. Using these techniques, Lis-1 was routinely detected in 1 oocyte or embryo, Bad in 5 oocytes and CREB in 15. Furthermore we demonstrated that it was possible to strip and reprobe membranes with different primary antibodies at least 3 times with little loss of sensitivity. The remarkable sensitivity achieved with these methods now allow the power of quantitative WB to be routinely used in the analysis of protein expression and protein interactions in the oocyte and preimplantation embryo.

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46. EFFECT OF GM-CSF ON THE IN VITRO DEVELOPMENT OF PORCINE EMBRYOS

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) is expressed in the female reproductive tract during early pregnancy and has been implicated in the regulation of preimplantation embryo development in several species. Culture of human 2- to 4-cell embryos in medium supplemented with recombinant human GM-CSF increased the number of blastocyst cells allocated to the inner cell mass (ICM) and reduced the number of apoptotic cells after 5 days (1). In this study we assessed the effect of recombinant porcine GM-CSF (rpGM-CSF) on the development of porcine embryos. One- and 2-cell embryos (n = 274) were surgically collected as described previously (2) from 18 Large White × Landrace gilts. In 5 replicates of the experiment, embryos from each donor were allocated across treatments. Serum-free culture medium of Tn5 cells infected with the AcPGM virus was the source of rpGM-CSF. The biological activities of rpGM-CSF in AcPGM-infected cell culture supernatants was previously demonstrated by porcine bone marrow cell proliferation and haematopoietic cell colony formation assays (3). Embryos were cultured for 7 days at 38.5°C in either North Carolina State University 23 (NCSU23) medium alone, or NCSU23 medium supplemented with supernatants to give a final GM-CSF concentration of 0, 1 or 10 ng/mL. The proportion of embryos developing to the hatching and hatched blastocyst stages was increased by the addition of supernatants containing 0, 1 and 10 ng/ml GM-CSF (62%, 59% and 59%, respectively) compared with NCSU23 medium alone (31%). Addition of 1 ng/mL GM-CSF increased the number of blastocyst cells allocated to the ICM (15.5 ± 1.9 cells) compared with 0 and 10 ng/ml GM-CSF (11.1 ± 1.7 and 8.2 ± 0.9 cells, respectively). The results indicate that factor(s) present in the supernatants other than GM-CSF affected blastocyst formation. Despite this, GM-CSF was found to influence the allocation of cells within the developing porcine embryo.

(1) Sjöblom *et al*. (2002) *Biol. Reprod.* **67**: 1817–1823. (2) Nagashima *et al*. (1994) *Biol. Reprod*. **51**: 618–622. (3) Inumaru *et al*. (1998) *Immunol. Cell Biol.* **76**: 195–201.

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47. RECOMBINANT HUMAN FSH INDUCED OVARIAN STIMULATION IMPAIRS IN VITRO EMBRYO DEVELOPMENT IN THE MOUSE

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Gonadotrophins are routinely used in animals and humans to induce multiple ovulations and thus increase the number of oocytes available for techniques such as in vitro fertilisation (IVF). Studies in the mouse, using equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (hCG), have reported a reduction in embryo quality compared to naturally conceived embryos. The impact of recombinant human follicle stimulating hormone (rhFSH), which is routinely used during human infertility treatment, on subsequent embryo development and quality is largely unknown due to its limited use in animal models. The aim of this study therefore was to develop a novel model of rhFSH induced ovarian stimulation in the mouse and investigate the impact of rhFSH on embryo development. One-cell embryos were collected from adult female C57Bl/6 × CBA F1 mice treated with rhFSH (0, 2.5, 5.0, 10.0 or 20.0 IU) or 5 IU eCG. All groups received 5 IU hCG 48 h after the start of gonadotrophin treatment. One-cell embryos were also recovered from non-treated control mice. Embryos were cultured in vitro for 88 h under 5% O_2, 6% CO_2, 89% N_2 and the stage of development was morphologically assessed. Differences between groups were determined by one-way ANOVA and Bonferroni's test for multiple comparisons. We found an increased proportion ($P<0.05$) of abnormal one-cell embryos recovered from mice treated with 10 IU (13.1 ± 3.6%) and 20 IU (11.5 ± 3.6%) rhFSH and eCG (19.7 ± 2.0%) compared to control embryos (0.7 ± 0.5%). Furthermore, blastocyst development was reduced in the 10 IU (72.3 ± 5.1%) and 20 IU (77.3 ± 5.6%) rhFSH groups compared to the control group (96.7 ± 1.0%). In conclusion, ovarian stimulation with rhFSH and eCG impairs the in vitro development of preimplantation mouse embryos. These results have potential implications for clinical ovarian stimulation during infertility treatment and subsequent embryo quality. L Edwards is supported by an NHMRC Peter Doherty Fellowship.

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48. CLONING AND CHARACTERISATION OF MOUSE GLUT12 IN PREIMPLANTATION EMBRYOS

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Glucose transport in preimplantation mouse embryos is mediated by a family of facilitative glucose transporters known as GLUT. Whilst several isoforms are expressed and critical during early embryonic development (1), the recent identification of novel mammalian GLUTs and their classification into three sub-classes necessitates re-evaluation of embryonic transporter expression. Here we report the cloning and characterisation of the murine homologue of GLUT12 from preimplantation embryos. Using an antiserum against the human C-terminal GLUT12 dodecapeptide (2), positive immunoreactivity was observed in mouse 2-cell embryos by western immunoblotting. To confirm this observation and identify GLUT12 mRNA transcripts, the mouse genome and the expressed sequence tag (EST) databases were searched and an EST clone (ID 6542091, Genbank Acc: BF139811) was identified that corresponded to the antigenic sequence. RNA from 2-cell embryos was subjected to 5' RACE RT-PCR using primers designed against this EST clone. The results indicate that the mGLUT12 gene contains an open reading frame of 1869 base pairs, potentially encoding a polypeptide of 622 amino acids, which shows 83% sequence homology to hGLUT12. Like its human homologue, GLUT12 mRNA is found predominantly in skeletal and cardiac muscle and fat. However, it is also found in the uterus and embryos. GLUT12 expression is apparent during early development to the 2-cell stage and declines thereafter until E11. GLUT12 expression in classically insulin-responsive tissues such as muscle and fat has led to the suggestion that this may be an insulin responsive transporter (2). However the significance of GLUT12 expression in oocytes and 2-cell embryos in the absence of insulin receptor expression and the decline of its expression following genome activation and insulin receptor expression is unclear.
(1) Pantaleon & Kaye (1998) *Rev Reprod* **3**: 77–81. (2) Rogers *et al.* (2002) *Am. J. Physiol. Endocrinol. Metab.* **283**: E733–E738.

10.1071/SRB03Ab49

49. TREATMENT OF THE PREGNANT MOUSE WITH IGF-II IN EARLY PREGNANCY ENHANCES FETAL AND PLACENTAL GROWTH

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Gene deletion studies have shown that insulin-like growth factor-II (IGF-II) null mutation reduces both placental and fetal growth while IGF-I deficiency reduces fetal growth only. In addition, ablation of the placental labyrinth specific IGF-II transcript (P0) reduces placental growth and alters placental transport capacity resulting in fetal growth restriction. This study aimed to determine whether treatment with exogenous IGF-II during the first half or throughout pregnancy increases placental and fetal growth and alters placental structural maturation. Two cohorts of C57Black female mice of similar weight were mated with Balb/C males. On day 2 of pregnancy (day of plug = day 1), a mini osmotic pump set to deliver either 0, 12.5 or 25 μg IGF-II/day in 0.1 mmol/L acetic acid for either 8 or 16 days was inserted subcutaneously. Females were killed on day 18 of pregnancy (term = 19 days), blood was taken and placental and fetal weights were recorded. Placentas were either frozen for mRNA analyses or fixed for morphometric analyses. Plasma IGF-II in mice treated throughout pregnancy was assayed. IGF-II mRNA expression relative to 18S rRNA was assessed by real time RT-PCR. Placental weight was increased by 9.6% and 7.5% (P<0.05) in mice treated with low and high dose IGF-II respectively from days 2-10 of pregnancy, while fetal weight was increased by 4.1% (P<0.05) in the higher dose group. The volume of the maternal blood space in the placental labyrinth was increased by 11.8% with low dose IGF-II treatment. IGF-II mRNA expression in the higher dose group of mice treated from days 2-18 was 180% of controls following exclusion of animals in which the mini pump had ceased to deliver IGF-II (P=0.01). Maternal net carcass weight was similar in all groups from both cohorts. In conclusion, treatment with exogenous IGF-II in the first half of murine pregnancy enhances both fetal and placental weights late in gestation but has small effects if administered throughout gestation. This suggests that IGF-II is most important in the invasive phase of placental development. IGF-II treatment of individuals with a poor capacity to synthesise IGF-II early in pregnancy may improve placental function and enhance fetal growth in pregnancies at risk.

10.1071/SRB03Ab50

50. FSH-REGULATED GENES IDENTIFIED BY MICROARRAY ANALYSIS OF AN ACUTE FSH WITHDRAWAL MODEL IN JUVENILE RATS

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Follicle stimulating hormone (FSH) affects testicular development and function, acting through receptors on Sertoli cells. We are interested in events that occur during the first wave of spermatogenesis, as during this period, adult sperm output is fixed by factors, including FSH, that influence the total number of Sertoli cells available to support developing germ cells. Acute FSH withdrawal was achieved by immunoneutralisation of FSH in rats for 4 days, and testes were collected from animals at 18 days postpartum (dpp). We previously observed that Sertoli cell proliferation and apoptosis were unaffected by this treatment, as was germ cell proliferation. However, a significant increase in apoptotic germ cell numbers was revealed by TUNEL staining. To identify genes regulated by FSH at this developmental interval, we isolated RNA from these samples and performed microarray analysis on Affymetrix rat genomic U34A chips. Two individual animals were examined for both control and FSH neutralised samples. Genes with at least a 1.5-fold level of expression difference between groups (using GeneSifter analysis program) were considered as significant candidates. Our study identified genes found in a similar analysis of cultured Sertoli cells ± FSH from 20 dpp rats (McClean 2003 *Mol. Endocrinol.*). The steroid acute-regulatory (STAR) protein and endothelin genes were down- and up-regulated, respectively, in the absence of FSH. We will next examine other time points following *in vivo* FSH withdrawal and during other stages of testis development when the cellular response to FSH is known to differ.

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51. FSH REGULATES SERTOLI CELL AND SPERMATOGONIAL POPULATIONS IN THE ADULT DJUNGARIAN HAMSTER

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The hormones that regulate spermatogonial (Sg) development are ill defined; in part owing to lack of appropriate Sg-enriched experimental models. The photo-inhibited hamster model provides a rich source of Sg, thus making it an ideal model to study their control. This study aimed to assess the effects of FSH and testosterone on the re-initiation of Sertoli cell and Sg development in the gonadotrophin-deplete Djungarian hamster, as induced by photo-inhibition. Long day (LD) photoperiod (16L:8D) adult hamsters were exposed to a short day (SD) photoperiod (8L:16D) for 11 weeks to suppress gonadotrophins, resulting in a Sg only testis. Animals then received FSH alone or in combination with either testosterone or the anti-androgen, flutamide, for 7 days. Another group received testosterone alone. Bouin's fixed testes embedded in resin were used for the determination of Sertoli and early germ cell number using the optical disector stereological technique. The number of Sertoli cells, type A Sg, type B Sg/preleptotene spermatocytes (S'cytes) and leptotene/zygotene S'cytes were suppressed in SD controls, to 66%, 34%, 19% and 10% (all $P<0.01$) of LD control values, respectively. Later germ cell types were not observed. FSH treatment, in the absence/presence of testosterone increased Sertoli cell number ($P<0.01$) to normal LD values. Similarly, FSH treatment in the absence/presence of testosterone increased type A Sg, type B Sg/preleptotene S'cytes and leptotene/zygotene S'cytes to ~85%, 69% and 80% (all $P<0.001$) of LD controls, respectively. Testosterone alone did not affect Sertoli and germ cell numbers and remained at SD controls values. These data demonstrate that the re-initiation of Sg is dependent on FSH, with testosterone playing no role. Surprisingly the adult Sertoli cell population in this model is hormonally dependent. This naturally occurring model now provides an extraordinary opportunity to understand the mechanism (apoptotic and or proliferative) by which FSH regulates Sertoli and germ cell development.

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52. PHYTOESTROGEN EXPOSURE REDUCES FERTILITY OF MALE RATS

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Phytoestrogens are plant-derived compounds able to bind to and activate oestrogen receptors α (ERα) and β (ERβ). Exposure to phytoestrogens, in particular soy, through diet is very common. ERα and ERβ are present throughout the male reproductive tract, but the exact role of oestrogen in male reproductive biology is unclear. Male and female Wistar rats used for this study were offspring of female rats maintained on a low soy diet (containing 112 μg/g isoflavanoid) prior to conception through to weaning. After weaning, the juvenile rats were fed the same low soy diet into adulthood. Six adult male rats were transferred to a high soy diet (containing 465 μg/g isoflavanoid) ($n = 6$); the remaining male rats were continued on the low soy control diet ($n = 8$). On days 3, 6, 12 and 25 following the commencement of the high soy diet, the male rats were housed overnight with pro-oestrus female rats (1:1). The female rats were housed separately until parturition. The size and sex ratio of the litters were recorded. After the final mating, the male rats were killed and the epididymides were removed. Sperm counts were performed on the initial segment, caput, corpus and cauda of one epididymis from each rat. Sperm counts showed fewer sperm in the initial segment ($P<0.05$), corpus ($P<0.05$) and cauda ($P<0.01$) epididymides of high soy rats, as compared to the low soy rats. The litter sizes for the treatment groups showed an exposure-dependent response. The litter sizes of the day 3 ($P<0.01$) and day 6 ($P<0.05$) high soy groups was significantly lower than the low soy group, while litter sizes of the day 12 and 25 groups were not significantly different to low soy fed animals. The sex ratios of the litters from both groups were not significantly different. In conclusion, short-term exposure to high phytoestrogen levels reduces male fertility. The mechanisms involved in these changes are being investigated.

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53. LEYDIG CELL RESPONSE TO HCG IN AGEING MEN

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Serum total testosterone (TT) levels decline by ~1% per year from the third decade. The associated rise in serum LH suggests an underlying primary testicular defect, but changes in the hypothalamo-pituitary axis have also been suggested. In this study we aimed to characterise further the effects of ageing on testosterone production in 108 non-obese men aged ≥55 years, and with symptoms suggestive of androgen deficiency, taking part in studies of testicular function and testosterone replacement. hCG (5000 IU IM), as an LH substitute, was administered to assess testicular reserve; TT was measured at baseline and on day 3. Ten reproductively healthy young men (23-35 years) acted as controls (to date 6 have completed testing). Compared to younger men, older men had a lower baseline TT (15.5 ± 0.5 nM *v.* 21.0 ± 1.1 nM, mean ± SEM; P=0.004) and higher LH levels (5.3 ± 0.5 IU/L *v.* 3.0 ± 0.4 IU/L; P=0.004). Following hCG the older men achieved a TT of 28.0 ± 0.9 nM and the younger men 36.2 ± 2.8 nM (P=0.01). The absolute increase in TT was of borderline significance (12.5 ± 0.7 nM *v.* 15.2 ± 0.5 nM; P=0.059) but the percentage rise from baseline was not different. When older men with baseline TT<15 nM (n = 55; TT 11.8 ± 0.3 nM) were compared to those with baseline TT>15 nM (n = 53; TT 19.5 ± 0.5 nM) no difference was seen in the absolute rise in TT achieved (12.5 ± 0.7 nM *v.* 12.7 ± 1.3 nM) while the percentage rise was greater in the lower TT group (112 *v.* 67%, P<0.0001). Across all older men the % rise in TT was greater with lower baseline TT levels (P<0.0001). We conclude (1) overall older men have baseline features of Leydig cell dysfunction (lower TT, higher LH) and a borderline diminished absolute secretory response to hCG compared to younger men; (2) older men with lower baseline T levels did not have increased LH levels and showed a comparable absolute Leydig cell response to hCG leading to a greater percentage rise in TT. These data may indicate a relative paucity of LH secretion in these older men consistent with a degree of hypothalamo-pituitary dysfunction. Further studies of the hypothalamo-pituitary-testicular axis are in progress.

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54. IDENTIFICATION AND CELLULAR LOCALISATION OF CYCLOOXYGENASE-1 AND -2 IN THE ADULT RAT TESTIS

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Although the testis undergoes qualitatively normal inflammatory responses to infection and other stimuli, it is also considered to be an immune privileged site supporting prolonged graft survival. The unique immune environment of the testes appears to be due, in part, to suppression of the pro-inflammatory functions and up-regulation of anti-inflammatory functions of the testicular macrophages. In macrophages from other tissues, regulation of this phenotype has been shown to be a consequence of prolonged prostaglandin (PG) synthesis by the macrophages itself. Although PGs are present at significant levels in the testis even under normal conditions, little is known about their cellular origin or regulation. Synthesis of PGs involves one of two distinct forms of cyclooxygenase (COX): the constitutively expressed COX-1, and the inducible COX-2, which promotes inflammation. Expression of COX-1 and -2 was examined in cultures (3 h, 37°C) of isolated rat testicular cells (macrophages, Sertoli cells, Leydig cells), seminiferous tubules, whole testis fragments and peritoneal macrophages (as control) with or without lipopolysaccharide (LPS; 10 μg/mL), using real-time PCR and/or Western blot analysis. Both COX-1 and -2 were detected in all testicular cells and fragments. However, following stimulation with LPS, COX-2 was significantly up-regulated in testicular and peritoneal macrophages only. As expected, COX-1 showed no response to LPS in any cell type. These data describe, for the first time, the cellular distribution of both COX forms in the rat testis. Both COX forms are expressed in a wide range of testicular cell types, including the testicular somatic cells, macrophages and germ cells, but only the macrophages show an increase in the inflammatory COX form in response to LPS-stimulation. These data provide an explanation for the endogenous levels of PGs in the normal testis, and suggest that production of PGs by testicular cells other than the macrophage may influence the anti-inflammatory/immunosuppressive phenotype of the testicular macrophage.

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55. PENILE DEVELOPMENT IN TAMMAR WALLABIES: A CONTINUING ENIGMA

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Androgens from the developing testes induce differentiation of the phallus in all mammals. In keeping with this concept, administration of androgen to (1) or transplantation of testes into (2) female tammar wallaby pouch young (PY) causes development of a male phallus. Phallic development in tammar males begins relatively late (3) and at a time (after day 60 of pouch life) when there is no sexual dimorphism in levels of plasma androgens (4). To address this dichotomy, we performed two studies. To determine if the late onset of phallic development is due to inactivation of testosterone (by conversion to androstenedione) at earlier stages, we compared the effects of methyltestosterone enanthate (MTE) (which cannot be oxidized to methylandrostenedione) and testosterone enanthate (TE) in female PY beginning at day 20. MTE did not accelerate phallic development whereas TE accelerated phallic growth, suggesting that the delay in male phallic development is due to limiting levels of endogenous androgens. Secondly, to address the fact that levels of androgens (testosterone (T), dihydrotestosterone (D), and androstanediol (A)) are not different in male and female PY between days 60 and 150, we considered the possibility that these hormones are released in a pulsatile fashion missed on routine sampling. Administration of a GnRH analogue to day-104 PY caused only a 55% increase in plasma T with no change in D or A, and we found no evidence of diurnal or pulsatile secretion (levels of T, D, and A averaged 58, 17, and 8 ng/dL) in day 90-115 male PY bled hourly around the clock. In summary, the mechanism by which androgens virilize the phallus is unclear. Possible explanations include the presence of some unidentified androgen in plasma (such as a conjugate), or action of unbound testosterone (circumventing the absence of a high affinity transport protein in this species) that is difficult to measure with available techniques.

(1) Leihy *et al*. (2003) *Endocrinology* **143**: 2643–51. (2) Tyndale-Biscoe and Hinds (1989) *Reprod. Fertil. Dev.* **1**: 243–54. (3) Butler *et al*. (1999) *Anat. Embryol*. **199**: 451–457. (4) Wilson et al. (1999) *Biol. Reprod*. **61**: 471–475.

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56. INVESTIGATION OF ANDROGEN ACTION IN SKELETAL MUSCLE GROWTH AND FUNCTION

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The anabolic effects of androgens on skeletal muscle have long been recognised and exploited by athletes, but the definitive mechanism of androgen action in muscle growth and maintenance remains poorly understood. Our aim is to investigate the physiological effect of androgens on skeletal muscle growth and function using an *in vivo* androgen withdrawal/replacement mouse model. Orchidectomised (Orx) male mice received 3 intraperitoneal 0.1 mg testosterone (T) implants (Orx+T, $n = 6$) or vehicle (Orx, $n = 5$) for 11 weeks. Serum testosterone levels were measured and the following muscles were excised and weighed: extensor digitorum longus (EDL), soleus (SOL), quadriceps, plantaris, gastrocnemius, tibialis anterior and levator ani (LA). LA muscle sections were assessed for fibre cross-sectional area. Following 11 weeks treatment Orx mice had negligible serum T levels (mean ± SE; 0.4 nM ± 0.1) compared to Orx+T mice (31 nM ± 7.1, $P<0.001$). Orx+T mice showed a statistically significant increase in muscle mass compared to controls for fast-twitch (EDL; Orx = 9.1 mg ± 0.3, Orx+T = 11.4 mg ± 0.5, $P<0.005$) and slow-twitch (SOL; Orx = 7.8 mg ± 0.3, Orx+T = 10 mg ± 0.5, $P<0.01$) muscle types, as well as all other muscle groups analysed. These mice also displayed a marked increase in LA fibre area (81% increase compared to Orx, $P<0.001$). We have observed significant muscle degeneration upon androgen withdrawal *in vivo* as reflected by a decrease in muscle mass and fibre size. Androgen replacement via testosterone implant prevented muscle atrophy by promoting hypertrophy of fibres. We are currently assessing the impact of androgen withdrawal/replacement on muscle function parameters such as maximum force, power output and fatigue to determine the relationship between androgen-induced muscle hypertrophy and strength.

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57. TEMPORO-SPATIAL ALTERATIONS IN PROSTATE BRANCHING MORPHOGENESIS IN ESTROGEN-DEFICIENT AROMATASE KNOCKOUT (ArKO) MICE

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The development and growth of the prostate gland requires branching morphogenesis, a process that continues during the neonatal period. Estrogen is involved in prostate development but its temporo-spatial effects during early development are not understood. Using new methods of confocal microscopy and volume-rendering image analysis, the aim was to detect and quantify the temporo-spatial changes in prostate branching morphogenesis in neonatal estrogen-deficient ArKO mice. In wild type animals, the lateral and medial ducts of anterior prostatic lobes reveal developmental asymmetry with significant increases in branching morphogenesis of the lateral duct, which is retained during day 0-3. In ventral lobes, similar asymmetrical growth is observed but increases in branching occur randomly in lateral or medial ducts. At day14, ArKO mice exhibit prostatic hypertrophy and significant increases in the stromal, epithelial and lumenal volume. To determine whether estrogen deficiency has specific immediate effects on neonatal prostate branching morphogenesis, prostatic lobes were analysed at days 1 and 3. At day 1, anterior lobe from knockout mice exhibits significant increase in the duct volume only. At day 3, all events of branching morphogenesis are significantly accelerated in both knockout and heterozygous mice. In the ventral lobe, the effect of estrogen deficiency results in significant increases in some branching parameters in knockout animals at day 3 only. To determine the spatial distribution of these alterations within prostatic lobes, branching events were assessed in individual ducts. In the anterior lobe, all parameters are significantly increased in both lateral and medial ducts in knockout animals with significantly pronounced increases in the medial ducts, resulting in less asymmetry. In the ventral lobe, however, no significant alteration is found in either duct. In summary, estrogen deficiency in ArKO mice leads to significant neonatal age-related lobe- and duct-specific alterations; being more detrimental in anterior than in ventral lobes and more pronounced in medial than in lateral ducts. We conclude that estrogen is a critical regulator of prostate gland development, exerting significant early effects on gland growth. Therefore, the detection and quantification of aberrant branching morphogenesis within the first 4 days of neonatal life predicts prostate pathology that is known to occur in adulthood.

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58. ASPECTS OF LUNGFISH DEVELOPMENT

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The first vertebrates recognizable as tetrapods appeared in the mid-Devonian. It is generally agreed that their ancestors were lobe-finned fish. What is not agreed is how close either of the extant groups of lobe-finned fish, lungfish and coelacanths, is to the actual ancestor of the tetrapods. The soft anatomy of living lungfish shares many similarities with that of living amphibians. These similarities are not present in either coelacanths or any members of the other extant bony fish, the ray-finned fishes. Many very well preserved lungfish from the Devonian possess specialized features that would appear to exclude them from being ancestral to the tetrapods. I am hypothesizing that lungfish in the Devonian may have included metamorphosis in their life cycle and that neoteny may have been an early corollary. These reproductively mature larval lungfish would not have had the specialized features of the metamorphosed adults. They may have had a close relationship with the ancestral lobe-finned fish, which is currently believed to be a panderichthiad fish. There are a number of larval features of living lungfish that suggest paedamorphosis. Also of interest is the very large genome of living lungfish, which they share with some families of urodele amphibians and which is highly correlated with neoteny in these groups. I and my students and colleagues are exploring several lines of research to test this hypothesis. First, we are looking for deficiencies in the lungfish thyroid axis that might be comparable to those found in neotenic amphibians. Second, we are probing the lungfish genome for highly repeated transposable elements that might account for its very large size. And third, we are looking at the patterns of gene expression associated with the development of lungfish fins, pectoral girdle, axial skeleton and head that can be compared with living tetrapods and other groups of living fish such as the more primitive ray-finned fish (*Polyodon, Polypterus*) and cartilaginous fish (*Heterodontus*). This is a ‘work-in-progress’. Some of our current data will be presented.

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59. WHY THE ELEPHANT HAS INTRA-ABDOMINAL TESTES

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The intra-abdominal testes of the elephant have been cause for comment since the time of Aristotle, in 350 BC, but no satisfactory explanation has been forthcoming. Our recent work on the embryology of the African elephant I think provides the answer; elephants are aquatic mammals that have only relatively recently colonised dry land. There are many facts that point to this simple but surprising conclusion. The paleontological evidence leaves no doubt that the elephant's earlier ancestors were aquatic. Fossils of some recent ancestral elephants are found on islands that have never had any connection with the mainland, such as those off the West coast of California. Mitochondrial DNA sequencing of the elephant's aquatic relatives, the dugong and the manatees of Africa and America, confirm their close relatedness. We have recently come to realize that the elephant's trunk almost certainly evolved as a snorkel, and the complete obliteration of the pleural cavity before birth enabled it to snorkel without rupturing its parietal pleural blood vessels. Even the infrasonic vocalizations of elephants, using a frequency similar to whales, and the fact that they continue to grow in height throughout most of their lives, also fits with this aquatic ancestry. But the testes are the icing on the cake. Physiologically, the testes are more threatened by cold stress than heat stress. Thus the Cetaceans (whales and dolphins), whose terrestrial ancestor entered the water 60 million years ago, were forced to retract their scrotal testes back into the abdominal cavity, together with the epididymis and pampiniform plexus. The elephant embryo (like the dugong and manatee) has no gubernaculum, no processus vaginalis, no pampiniform plexus, no scrotum, and hence no means of effecting testicular descent. In a coldwater environment, it made sense to keep its testes warm at all times, and spermatogenesis occurs quite normally at a body temperature of around 37 degrees Centigrade.

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60. SPRASA, A NOVEL HIGHLY CONSERVED SPERM PROTEIN

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Antisperm antibodies (ASA) may be an important cause of infertility, but current tests for the detection of ASA have poor diagnostic value. The inadequacy of current tests, in part, reflects their inability to define the antigenic specificity of the sperm proteins that the ASA react with. Identification of the sperm proteins that ASA bind to is a necessary preliminary step to the development of more useful diagnostic tests for ASA. Identification of sperm proteins that are the antigens for ASA may also lead to a greater understanding of the basic biology of fertility. We have used two-dimensional electrophoresis and western blots to identify a 16 kDa sperm protein as the antigen for ASA from infertile men. Amino acid sequencing by mass spectrometry of tryptic peptides from the protein identified it a previously uncharacterised protein of the α-lactablumin/c-type lysozyme family which we have named SPRASA. A polyclonal antiserum reactive with SPRASA indicated that SPRASA is localised to the acrosome of human, bovine, ovine and cervine sperm. SPRASA and a murine orthologue of SPRASA appear to be expressed primarily in the testis. We conclude that SPRASA is a highly conserved sperm protein that is the antigen for ASA from infertile men and that it is likely to be important in the fertility of humans and other species.

10.1071/SRB03Ab61

61. PRESENCE OF ACTIN AND GTPASE RHO A IN PIDIDYMAL SPERM FROM THE FAT-TAILED DUNNART (*SMINTHOPSIS CRASSICAUDATA)*

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Marsupial sperm are released from the testis with an immature morphology. Whilst in transit through the epididymis the sperm cell undergoes radical structural changes (1). In somatic cells morphological change occurs through reorganisation of the actin cytoskeleton, a process regulated by RhoA protein. It is known that actin is found in the sperm head of a range of mammalian species, and there is evidence that actin is involved in sperm maturation in the tammar wallaby (2). To localise F-actin and RhoA, the epididymis from freshly killed fat-tailed dunnarts was dissected into caput, corpus and caudal regions. Sperm were diffused into PBS and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, and membranes were permeabilised in -20°C acetone. Presence and distribution of F-actin on sperm was tested by incubation with 50 µg/mL Phalloidin-FITC conjugate in PBS with 1% DMSO for 60 min at RT. Presence of RhoA was tested by immunofluorescence: Binding sites were blocked with 10% BSA and 5% NGS in PBS, before exposure to the primary antibody – 1:50 mouse monoclonal anti-human RhoA. Secondary antibody was 1:100 FITC-conjugated anti-mouse IgG. F-actin was found in epididymal sperm of the fat-tailed dunnart. Sperm from the caput and corpus epididymis displayed bright fluorescence on the head and midpiece of the cell whereas fluorescence subsided from all regions but the midpiece of cauda sperm. RhoA distribution coincided with F-actin, and strong fluorescence was seen in the head and midpiece of caput and cauda sperm. Low levels of RhoA were also detected along the flagellum of mature sperm. Control treatments showed that the fluorescence was not due to autofluorescence or non-specific binding of the FITC conjugate. F-actin and RhoA were spatially and temporally localised to those regions of the sperm that are undergoing morphological change. It appears that the proteins may be involved with the processes of sperm epididymal maturation. RhoA found on the flagellum is thought to act through Rhophillin, Roporrin and ACAP3 on the microtubules to regulate motility (3).

(1) Harding, H.R. *et al.* (1979) 'The Spermatozoan.' (Urban & Schwarzenberg: Baltimore.) (2) Lin, M. *et al.* (2002) *Reproduction* **124**: 107–117. (3) Eddy, E.M *et al.* (2003) *Micros. Res. Tech*. **61**: 103–115.

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62. SPERM PROACROSIN/ACROSIN SYSTEM IN TWO MARSUPIAL SPECIES, THE BRUSHTAIL POSSUM (*TRICHOSURUS VULPECULA*) AND THE TAMMAR WALLABY (M*ACROPUS EUGENII*)

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A zymographic procedure using gelatin-sodium dodecylsulphate polyacrylamide gel electrophoresis (gelatin-SDS PAGE) has been optimized to study proacrosin/acrosin system in two species of marsupial, the brushtail possum (*Trichosurus vulpecula*) and the tammar wallaby (*Macropus eugenii*). One major protease digestion band at 50 kDa was detected on a gelatin-SDS PAGE for epididymal brushtail possum sperm acid extract compared to three major bands, 46, 38 and 32 kDa approximate weight for epididymal and ejaculated tammar wallaby sperm acid extract. A minor protease band of digestion at 66 kDa was also present in both these species. Preincubating the gels with 50-mM benzamidine completely inhibited the protease digestion, indicating that these are trypsin-like proteases. A zymogen form of acrosin, proacrosin, was detectable using a spectrophotometric assay to study the activation profile of the acid extracts in both species. Proacrosin activation to acrosin occurs maximally within 30 min and at pH 8.0. The total acrosin activity of sperm present in both species was found to be several-folds the activity found in eutherian sperm. No differences were found in proacrosin activation profile or total acrosin activity isolated from either epididymal or ejaculated spermatozoa in both these species. The importance of the study in relation to fertilization process in these species is discussed.

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63. WHEAT GERM AGGLUTININ INDUCED TYROSINE PHOSPHORYLATION OF HUMAN SPERMATOZOA

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Acquisition of the ability to fertilise the egg is conferred on mammalian spermatozoa during their transit through the female reproductive tract. This process, termed capacitation, represents the culmination of a series of complex physiological and biochemical changes. Among the correlates of this process, the phosphorylation of tyrosine residues on multiple sperm proteins appears to be critically important for subsequent sperm-egg interaction. In this study, we report the novel finding that the binding of the lectin, wheat germ agglutinin (WGA) to human spermatozoa elicits a dose-dependent increase in tyrosine phosphorylation. Interestingly, this response appears to be specific to the WGA lectin, which possesses dual binding specificity for terminal N-acetylglucosamine (GlcNAc) and sialic acid residues. Other lectins, which possess binding specificities for either GlcNAc or sialic acid alone, were not capable of eliciting a similar response. In addition, this response appears to display some species specificity given that we were unable to elicit a similar effect in mouse spermatozoa. The mechanism through which WGA elicits an increase in tyrosine phosphorylation and the physiological significance of the process remains to be elucidated. However, preliminary evidence has led to the hypothesis that the lectin mimics a protein(s) present within the female reproductive tract. The binding of such a protein to human spermatozoa presumably induces an aggregation of a specific receptor(s) on the surface of these cells. This aggregation, in turn, activates a cyclic adenosine monophosphate (cAMP) dependent signal transduction cascade that involves Src family kinases. Future work will be directed to elucidating the identity of the sperm receptor(s) and the precise nature of the signal transduction pathway involved.

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64. ANALYSIS OF THE MECHANISM BY WHICH CALCIUM NEGATIVELY REGULATES THE TYROSINE PHOSPHORYLATION CASCADE ASSOCIATED WITH SPERM CAPACITATION

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Controversy surrounds the impact of extracellular calcium on tyrosine phosphorylation during capacitation of mammalian spermatozoa, with positive (1,2) and negative (3) effects recorded in independent publications. Here we demonstrate that presence of calcium in the external medium decreases tyrosine phosphorylation in both human and mouse spermatozoa. Under these conditions, a correlative rise in intracellular pH was also noted; however, this event does not regulate tyrosine phosphorylation. The regulation of tyrosine phosphorylation in sperm incubated in calcium-depleted medium appears to lie at the level of ATP. We found that the amount of ATP in both human (57 ± 3 μg ATP/10^6 cells) and mouse (400 ± 50 μg ATP/10^6 cells) spermatozoa incubated in the presence of external calcium significantly changed when compared to sperm bathed in calcium-depleted conditions (human 47 ± 2 μg ATP/10^6 cells; $P < 0.05$; mouse 1000 ± 100 μg ATP/10^6 cells, $P < 0.001$). Furthermore, the removal of glucose, or addition of 2-deoxyglucose, decreased ATP levels within human spermatozoon populations and induced a corresponding decline in phosphotyrosine expression. The mitochondrial inhibitor rotenone had no effect on either ATP levels, or the amount of tyrosine phosphorylation. Addition of the affinity-labeling probe 8-N_3 ATP confirmed our prediction that spermatozoa have many calcium-dependent ATPases. However, quercetin, a plasma membrane ATPase inhibitor, did not increase tyrosine phosphorylation in human spermatozoa. Based on these findings, the present study indicates that extracellular calcium suppresses tyrosine phosphorylation by decreasing the availability of intracellular ATP, and not by activating tyrosine phosphatases or inhibiting tyrosine kinases as previously suggested (3).

(1) Visconti, P.E., Bailey, J.L., Moore, G.D., Pan, D., Olds-Clarke, P. and Kopf, G.S. (1995) *Development* **121**, 1129–1137. (2) Leclerc, P., de Lamirande, E. and Gagnon, C. (1998) *J. Androl.* **19**, 434–443. (3) Luconi, M., Krausz, C., Forti, G. and Baldi, E. (1996) *Biol. Reprod.* **55**, 207–216. (4) Carrera, A., Moos, J., Ning, X.P., Gerton, G.L., Tesarik, J., Kopf, G.S. and Moss, S.B. (1996) *Dev. Biol.* **180**, 284–296.

10.1071/SRB03Ab65

65. LOCALISATION OF TYROSINE PHOSPHORYLATED PROTEINS ON MOUSE SPERMATOZOA DURING ZONA PELLUCIDA INTERACTION AND CHARACTERISATION OF SPERM SURFACE PHOSPHOPROTEINS

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Prior to fertilisation, mammalian spermatozoa undergo a series of molecular and biochemical events collectively termed capacitation. Although phosphorylation of sperm proteins on tyrosine residues has been recognised as an important correlate of this process, the precise relationship between protein phosphorylation and fertilizing ability has not previously been appreciated. This study demonstrates a direct association between tyrosine phosphorylation and zona pellucida binding affinity of mouse spermatozoa. Tyrosine phosphoproteins were localised to internal flagellar targets on 30% of capacitated spermatozoa and to plasma membrane head antigens on approximately 10% of capacitated cells. Further investigation revealed that almost all spermatozoa bound to the zona pellucida displayed phosphorylation of both head and flagellar proteins, suggesting that it is this sub-population of cells that are functionally competent. While these data implicate tyrosine phosphorylated sperm proteins in gamete interaction, our findings suggest that the zona pellucida recognition epitope itself is not phosphorylated. Rather, we propose that the sperm surface receptor may be a multimeric complex encompassing a zona pellucida recognition region and one or more tyrosine phosphoproteins. Activation of the complex by phosphorylation during capacitation may trigger changes in the sperm surface architecture that facilitate the formation of a functional zona receptor complex and allow recognition and adhesion to the oocyte. The current data imply an important role for phosphorylation as a post-translational mechanism of regulation that may be instrumental in the acquisition of sperm fertilizing ability. Phosphoproteome analysis of murine spermatozoa has revealed the identities of several proteins that may play an active role gamete interaction. Current research is focussed on elucidating the precise function of these candidates. These results contribute to our understanding of mammalian fertilisation and may provide insight into the fields of contraception and male infertility.

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66. FLUID FORMATION AND CYTOKINE EXPRESSION IN THE NORMAL AND INFLAMED RAT TESTIS FOLLOWING LEYDIG CELL DEPLETION

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Leydig cells play an important role in several inflammation-related responses of the testis, stimulating testicular macrophage recruitment, producing the inflammatory cytokines interleukin-1 (IL-1) and IL-6, and regulating interstitial fluid (IF) formation through an androgen-dependent mechanism mediated via the seminiferous tubules. In contrast to other tissues, inflammation in the testis results in a reduction in IF formation. A similar decline in IF follows withdrawal of testosterone (T). In the present study, adult male rats received the Leydig-cell-specific toxin, ethane dimethane sulphonate (EDS; 75 mg/kg i.p.) or carrier alone. Seminiferous tubule function was supported in some EDS-treated animals by 24 cm subcutaneous T-implants. After 10 days, animals received an inflammatory stimulus, lipopolysaccharide (LPS; 0.1 mg/kg i.p.) or saline and were killed 3 h later. Testes were collected for measurement of cytokine mRNA by RNase protection assay (RiboQuant^{TM}), IF volume and testicular T levels. Both depletion of Leydig cells by EDS and LPS-treatment caused a decrease in IF to about 35% of control, but the effects were not additive. Maintenance of T prevented the IF decrease following EDS-treatment, but not the LPS-induced effects. Transforming growth factor-β1 (TGFβ1), migration inhibitory factor (MIF) and interferon-γ (IFNγ) were strongly expressed in the normal and inflamed testis, whereas other cytokines (IL-1α, IL-1β, IL-1ra, TNFα, IL-6 and IL-10) were close to or below the limit of detection in all groups. EDS caused a significant decline in TGFβ1, MIF and IFNγ expression, which was prevented by the T-implants. These data indicate (i) that testis expression of TGFβ1, MIF and IFNγ is not dependent on intact Leydig cells, but is under T control, and (ii) the decline in testicular IF during inflammation involves the Leydig cells, but this regulation is T-independent (i.e. mediated by non–androgenic Leydig cell secretions).

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67. INHIBIN IN THE MALE TAMMAR WALLABY, *MACROPUS EUGENII*

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The relationship between the gonadal peptide inhibin and other hormones is well understood in both sexes of eutherian mammals. Inhibin has been barely investigated in marsupials: the inhibin α-subunit gene has been characterised and its expression studied [1]. This study reports the results of two experiments examining the relationship between inhibin, FSH and testosterone in mature male wallabies. Experiment one (N = 7) determined the level of inhibin [2] in male plasma over 18 months, in relation to testosterone [3] and testis volume [3]. Experiment two examined the source of inhibin, its relationship with FSH [4] and testosterone, from non-breeding (May/June) to peri-breeding season (December) in sham-operated (N = 3) and castrated males (N = 3). Male wallabies are capable of inseminating females throughout the year, and seasonality is induced by female receptivity [5]. Inhibin levels rose gradually before the breeding season began (Exp 1), peaking in February and following a seasonal decline (March to May) with testosterone until November, changes unrelated to changes in testicular volume. Like Exp 1, Exp 2 sham males had depressed inhibin and testosterone from May to November, while FSH levels became elevated in June after being low in late breeding season. After castration (Exp 2) inhibin levels fell but did not vanish below assay sensitivity like testosterone, suggesting there are also non-gonadal sources. FSH levels rose quickly post-castration, remaining elevated. These data suggest the testis is a significant source of inhibin in male wallabies, and the role of inhibin in modulating FSH levels requires further detailed study.

(1) Vanmontfort, D., *et al*. (1998) *J. Molec. Endocrinol.* **21**: 141–152; (2) Robertson, D.M., *et al*. (1988) *Mol. Cell. Endocrinol.* **58**: 1–8; (3) Williamson, P., *et al*., (1990) *J. Reprod. Fertil.* **88**: 549–557; (4) Moore, L.G., *et al*., (1997) *Gen. Comp. Endocrinol.* **106**: 30–38; (5) Tyndale-Biscoe, C.H. and M.B. Renfree (1987) 'Reproductive Physiology of Marsupials.' (C.U.P.: Cambridge.)

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68. SOURCE OF INHIBIN IN THE TAMMAR WALLABY, *MACROPUS EUGENII*

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The relationship between the gonadal peptide inhibin and other hormones is well understood in both sexes of eutherian mammals. Inhibin has been barely investigated in marsupials: the inhibin α-subunit gene has been characterised and its expression studied in possums [1], and the first account of circulating inhibin in male wallabies is reported in these Proceedings [2]. This study reports the results of an experiment examining the source of inhibin in tissues and fluids from adult female wallabies at post mortem. Tissues were snap frozen, homogenised in buffer to produce an extract assayed for total inhibin [3] ± 6% PEG and antisera to determine antibody specific binding. Inhibin is present in a wide range of tissues. Poor inhibin binding was observed in neural tissues, except the pituitary. Most body tissues, e.g. muscle, spleen, heart, bladder, were all negative for inhibin. High inhibin levels were seen in adrenal tissue sampled during gestation and lactation but whether this was from adrenal medulla or cortex was not determined. Median vagina, cervix, endometrium and myometrium had no inhibin. Oviductal tissue was positive, but levels were not as high as ovarian tissue. Ovarian tissue was divided into component parts (corpus lutea and follicles of different ages, interstitial and ovarian tissue excluding the CL or large follicle) and the inhibin levels were ranked: gestating ovary > CL ≥ follicle > follicle drained of fluid > old CL > CL in lactation > corpus albicans > active CL. Follicular fluid (wFF) is the highest source of inhibin. Both allantoic and yolk-sac fluid contained inhibin, presumably from a fetal source. Tissues in the female wallaby produced inhibin, predominantly from ovarian tissue, with the levels varying as the pregnant cycle progresses. These results are consistent with the tissue distribution of inhibin in the possum [1]; however, the form inhibin takes in different tissues requires further study.

(1) Vanmontfort, D., *et al*. (1998) *J. Mol. Endocrinol*. **21**: 141–152; (2) Borchers, C.E., *et al*. (2003) *Reprod. Fertil. Dev*. Suppl. **15**: Abs. 67; (3) Robertson, D.M., *et al*. (1988) *Mol. Cell. Endocrinol.* **58**: 1–8.

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69. SEASONAL CHANGES IN PROSTATIC MESOTOCIN IN THE BRUSHTAIL POSSUM *(TRICHOSURUS VULPECULA)*

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In eutherian mammals, oxytocin is locally synthesized in the prostate, and has been implicated in prostate growth. This study investigates the presence of an oxytocin-like hormone, mesotocin, in the prostate of the brushtail possum in relation to seasonal changes in prostate size. Male possums were sacrificed throughout the year during the breeding and nonbreeding periods, and blood samples and prostate tissue collected. Prostates were divided into cranial and caudal areas and either fixed in 10% neutral buffered formalin for immunocytochemistry of mesotocin and its carrier protein, neurophysin, or frozen in preparation for measurement of mesotocin by radioimmunoassay. Significant changes ($P<0.05$) in prostate weight occurred throughout the year, with the largest prostate weights occurring in the main breeding season months of March (26.52 ± 8.25 g), April (19.61 ± 6.42 g) and May (23.03 ± 6.62 g), and lowest in the nonbreeding month of January (7.54 ± 1.48 g). Mesotocin and neurophysin-like immunoreactivity were identified in the cranial and caudal prostate. The peptides were immunolocalised to all the epithelial cells of the glandular acini. The concentration of mesotocin in the cranial prostate changed throughout the year in relation to changes in prostate weight, and was significantly higher ($P<0.05$) in March (156 ± 9 pg/g) than at other times of the year. The concentration of mesotocin in the caudal prostate also changed throughout the year, displaying the same pattern as the cranial prostate, with the highest concentration present in March (219 ± 16 pg/g). The levels of plasma mesotocin were lower than those present in the prostate and did not change throughout the year. The seasonal increases in prostate weight in the months of March, April and May correspond to part of the main period in which mating occurs. The concentration of prostatic mesotocin closely mimics changes in prostate weight, supporting an involvement for the hormone in prostate growth in the marsupial in addition to the eutherian. The higher levels of mesotocin present in the prostate than the peripheral circulation and the localisation of a neurophysin-like peptide in the prostate also support the local synthesis of mesotocin in the possum prostate.

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70. THE LOCALISATION OF OXYTOCIN AND OXYTOCIN RECEPTOR IN NORMAL HUMAN PROSTATE CELLS

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Oxytocin has been demonstrated in the reproductive tissues of male mammals but has not previously been localised in human prostate tissue. Oxytocin has been shown to increase prostatic muscle tone and prostatic growth. It has been found in high levels in the prostate of aging dogs with benign prostatic hyperplasia (BPH). This may be of significance in this species and in humans, who both suffer from BPH, an androgen dependent condition that develops with age. Dihydrotestosterone (DHT) is the active hormone in the prostate and is produced from testosterone by the enzyme 5α-reductase. This conversion has been shown to be augmented in the presence of oxytocin. The aim of this study was to investigate the presence of oxytocin and oxytocin receptor in normal human prostate cells and correlate findings with the presence of 5α-reductase. Normal human prostate stromal epithelial cells (Clonetics) were grown in either prostate stromal cell media or prostate epithelial media (Clonetics). Stromal cells were at passage six and epithelial cells at passage four. Cells were fixed in Bouins and stained with antibodies for oxytocin, oxytocin receptor and 5α-reductase I and II. Western blot analysis was performed on the same cell types for the presence of oxytocin receptor. The presence of oxytocin and oxytocin receptor was confirmed in both epithelial and stromal cells by immunocytochemistry. Oxytocin receptor was confirmed by Western blot with a band present at 66 kDa. 5α-reductase I and II were also localised in both cell types by immunocytochemistry. These results demonstrate for the first time the expression of oxytocin and oxytocin receptor in both stromal and epithelial cells from normal human prostate. The demonstration of oxytocin, oxytocin receptor and 5α-reductase I and II in the same cell types suggest that oxytocin may play a role in the regulation of DHT in the human prostate.

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71. GRAFTING OF OVARIAN TISSUE IN A NON-HUMAN PRIMATE MODEL

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Ovarian tissue can contain a large number of immature oocytes. In species such as the mouse and sheep these remain functional and can give rise to live young following frozen storage and grafting. Frozen storage of ovarian tissue has therefore been suggested as a strategy for cryobanking female germ cells for rare or endangered species and for young women who are at high risk of premature menopause. Trials on a small number of women and non-human primates show that grafts of frozen–thawed ovarian tissue can restore menses, but have not ascertained how long the grafts remain functional or whether normal, fertilizable, oocytes can be collected from these grafts. In this study, frozen–thawed ovarian tissue were autografted to eleven previously ovariectomised female macaques (*M. fascicularis*) aged between 4 and 15 years at the start of the study. The females were studied for at least 6 months following graft replacement to a subcutaneous site on the abdomen to ascertain: the delay until menses were restored, the regularity and length of the cycles and whether cycles ceased. Oocyte retrievals were performed in a hormonally stimulated cycle to assess the number, quality and fertilizability of oocytes from the grafts. *Results*. Menstrual cycles were restored to all females usually within 3 months of graft replacement, but only 6 of the females developed regular cycles. Oocytes (including MII oocytes) could be recovered from some, but not all grafts, and some, but not all, females. There was evidence that graft function (regularity of cycles) declined with time. The number of oocytes recovered from the equivalent of one grafted ovary was significantly lower than the number recovered from an intact single ovary. We conclude that ovarian autografting to a subcutaneous site does permit maturation of oocytes and oocyte retrieval for assisted reproductive techniques, the efficiency is, however, low.

10.1071/SRB03Ab72

72. STEREOLOGICAL ASSESSMENT OF GONADOTROPIN EFFECTS ON OVARIAN FOLLICLE DEVELOPMENT IN MOUSE OVARY

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Although ovulation requires concerted action of gonadotrophins, the specific effects of FSH on ovarian follicle populations remains difficult to isolate from LH effects in vivo. We therefore aimed to study the effects of FSH and LH in the gonadotrophin-deficient *hpg* mouse using unbiased stereology to study gonadotropin effect on ovary. Female *hpg* mice were treated for 20 days (days 21-41 days of age) with daily i.p. injections of hFSH (10 IU/day) or hCG (1 IU/day) alone or combined. Ovaries were fixed in paraformaldehyde and embedded in glycol methacrylate; thick sections (25 μm), stained with PAS, were subjected to stereological counting of follicle number (primordial, primary, secondary, antral, preovulatory) using the oocyte nucleolus as index particle. Sections and frames for counting were selected by random uniform sampling and the particle count obtained through the middle 18 μm of the section (CAST-grid system, Olympus). Follicle number per ovary was obtained by combining particle density by optical disector with ovarian volume by Cavalieri's principle. The number of primordial follicles was higher in untreated *hpg* compared with phenotypically normal littermates (2771 ± 415 *v.* 1800 ± 216) and was reduced by FSH treatment alone (1793 ± 186) or with hCG (1147 ± 273) but not by hCG alone (2715 ± 600). By contrast, primary follicle numbers were increased by hCG treatment alone (994 ± 62), or with FSH (889.5 ± 104.0) compared with untreated (325 ± 52), FSH-treated *hpg* mice (426 ± 79) and normals (403 ± 27.09). Antral follicles were absent in the untreated *hpg* and hCG-alone-treated animals, but appeared following treatment with FSH alone (101 ± 20) or with hCG (187 ± 34) and in normals (116 ± 21.42). Preovulatory follicles were only seen in the wild type (5 ± 3.58) and combined treatment groups (10 ± 6.86). We conclude that, using unbiased quantitative methods, gonadotrophins have distinct effects on early follicle recruitment with prolonged FSH reducing primordial follicle number, whereas hCG had no effect, while the reverse effects were evident in primary follicle numbers. Nevertheless, antral, pre-ovulatory and ovulated require joint action of both LH and FSH. Further studies are required to investigate the mechanisms involved. Supported by NHMRC.

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73. CROSSTALK BETWEEN PROGESTERONE AND INTERLEUKIN 11 SIGNAL TRANSDUCTION PATHWAYS IN HUMAN ENDOMETRIAL STROMAL CELLS DURING DECIDUALIZATION

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Decidualization of endometrial stromal cells is critical for embryo implantation and establishment of pregnancy. IL-11 is one of the few molecules known to be obligatory for decidualization and implantation in the mouse and enhances progesterone (P)-induced human endometrial stromal cell (HESC) decidualization[1,2]. IL-11 signals via a heterodimeric complex composed of an IL-11 receptor alpha (IL-11Rα) chain and gp130 and signal transduction occurs via the Janus kinase/signal transducer and activator of transcription (STAT) pathway. This study examined the regulation of STAT3 in HESC during P-induced decidualization and the effect of IL-11 on activation of STAT3 in HESC. The decidualization of HESC was assessed using an *in vitro* model in which P was administered for 10 days to cells cultured in serum-free conditions with added estrogen (E). Medium was changed every 48 h for measurement of prolactin (PRL) as a decidual marker, and cellular protein was extracted at each medium change for Western analysis. HESC were also cultured in serum free conditions for 30 min with added IL-11 and cellular protein extracted at 5 min intervals for Western analysis. Treatment of HESC with P increased the abundance of STAT3 protein from day 6 of culture coinciding with an increase in PRL secretion. Co-treatment of HESC with antiprogestin (onapristone) after decidualization was in process reduced the abundance of STAT3, but had no influence on STAT3 phosphorylation. Addition of IL-11 to HESC resulted in the phosphorylation of STAT3 from 5 min. Phosphorylation was abolished following co-treatment with IL-11 neutralising antibody while STAT3 levels remained stable. Our observations demonstrate that P regulates STAT3 protein expression in HESC possibly via P receptor and IL-11 phosphorylates STAT3 in HESC. The data provides evidence of synergy between the P and IL-11 signal transduction pathways during decidualization of HESC. This knowledge is important in understanding the formation of decidua and a functional placenta, and regulation of fertility.

(1) Robb L, Li R, Hartley L, Nandurkar HH, Koentgen F, Begley CG (1998) *Nat. Med.* **3**: 303–308. (2) Dimitriadis E, Robb L, Salamonsen LA (2002) *Mol. Hum. Reprod.* **8**: 636–643.

10.1071/SRB03Ab74

74. NOVEL ERα LIGANDS, PPT AND R,R-THC, PROMOTE ANGIOGENESIS IN HUMAN MYOMETRIAL MICROVASCULAR ENDOTHELIAL CELLS

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Angiogenesis is the growth of new blood vessels from pre-existing vessels and involves proliferation of microvascular endothelial cells (MEC). VEGF is a major promoter of angiogenesis and mediates angiogenic effects through interaction with VEGF receptor-2 (VEGF-R2). We have demonstrated that MEC derived from human myometrium (MMEC) constitutively express estrogen receptor-β (ERβ), while ERα varies between subjects and is only expressed in approximately 60% of MEC isolates[1]. 17β-estradiol (E) upregulates VEGF-R2 and promotes MEC proliferation in the ERα-expressing isolates, but not in ERα negative MMEC[2]. The aim of the present study was to determine whether ERα mediates upregulation of VEGF-R2 and the angiogenic effects of E in ERα-expressing adult human MEC using the novel ERα-selective ligands, PPT and R,R-THC[3]. Myometrial MEC were isolated from hysterectomy tissue obtained from ovulating women, cultured and used between passages 1-3 (purity >98% CD31+ cells)[4]. ERα and VEGF-R2 expression were measured by flow cytometry using an ERα antibody and biotin-rhVEGF$_{165}$ binding respectively[2]. MEC proliferation was determined by MTS bioassay[2]. We first tested the activity of PPT and R,R-THC on a breast tumour cell line known to express wildtype ERα (MCF-7) and demonstrated that both ligands significantly increased proliferation in a similar manner to E, an effect blocked by the nonspecific ER antagonist, ICI 182,780. Neither PPT or R,R-THC stimulated proliferation of the ERα negative cell line MDA-DB-453. In ERα+ MMEC, PPT and E increased VEGF binding in a dose-dependent manner, but had no effect on ERα negative MMEC samples. PPT, R,R-THC and E significantly augmented VEGF-induced MMEC proliferation in ERα positive MMEC ($P< 0.05$) but not ERα negative MMEC. These data confirm that the angiogenic effects of E on MMEC are due to upregulation of VEGF-R2 and are mediated by ERα rather than ERβ.

(1) Gargett CE *et al.* (2002) *Mol. Hum. Reprod.* **8**: 770–775. (2) Gargett CE *et al.* (2002) *J. Clin. Endocrinol. Metab.* **87**: 4341–4349. (3) Kraichely DM *et al.* (2000) *Endocrinol.* **141**: 3534–3545. (4) Gargett CE *et al.* (2000) *Hum. Reprod.* **15**: 293–301.

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75. THE AROMATASE KNOCKOUT (ArKO) MOUSE AS A MODEL TO STUDY THE OESTROGENIC ACTIONS OF TIBOLONE

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Tibolone (ORG OD14) has oestrogenic, progestogenic and/or androgenic activity depending on the tissue. The aromatase knockout (ArKO) mouse provides an ideal model to study the multiple properties of Tibolone given its inability to synthesise endogenous oestrogens. In this study, we examined the effect of Tibolone administration on the ovariectomised (ovx) ArKO mouse. Ovx or sham-operated ArKO and wildtype (WT) mice were orally administered Tibolone (2 μg/g body mass), ethinyl estradiol (EE) (0.05 μg/g body mass) or vehicle once daily for 6 weeks. As expected, ovx increased body weight gain. Tibolone produced a precipitous decline in body mass in ovx ArKO mice. Within 3 weeks of administration, mice lost a mean of 25% of initial body mass, versus 10% for comparative WT mice, likely a reflection of adipose tissue loss. Control EE-induced body mass loss was not as great in ovx ArKO (11.2%). Uterine mass increase was significantly greater in Tibolone-treated ArKO and WT groups compared to EE replacement (13.5- *v.* 7.7-fold increase Tibolone; 3.9- *v.* 2.4-fold increase EE, ArKO *v.* WT respectively, compared to ovx + vehicle). In line with these effects, Tibolone showed an oestrogenic effect on bone yielding an increased bone mineral density in the distal femur. Ovx or intact vehicle-treated ArKO mammary glands were rudimentary, consistent with their oestrogen-naïve background. In contrast, EE-treated ArKO mammary glands had ducts extending from the nipple to beyond the lymph node, relatively small terminal end buds (TEBs), and a bifurcated branching pattern. Tibolone-replaced ovx ArKO mammary glands displayed extensive side branching. TEBs were prominent beyond the lymph node where the duct spread into the fat pad. Clearly Tibolone expresses both oestrogenic and progestogenic activity on breast development. Furthermore it is evident that Tibolone does not need to be aromatised to produce oestrogenic activity as observed on ArKO body-, adipose tissue-, uterine- and bone-mass. The effect is likely being mediated by the two Tibolone metabolites 3α- & 3β-hydroxytibolone. These results demonstrate that the ArKO mouse provides a useful model for assessing the composite steroidal activities of Tibolone.

10.1071/SRB03Ab76

76. PROGESTERONE RECEPTOR EXPRESSION IS MODULATED BY PROSTAGLANDINS IN HUMAN MYOMETRIAL CELLS

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Progesterone withdrawal transforms the myometrium to a highly contractile state required for parturition. In human pregnancy, progesterone withdrawal is mediated functionally by a decrease in myometrial progesterone responsiveness. This is attributed to increased expression of progesterone receptor type A (PR-A), a repressor of progestin actions. Prostaglandins (PGs) are potent endogenous uterotonins and their administration at any stage of human pregnancy induces the full parturition cascade. We hypothesized that in human pregnancy PGs act, at least in part, by inducing functional progesterone withdrawal by modulating myometrial expression of PR-A relative to PR-B. To test this hypothesis, we determined whether PGE_2 and $PGF_{2\alpha}$ influence PR-A and PR-B expression in the PHM1-31 human myometrial cell line. PHM1-31 cells were exposed to PGE_2 and $PGF_{2\alpha}$ (1 pM to 10 nM each) for 24 h. Relative abundance (normalized to 18S rRNA) of mRNAs encoding total PR-A and PR-B were determined by real-time quantitative RT-PCR. Abundance of PR-A and PR-B mRNAs were differentially and dose dependently increased by PGE_2. PGE_2 more efficiently induced PR-A than PR-B expression. Consequently, the PR-A/PR-B expression ratio, which is thought to reflect the extent to which PR-A suppresses progesterone responsiveness, increased in response to low doses of PGE_2 (0.01 to 1 nM) and returned to basal levels in response to higher PGE_2 levels (1-10 nM). $PGF_{2\alpha}$ stimulated expression of PR-A but had no effect on PR-B. This increased the PR-A/PR-B expression ratio. These data show that PGE_2 and $PGF_{2\alpha}$ regulate PR-A and PR-B expression in human myometrial cells. Since progesterone responsiveness is inversely related to the PR-A/PR-B expression ratio, the increase in this ratio by PGE_2 (at low concentrations) and $PGF_{2\alpha}$ may cause functional progesterone withdrawal. These data suggest that PGs induce parturition in part by inducing functional progesterone withdrawal. Such a mechanism would explain why PGs alone can induce the full parturition cascade.

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77. A ROLE FOR PLATELET-ACTIVATING FACTOR IN LUTEOLYTIC $PGF_{2\alpha}$ PRODUCTION BY THE OVINE ENDOMETRIUM

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Pulsatile release of endometrial prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) induces luteolysis in ruminants (1). It has long been known that this pulsatility is driven by the pulsatile release of ovarian oxytocin, and that the two mediators form a positive feedback loop (2). However, several studies have shown that pulsatility persist in the absence of either the ovary, corpus luteum or pituitary (2, 3). It was hypothesized that uterine $PGF_{2\alpha}$ pulses are generated by a uterine loop of platelet-activating factor (PAF) signaling (4). PAF induces a uterine sex-steroid dependent $PGF_{2\alpha}$ response that is augmented by oxytocin and inhibited by embryonic interferon tau (4). The aim of this study was to investigate whether the maturation of a uterine PAF signaling loop in the ovine endometrium occurs at the time that $PGF_{2\alpha}$ pulsatility is first observed. Caruncular endometrium and uterine luminal fluids were collected from ewes on days 10, 12, 14 and 16 of the estrous cycle. The PAF content in tissue and the amount released into media increased significantly ($P < 0.01$) between days 10–16. PAF-receptor mRNA increased in a steroid-dependent manner, with immunolocalisation of the PAF-R protein showing that it was present in stratum compactum stroma and glandular epithelium. By day 14 there was a marked increase in PAF:acetylhydrolase activity in the luminal fluids ($P < 0.001$) and endometrial tissue ($P < 0.05$). Plasma PAF:acetylhydrolase protein immunolocalisation showed that it was present in the lumen of the glandular epithelium. This may serve to prevent accumulation of PAF outside of endometrial tissue. This study shows that the components of a localized loop of PAF signaling were assembled in the ovine endometrium at the time that the onset of uterine $PGF_{2\alpha}$ pulsatility was expected.

(1) McCracken, J.A., *et al*. (1972). PGF 2 identified as a luteolytic hormone in sheep. *Nature* **238**(83): 29–34. (2) Silvia, W.J. and Raw, R.E. (1993) Regulation of pulsatile secretion of PGF_{2a} from the ovine uterus by ovarian steroids. *J. Reprod. Fertil*. **98**(2): 341–7. (3) Denamur, R.J., *et al.* (1973) Pituitary control of the ovine corpus luteum. *J. Reprod. Fertil*. **32**(2): 207–20. (4) Chami, O., *et al.* (1999) PAF may act as an endogenous pulse generator for sheep of luteolytic PGF_{2a} release. *Am. J. Physiol*. **276**: E783–92.

10.1071/SRB03Ab78

78. MACROPHAGE MIGRATION AND LUTEAL REGRESSION IN OVARIES OF LEUKOCYTE ADHESION MOLECULE-DEFICIENT (ICAM-1-/-) MICE

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Luteal regression is an ovarian remodeling event involving apoptosis of luteal cells, an influx of macrophages thought to phagocytose luteal cell debris, and resorption of the corpus luteum (CL). Macrophage adhesion and migration in many tissues is mediated by ICAM-1 (a cellular adhesion molecule which acts as a counter-receptor for leukocyte β2-integrins); and increased ICAM-1 expression has been associated with macrophage infiltration in regressing CL of the rat. To test whether ICAM-1 mediates macrophage infiltration during regression, CL of ICAM-1 null (ICAM-1-/-) mice were analysed by immunohistochemistry for markers of macrophages (F4/80) and luteal cell apoptosis (caspase-3). Ovaries of adult cycling ICAM-1-/- females showed abundant macrophages in regressing CL indicating that ICAM-1 is not required for macrophage migration during luteolysis. In older mice (6 months of age), wildtype (WT) ovaries consisted primarily of follicles and CL, however, ICAM-1-/- ovaries consisted almost entirely of stroma with only a few follicles and CL. These "stromal" cells were reminiscent of luteal cells (hypertrophied and eosinophilic) suggesting that CL regression failed to resolve normally resulting in accumulation with time. To test this, WT and ICAM-1-/- mice were hormonally primed to stimulate luteinization and subsequent regression, with PMSG/ hCG for 4 or 6 days, and the regressing CL were compared. Preliminary results showed that the CL of ICAM-1-/- mice are less regressed than WT at 4 d post-hCG, an effect which is most pronounced at 6 d post-hCG. Immunohistochemical staining patterns for F4/80 and caspase-3 were similar in WT and ICAM-1-/- ovaries indicating that macrophages are present and luteal cell apoptosis is occurring in the "regressing" CL. Concurrently, to test whether the lack of ICAM-1 and a potential defect in luteal regression would impact fertility, female ICAM-1-/- and WT littermates were housed with WT males. After 6 months ICAM-1-/- females had produced normal numbers of litters and pups. Thus, ICAM-1 is not essential for female fertility. That CL regression was delayed despite the presence of luteal macrophages in ICAM-1-/- ovaries indicates that during luteal regression ICAM-1 is likely to be more important for macrophage activation and/or phagocytosis than recruitment or migration.

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79. STUDYING THE GH/IGF/INSULIN AXIS USING KNOCKOUT AND TRANSGENIC APPROACHES

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Insulin-like growth factors (IGFs) are involved in normal growth and development. Initially, it was thought that IGF-I was produced solely by the liver in response to growth hormone (GH), and that this liver-produced, circulating IGF-I was the mediator of GH-effects on pubertal growth. More recently, it has been established that virtually all tissues produce IGF-I. Thus two distinct systems were recognized, the "endocrine" circulating IGF-I and the locally produced "autocrine/paracrine" IGF-I. Since the endocrine form of IGF-I is extremely sensitive to GH, it was believed that GH stimulates growth via the circulating IGF-I and locally produced IGF-I has some local tissue-specific effects. We have re-examined this hypothesis by ablating the liver production of IGF-I in a tissue-specific manner (LID mice), utilizing the cre/loxP system. Circulating IGF-I levels are significantly reduced (by 70%) and GH levels are markedly elevated; proving that circulating IGF-I is primarily derived from the liver and controls GH secretion. Surprisingly the (LID) mice had normal growth and development. However, when we crossed these mice with acid-labile subunit knockout (ALSKO) mice, we were able to reduce circulating IGF-I levels even further and these mice showed postnatal growth retardation and osteopenia, suggesting that circulating IGF-I is important for post-natal growth and development. Circulating GH levels were elevated in the LID mice and this was associated with insulin resistance as determined by hyperinsulinemia in the face of normoglycemia and using the hyperinsulinemic-euglycemic clamp technique. The insulin resistance could be corrected by injecting rhIGF-I, a GHRH antagonist, and most convincingly by crossing these animals with a GH antagonist transgenic mouse. Thus it appears that, at least in the case of this mouse model, GH is the most proximal cause for the insulin resistance seen in the face of low circulating IGF-I levels.

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80. EMBRYO IMPLANTATION, STEM CELLS AND SOCIETY

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The purpose of these studies (1) was to achieve an *in vitro* system to study the endocrinology of implantation in primates. Embryos were recovered non-surgically at morula and blastocyst stages from marmoset and rhesus monkeys. Successful establishment of the culture system led in turn to research on the embryonic secretion of chorionic gonadotrophin (CG) during the peri-implantation period, and to the discovery that blastocyst stage embryos secreted gonadotrophin releasing hormone (GnRH). The ability to culture embryos routinely to yolk-sac stage also enabled the early isolation and characterisation of stem cells from the inner cell mass, laying the basis for application of the same experimental protocols to the human. Over a thousand individually recovered and cultured embryos from marmoset and rhesus monkeys were studied. The successful recovery rate in latter periods of the study approached 70% on days 5-6 after ovulation. The growth rates of embryos and the profile of secretion of GnRH and CG were established. The effects of culture of embryos in the presence of agonist and antagonist to GnRH showed that agonist accelerated endocrine profiles but effectively disaggregated *in vitro* development, while antagonist slowed development. Culture with natural GnRH showed enhanced growth and development of the embryo, with a very high proportion surviving to yolk-sac stages. This result suggests potential therapeutic applications in enhancing the success of some IVF procedures. Embryonic stem cells also secreted GnRH and CG once they began differentiation. One aspect of this research was its rapid transition to the broader research, ethical and regulatory developments in international management of embryonic stem cell biology. The significant attention afforded to the stem cell debate not only engaged legislators, government departments and the public in learning about the issues and the nature of experimental research, but also translated to legislation in many countries with analysis and policy statements from several international agencies. The variations in approach between, for example, the UK and France, Germany and Ireland, the public and private sectors in the USA and in Australia, results in a continuing debate on the acceptable limits of stem cell research and the use or formation of embryos for this and other research purposes.

(1) Hearn, J.P. (2001) Embryo implantation and embryo stem cell development in primates. *Reprod. Fertil. Dev.* **13**, 517-522.

10.1071/SRB03Ab81

81. THE CHALLENGES OF GLOBAL REPRODUCTIVE HEALTH

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The latest UN estimates of growth rates of global population in the next 150 years point to a decrease, no doubt due in part to the increase in contraceptive prevalence and to socioeconomic development in many countries. Using the medium fertility scenario of two children per family, total world population should level out at about 10 billion by 2050. Compare this to a population of 27 billion by 2150 if the fertility rate rises to 2.5–2.6 per family. However, the estimates are uneven and there is more to the demographic challenge than total population numbers. It is important to consider the reproductive health of individuals and populations. The proportion of adolescents and people over 60 years will increase, requiring special consideration of their reproductive health needs. It is estimated that over 120 million couples do not have access to safe and effective methods of family planning. The burden of maternal and perinatal ill-health remains persistently high in many developing countries, with maternal deaths that could be avoided in excess of 500,000 per annum. It is estimated that 340 million new cases of sexually transmitted infections occur annually and are disproportionately found in adolescents and in developing countries. Many of these STIs are curable, but not in the case of HIV/AIDS, of which there are over 15000 new cases a day. Much of this burden falls on women and children in developing countries. The International Conference on Population and Development held in 1994 articulated a comprehensive approach to population and development including poverty alleviation, provision of health care and education, and preservation of the environment. Its emphasis on gender equality and equity and on meeting individual reproductive health needs remains as pertinent today as it was in 1994.

10.1071/SRB03Ab82

82. THE PRODUCTIVITY IMPERATIVE – RESEARCH AND ITS CONTRIBUTION TO THE COMPETITIVENESS OF RURAL INDUSTRIES

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To remain globally competitive, rural industries must respond and adjust to several dimensions of change, aspects of which have been intensifying in recent times. These dimensions may be broadly categorised as follows – globalisation of markets and associated intensifying competition, terms of trade, environmental sustainability, food safety, supply chain transformation and advancing technologies. These pressures are not new and rural industries have been responding to them since commodity trading began. For example, during the period 1980 to 2000 the following changes occurred in the Australian dairy industry – the number of farming enterprises halved from 25,000 to 12,000; milk output per cow rose from 3500 L to 5000 L p.a.; total milk output rose from 6 bn L to 11 bn L p.a. During this same period terms of trade declined by 1.2% p.a.; to offset this total factor farm productivity rose by 1.6% p.a. There are 3 core factors contributing to improved economic productivity – these are scale economies (i.e. bigger farms, more cows per proprietor), bought-in economies (e.g. bigger cow sheds, tractors, etc) and improving the output yield from farm resources such as pastures and cows. It is in this latter grouping that science contributes. For example, the genetic merit of the national herd rose significantly in the above 20-year period, although the fertility of the herd actually declined in the same period. The challenge to rural industries to remain competitive is intensifying – there are a number of causes – a major one is deregulation of markets and intensifying competition and speed of change. Others are environmental sustainability and food safety – both of which place considerable demand on supply chain transformation. Continuing declining terms of trade means farm enterprises will have to continue to improve productivity – science will have to provide the underlying technologies that will deliver performance improvements. For example, there is ample opportunity for the reproductive and the genetic sciences to jointly deliver faster better directed genetic gain. To remain relevant and competitive, both the farming and the science industries have to adjust rapidly to the changing science paradigm, which includes principally the rapid evolution of the molecular sciences and issues of critical mass and integration, but also evolving policy and economic paradigms in governments and commerce. The capacity of science to aid the transformation of industry will be sorely tested in the period ahead. Aspects of the above will be illustrated and discussed.

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83. THE EFFECT OF SEMINAL VESICLE ABLATION ON REPRODUCTIVE OUTCOME IN MICE

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The molecular and cellular events occurring within the uterus preceding embryo implantation are critical for the future development of the embryo and placenta. A key regulator of the pre-implantation uterine environment is hypothesised to be seminal plasma, derived primarily from the seminal vesicle glands. To investigate the importance of seminal plasma in embryo implantation and fetal development, male mice deficient in seminal plasma were prepared by surgical removal of the seminal vesicles. Females mated with seminal vesicle deficient (svx) males exhibited a reduction in pregnancy rate compared to females mated with intact males, with 43% (10/23) compared to 100% (9/9) matings resulting in pregnancy respectively. A 4.6-fold reduction in implantation rates was also seen between females mated with svx and intact males (P<0.005). Fetal and placental weights were assessed at day 18 of pregnancy to evaluate the importance of seminal plasma in post implantation development. Fetal weight was not significantly altered at day 18, but placentas derived from svx matings showed a 17% increase in weight compared to placentas taken from females mated with intact males (P=0.005). This resulted in a 15% reduction in the ratio of fetal to placental weights (P<0.005), a measure of placental transport function. Morphometric analysis was performed on placentas to assess the impact of seminal plasma on placental structure. The mid-saggital area of placentas obtained from svx-mated females was significantly increased (P=0.02), due to a relative increase in both the junctional and labyrinthine areas. These data show that factors in seminal plasma contribute to establishing early pregnancy and facilitating optimal placental development. Exclusion of seminal plasma from the insemination process can reduce the quality of placental function, possibly impacting on the conceptus in adult life. This finding is relevant to assisted reproductive techniques such as IVF where pregnancy may be initiated in the absence of female tract exposure to semen.

10.1071/SRB03Ab4

84. CLONOGENICITY OF HUMAN ENDOMETRIAL EPITHELIAL CELLS

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The human endometrium regenerates from the lower basalis layer, a germinal compartment that persists after menstruation to give rise to the new upper functionalis layer. We hypothesise that epithelial stem cells (ESCs) reside in the endometrial basalis from which new endometrial glands grow with each menstrual cycle. One property of stem cells is their clonogenic ability. The aims of this study were to determine 1) the clonogenic activity of human endometrial epithelial cells, 2) which growth factors support clonogenic activity and 3) compare clonogenic activity of various endometrial states. Endometrial tissue was obtained from 21 women undergoing hysterectomy from proliferative, secretory or inactive states. The entire endometrium was dissociated with collagenase to achieve single cell suspensions. Glandular epithelial cells were selected using Ber-EP4 Dynabeads and cultured at low seeding density (500 cell/cm^2) for 15 days in serum-containing medium (SM), or serum-free medium (SFM) containing either PDGF-BB, TGF-α, EGF, IGF-1, LIF, FGF-2, HGF or SCF on mouse fibroblast feeder layers. Colonies were stained and the cloning efficiencies were determined. Two types of colonies were observed: small loosely packed colonies (SC) containing large cells (<4000 cells/colony) and large colonies (LC) with small, densely packed cells (>4000 cells/colony). In SM, the clonogenicity was 0.20 ± 0.05% (n = 21), 0.08 ± 0.02% for LC and 0.12 ± 0.03% for SC. In SFM, TGF-α, EGF and PDGF strongly supported clonogenicity of epithelial cells in the proliferative phase 0.57 ± 0.20% (n = 7), 0.36 ± 0.14% (n = 8), 0.43 ± 0.14% (n = 7) respectively and in the secretory phase 0.47 ± 0.16% (n = 5), 0.60 ± 0.29% (n = 5), 0.53 ± 0.25% (n = 5). Clonogencity was generally lower in inactive endometrium for all growth factors. HGF, SCF and FGF-2 showed no support for clonogenic activity of epithelial cells from proliferative or inactive endometrium, while LIF and IGF-1 were weakly supportive. Variation between individual samples was high, possibly masking differences in clonogenicity between the endometrial states. This study provides preliminary evidence for the existence of ESCs in the human endometrium, identifies 5 growth factors supporting the clonogenic activity of ESCs and demonstrates some differences in clonogenic activity of epithelial cells from proliferative, secretory and inactive endometrium.

10.1071/SRB03Ab85

85. CO-EXPRESSION OF FRACTALKINE AND ITS RECEPTOR IN HUMAN ENDOMETRIUM SUPPORTS A ROLE FOR FRACTALKINE IN LEUKOCYTE RECRUITMENT AND ENDOMETRIAL REMODELLING

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Leukocytes are critical mediators of endometrial remodelling, particularly during embryo implantation and menstruation, through the production of inflammatory mediators, cytokines and enzymes. However, the mechanisms by which the different leukocyte subpopulations enter the uterus are unknown. The cyclical patterns are indicative of regulation by progesterone, but endometrial leukocytes do not posses progesterone receptors. We hypothesised that their migration is indirectly induced by progesterone-regulated chemokines. In another study (Jones *et al.* unpublished) we identified the most abundant chemokines in the endometrium at times of leukocyte recruitment. In the present study, fractalkine, one of the most abundant of these chemokines, and its receptor (CX_3CR1) were selected for detailed study. Fractalkine is a membrane-bound chemokine, which acts as an adhesion factor and chemoattractant for macrophages, T cells, neutrophils and natural killer (NK) cells. Fractalkine and CX_3CR1 protein production was assessed by immunohistochemistry in endometrial samples across the cycle, in early pregnancy and in women using progestin-only contraceptives. Fractalkine was localised predominantly to the glandular epithelial and decidualised stromal cells, with highest production in the secretory phase and in early pregnancy. Fractalkine production was also detected in subpopulations of endometrial leukocytes (identified as macrophages and uterine NK cells by serial immunostaining), with maximal numbers present in the proliferative phase and early pregnancy. CX_3CR1 was co-localised to the glandular epithelial and decidualised stromal cells, with highest expression in the secretory phase. However, leukocytes, identified as macrophages and neutrophils, possessing CX_3CR1 were in greatest abundance during the menstrual phase. Interestingly, in the presence of continuous progesterone, as in the endometrium of women using progestin-only contraceptives, immunoreactive fractalkine was markedly reduced in the glandular epithelium. In contrast increased immunostaining was observed in decidualised stroma and infiltrating leukocytes in these tissues. Such a pattern is suggestive of progesterone regulation. Co-localisation of ligand and receptor in the glands and decidua suggests a role for fractalkine in remodelling of the secretory glands and decidua. These findings support a role for fractalkine in the recruitment of leukocytes into the endometrium, especially in the secretory phase, in early pregnancy and in women using progestin-only contraceptives.

10.1071/SRB03Ab86

86. REDOX REGULATION OF HYPOXIA-INDUCIBLE FACTORS IN BOVINE BLASTOCYSTS

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Addition of the OXPHOS uncoupler 2,4-dinitrophenol (DNP) to culture media, to favour the production of ATP via glycolysis during compaction and blastulation, has a beneficial effect on bovine embryo development (1), most likely through a change in REDOX state. Hypoxia-inducible factors (HIFs) are transcription factors that mediate O_2-dependent regulation of numerous genes thought to be important for further embryo development, including glucose transporters (GLUTs), glycolytic enzymes and angiogenic growth factors. HIF DNA-binding is regulated by protein stabilisation and nuclear translocation in response to hypoxia or the Fe-chelator desferrioxamine (DFO). REDOX regulation of HIF activity is also postulated to occur. We have demonstrated O_2-regulated gene expression in bovine blastocysts (2). Here we test our hypothesis that addition of DNP or DFO post compaction alters the expression of HIFs and HIF-regulated genes. Bovine IVP embryos were cultured to Day 5 under 7% O_2. Morula were further cultured in ±10 μM DNP, or ±1 μM DFO, for 72 h. Resulting blastocysts were pooled and total RNA isolated. Real time RT-PCR was performed for quantification of reaction products, normalised by measuring 18S rRNA for each sample. Statistical analyses were performed by ANOVA, with significance determined at $\alpha = 0.05$. Real time RT-PCR analysis of HIF1α and HIF2α mRNA revealed that both subunits were altered by addition of either DNP or DFO. Following DNP treatment, HIF1α and HIF2α mRNA were significantly increased ($P<0.05$ respectively). HIF1α mRNA was increased following DFO treatment, although not significantly. In contrast, DFO decreased HIF2α mRNA levels ($P<0.05$). These findings have not been previously reported in either embryonic or somatic cells. GLUT1 expression was significantly increased by the addition of DFO to post-compaction culture media ($P<0.05$), and was non-significantly increased following DNP treatment. VEGF mRNA expression was not altered by either treatment. These results suggest that alterations in intracellular REDOX, associated with altered metabolic preference or induced expression of HIFs, regulates specific genes in bovine blastocysts. Supported by NHMRC.
(1)Thompson JG *et al.* (2000) *J. Reprod. Fertil.* **118**: 47–55. (2)Harvey AJ *et al.* (2003) *Theriogenology* **59**: 208.

10.1071/SRB03Ab87

87. IGF-2 MEDIATES THE EFFECT OF HYPOXIA ON HUMAN CYTOTROPHOBLAST OUTGROWTH

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Poor placental development has been associated with a variety of pregnancy-associated disorders, including preeclampsia. Placentation involves spatial and temporal regulation of cytotrophoblast (CTB) proliferation, differentiation and invasion. These processes, during early pregnancy occur in a relatively hypoxic environment and insulin-like growth factor (IGF)-2 is abundantly expressed by the invading extravillous CTBs. Therefore, this study aimed to investigate the interaction of O_2 concentration and IGF-2 on CTB morphology and IGF-2 and IGF-2 receptor (IGF2R) gene expression *in vitro*. First trimester (7-8 weeks gestation) human placental villous explants were cultured in serum-free media with and without the addition of 125 ng/mL IGF-2, in 1%, 5%, or 20% O_2 (6 groups). On day 6, explants were photographed and RNA was extracted and pooled (8 wells/treatment) for quantification of IGF-2, IGF2R and 18S RNA using real time RT-PCR. Photographs were scored for CTB column formation, the number of tips growing/explant and extravillous CTB phenotype (rounded or dendriform). Villous explants cultured in 1% and 5% O_2 formed 55% and 40% more CTB cell columns than those cultured in 20% O_2, respectively (P<0.001). Exogenous IGF-2 enhanced CTB outgrowth by 35% in 20% O_2 explants (P=0.001), but not in those cultured in 1% or 5% O_2. Less than half of extravillous CTBs exhibited a dendriform migratory phenotype in 20% O_2 compared to 1% O_2 (P=0.02). IGF-2 expression relative to 18S RNA, was increased 3-fold and 2.5-fold in explants cultured in 1% O_2 compared with 5% and 20%, respectively, but this was not significant. IGF-2 mRNA was positively correlated with extravillous CTB migration (r=0.38, P=0.009). There was no effect of treatment on IGF2R transcription in placental villous explants, but IGF2R expression was positively correlated with cell column formation (r=0.37, P=0.01) and extravillous CTB migration (r=0.56, P<0.000). In conclusion, culture in low O_2 promotes early CTB outgrowth and addition of IGF-2 mimics this effect. Our previous work found a positive correlation between IGF-2 mRNA synthesis and CTB cell column formation on an individual well basis. These data suggest that the effect of hypoxia may be mediated by IGF-2.

10.1071/SRB03Ab8

88. INSEMINATION INDUCES PRO-INFLAMMATORY CYTOKINE mRNA EXPRESSION IN THE HUMAN CERVIX

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In mice and other species, deposition of semen into the female reproductive tract elicits a local inflammation-like response, characterised by the recruitment of leukocytes into the uterine endometrial tissues. Recent studies in our laboratory have demonstrated that an equivalent inflammatory response occurs within the human cervical tissue following insemination. The purpose of this study was to investigate the molecular regulation of this response, specifically to examine the effect of insemination on expression of mRNA for pro-inflammatory cytokines and chemokines in cervix tissue. Biopsies were collected from the ectocervix during the peri-ovulatory stage of the menstrual cycle (LH0 to LH+1) and again 48 hours later, at 12 hours following either unprotected intercourse (n = 6), condom-protected intercourse (n = 5) or abstinence from intercourse (n = 7). Total RNA was prepared using standard techniques, reverse-transcribed and relative mRNA expression for pro-inflammatory cytokine and chemokines was quantified using real-time RT-PCR. The relative mRNA expression of GM-CSF, a potent stimulator of myeloid cell recruitment, was found to increase by 254 ± 72% (mean percent increase ± SD)(P = 0.004) following unprotected intercourse. There were trends towards increases after intercourse in expression of IL-6, a stimulator of antigen presentation by macrophages and dendritic cells (265 ± 107%; P = 0.11) and IL-8, a strongly chemo-attractive molecule regulating myeloid cell recruitment (1733 ± 1400%; P = 0.06) following intercourse. mRNA expression was not significantly changed following abstinence or condom-protected intercourse. These data demonstrate that chemotactic cytokines mediate the inflammatory-like response to insemination within the cervical tissues of women. Exposure to semen, as opposed to mechanical trauma appears to be responsible for generating this response, which is likely to be of importance in eliciting an immune response to sperm antigens and micro-organisms contained within the ejaculate.

10.1071/SRB03Ab89

89. THE EFFECT OF KIT LIGAND ON FOLLICLE GROWTH INITIATION IN CULTURED RABBIT AND MOUSE OVARIES

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The mechanisms by which primordial follicles initiate growth remain largely unknown, though evidence from mutant mice suggest that the cytokine, kit ligand and its receptor c-kit, play a central role in early follicle development. We investigated the effects of kit ligand on the initial recruitment of primordial follicles in the mouse and rabbit ovary. Whole 4-day-old mouse ovaries and 8-week-old rabbit ovarian cortex explants were maintained in our organ culture system for 8 days. Selected ovaries were co-cultured with 150 ng/mL recombinant mouse kit ligand and/or anti-recombinant mouse kit ligand antibody. Indices of follicle growth initiation (oocyte diameter, follicle diameter and the proportion of primordial follicles to total follicles) were compared with controls and between treatment groups for each species. Initial analysis of mouse ovaries indicates that kit ligand promotes the initiation of primordial follicular growth. Of the follicles present in untreated cultured mouse ovaries, 35% were in the primordial stage, but primordial follicles were found to make up only 6% of follicles in kit ligand treated ovaries ($P<0.05$). There was a concomitant increase in the proportion of early primary follicles from 55% in untreated cultured mouse ovaries to 73% in ovaries cultured with kit ligand ($p<0.05$). Kit ligand also promoted an increase in the diameter of small oocytes and follicles (mean untreated primordial oocyte diameter 18.9 ± 0.1 μm *v.* mean kit ligand treated primordial oocyte diameter 21.1 ± 0.2 μm, $P<0.01$). These effects were completely inhibited by co-culture with anti-recombinant mouse kit ligand antibody (43% primordial follicles, mean primordial oocyte diameter 18.8 ± 0.1 μm, $P<0.01$). In contrast, recombinant mouse kit ligand had no stimulatory effect on primordial follicle recruitment in the cultured rabbit ovarian explants. Further experimentation with homologous ligand is required to confirm the roles of kit ligand and c-kit in rabbit follicular development.

10.1071/SRB03Ab90

90. FGF9: A MALE SEX-DETERMINING FACTOR IDENTIFIED IN THE OVARY

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The presence of sex-reversed cells (Sertoli and Leydig cells) in the estrogen-deprived, aromatase knock out mouse (ArKO) ovary (1), suggests that estrogen is essential for the maintenance of the 'female' ovarian phenotype. In the course of investigating the expression of 'male'genes (MIS, Sox9, fgf9) by the ArKO ovary we found that fgf9 was present in normal ovary. Fgf9 has been implicated in testicular cord formation (2) and XY fgf9-/- gonads are sex reversed showing predominantly female reproductive structures (oviducts and fused uteri) and histology (3). The apparent pro-male role of fgf9 probably accounts for the lack of data on fgf9 expression by the ovary. The preliminary studies described here, were undertaken to establish if fgf9 mRNA and protein were present in rat ovaries and thus whether fgf9 is regulated by estrogen. Ovaries were collected from Sprague-Dawley rats, 4, 8 and 12 days of age, and 21- and 24-day-old animals that had been treated with DES for 4 or 1 days, respectively. At least 3 groups of ovaries were collected for each age or treatment. Adult rat testis was used as a positive control. RNA was extracted, reverse-transcribed and real time PCR performed. Fgf9 primers amplified a 222 bp cDNA fragment. GAPDH was used for data normalisation. Fgf9 protein was immunolocalised on formalin-fixed, paraffin-embedded sections of ovary (age and treatments as above) using a specific antibody. Levels of FGF9 mRNA were highest in the rat ovary 4 days after birth, declining to approximately 20% of these levels on postnatal days 8 and 12, which was similar to levels expressed by adult testis. DES treatment increased FGF9 mRNA expression by the ovary within 24 hours, ultimately reaching 2-fold after 4 days of treatment. Fgf9 protein was localised to theca and interstitial cells of postnatal and DES-treated ovaries. Fgf9 protein was undetectable in granulosa cells and oocytes. Based on its localisation to the ovary (mRNA and protein) and its regulation by estrogen, the data support our hypothesis that fgf9 is a local regulator of ovarian function. Supported by the NH&MRC of Australia (Regkey 241000).
(1) Britt *et al.* (2002) *FASEB J.* **16**: 1389–97. (2)Cotinot *et al*. (2002) *Semin. Reprod. Med*. **20**: 157–68. (3) Colvin *et al*. (2001) *Cell* **104**: 875–89.

10.1071/SRB03Ab91

91. EFFECT OF GONADOTROPHINS ON OVARIAN FOLLICULAR DEVELOPMENT AND OOCYTE MATURATION IN SOUTHERN HAIRY-NOSED WOMBATS

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Ovarian stimulation with exogenous gonadotrophins enhances follicular development, oocyte maturation and ovulation. The aim of this study was to compare the effect of porcine follicle stimulating hormone (pFSH) to pregnant mares serum gonadotrophin (PMSG) on ovarian follicular development and oocyte maturation in the southern hairy-nosed wombat (SHW), *Lasiorhinus latifrons*. Twenty-three SHW, caught in September 2002, were allocated to one control and two gonadotrophin experimental groups. Group 1 (n = 10) was given pFSH (8 × 25 mg) (Folltropin-V, Vetrepharm, Canada, Inc.) and group 2 (n = 5), PMSG (150 IU) (Vetrepharm, Canada, Inc.). Experimental groups were administered 25 mg of porcine luteinising hormone (pLH) (Lutropin-V, Vetrepharm, Canada, Inc.), 12 h after the final injection of pFSH or three days after PMSG. After euthanasia, 24 h after administration of pLH, ovaries were removed and antral follicles measured. The left ovary was fixed in 10% buffered formaldehyde and the morphology of antral follicles determined. Oocytes were aspirated from follicles of the right ovary, fixed in 10% gluteraldehyde, stained with DAPI and viewed by fluorescent microscopy. Although no oocytes (0/32 oocytes) from controls had undergone GVBD, approximately 40% (35/75 oocytes) of pFSH and 90% (14/17 oocytes) of PMSG treated animals had GVBD. There tended to be more large ovarian follicles (>3 mm) in animals primed with pFSH (7.4 ± 2.3) and PMSG (3.8 ± 1.8) than controls (0.9 ± 1.4). In addition, in all females administered exogenous porcine gonadotrophins (but not controls), some of the mural granulosa cells of large tertiary follicles had markedly enlarged granulosa cell nuclei (ca. ~14 μm). This study has shown that administration of both exogenous porcine gonadotrophins and PMSG to SHW results in an increased number of large follicles with some of the granulosa cell nuclei becoming enlarged, possibly due to polyploidy. Furthermore, exogenous gonadotrophins resulted in GVBD of many of the oocytes.

10.1071/SRB03Ab92

92. SEMEN-INDUCED LUTEAL PHASE IN THE KOALA

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The koala luteal phase is coitally induced (1; *J. Reprod. Fertil*. **120**: 57–64) but it is uncertain whether the mechanism is primarily a neural reflex caused by penile stimulation of the urogenital sinus or an ovulation-inducing factor present in the semen. To better understand the role of koala semen in induction of the luteal phase, sexually mature clinically healthy captive koalas were randomly allocated into one control and 3 treatment groups. The control group (–S/–GR; n = 9) was artificially inseminated (AI) (2; *Int. Zoo. Y.B.* **38**: 160-172) using 1 mL of sterile 0.9% saline. Treatment group 1 (+S/–GR; n = 9) was inseminated with approx 1 mL of koala semen. The urogenital sinus of the second treatment group (–S/+GR; n = 9) was manually stimulated with a purpose built glass rod that mimicked the koala penis. The glass rod was worked back and forth along the length of the urogenital sinus with a slight twisting motion to a depth of 40–60 mm; the stimulation protocol was based on previous descriptions of natural coitus (1). The final treatment group (+S/+GR; n = 9) received urogenital stimulation followed by AI of koala semen. All treatments were conducted during the breeding season on day 2 of the oestrous cycle. A luteal phase was confirmed by an elevated progesterone concentration on day 14 or day 28 greater than 0.67 ng/mL; this concentration represented the upper threshold progesterone concentration (99.99% confidence interval) from all 36 oestrous koalas on the day of treatment. Insemination of sterile saline without the glass rod (0/9) and glass rod stimulation without semen (0/9) failed to induce a luteal phase. Insemination of semen without rod stimulation resulted in a luteal phase occurring in 4/9 koalas, 3 of which produced pouch young. Insemination of semen in combination with rod stimulation induced a luteal phase in 7/9 koalas, 4 of which gave birth. Based on the 2 × 2 factorial design and using an exact logistic regression technique, semen was shown to have a significant effect on induction of the koala luteal phase (P<0.001); glass rod stimulation has no such effect (P=0.335). These results indicate that a component of semen must be involved in inducing ovulation in this species.

10.1071/SRB03Ab93

93. IMMUNONEUTRALIZATION OF GROWTH DIFFERENTIATION FACTOR-9 REVEALS IT PARTIALLY ACCOUNTS FOR OOCYTE MITOGENIC ACTIVITY

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Paracrine factors secreted by oocytes play a pivotal role in promoting early ovarian follicle growth and in defining a morphogenic gradient in antral follicles, yet the exact identity of these oocyte factors remains unknown. The objectives of this study were to generate a specific growth differentiation factor-9 (GDF-9) neutralising monoclonal antibody, and to utilise this antibody to determine the extent to which the mitogenic activity of oocytes can be attributed to GDF-9. To do this, anti-GDF-9 monoclonal antibodies were generated. Based on epitope mapping, a clone was identified with very low sequence homology with related TGF-β superfamily members, including GDF-9B. 3D-peptide modelling of mature mGDF-9 suggested that the binding motif lies at the C-terminal fingertip. As predicted by the modelling, the antibody detected GDF-9, but not GDF-9B in a Western blot, and GDF-9 protein in oocyte extract and oocyte-conditioned medium. In a mouse mural granulosa cell (MGC) bioassay, the anti-GDF-9 antibody completely abolished the mitogenic effects of GDF-9, but had no effect on TGF-b1 or activin A-stimulated MGC proliferation. An unrelated IgG at the same dose had no effect on mGDF-9 activity. This GDF-9 neutralizing antibody (NAb) was then tested in an established oocyte-secreted mitogen bioassay [1], where denuded oocytes co-cultured with MGC promote cell proliferation in a dose-dependent manner. An increasing dose of GDF-9 NAb (0-160 μg/mL) dose-dependently decreased the mitogenic activity of oocytes, but only by ~45% at the maximum dose of NAb. The NAb at just 5 μg/mL neutralised 90% of recombinant mGDF-9 mitogenic activity, but only 15% of oocyte activity. This study has characterised a mGDF-9 neutralising antibody. This antibody was able to neutralise ~½ of oocyte bioactivity, demonstrating that GDF-9 is an important oocyte mitogen, but also that GDF-9 accounts for only part of total oocyte bioactivity.

[1] Gilchrist RB *et al.* (2001) *Developmental Biology* **240**: 289–298.

10.1071/SRB03Ab94

94. KINETICS OF MEIOTIC MATURATION DIFFER BETWEEN PRE-PUBERTAL AND ADULT PORCINE OOCYTES

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Oocytes from pre-pubertal pigs have lower developmental competence (DC) than those from adult pigs during in vitro embryo production [1]. Oocyte DC depends on success of nuclear (meiotic) and cytoplasmic maturation. Comparison of these oocytes groups provides an excellent model to better understand these developmental competence differences. The objective of this study was to determine whether meiotic maturation kinetics differ between pre-pubertal versus adult oocytes and their response to dibutyryl cAMP (dbcAMP) meiotic inhibitor. Oocytes were aspirated from 3-8 mm follicles of pre-pubertal and adult pig ovaries, washed three times in maturation media (MM) and transferred to 50 μL droplets of MM. Oocytes were matured for 44 h in medium 199 supplemented as previously described [2], either in presence or absence of 1 mM dbcAMP. After 24 h oocytes were washed in MM without dbcAMP and transferred to 50 μL MM droplets without dbcAMP. Groups of oocytes were fixed at 0, 16, 22, 40 and 44 h of IVM in a solution of ethanol and acetic acid (3:1, v/v) for 48 h then stained with 1% (w/v) orcein. Nuclear structures were then assessed by phase contrast microscopy. Eight separate replicates were examined with approximately 25 oocytes examined per time point per replicate. Pre-pubertal oocytes progress faster throughout meiotic stages germinal vesicle (GV) and metaphase 1 (M1), finishing meiosis by reaching metaphase 2 at the same rate as adult. dbcAMP maintains higher rates of pre-pubertal and adult oocytes in GV and M1 meiotic stages, however pre-pubertal oocytes still reach these stages earlier than adult oocytes. In conclusion, increased time in meiotic stages appears to be important for attainment of developmental competence during in vitro maturation. Likewise, increased response of adult oocytes to dbcAMP indicates they are more equipped to utilize or receive more dbcAMP to increase meiotic time, hence DC.

(1) Torres CRL, Rath D (1992) *Theriogenology* **37**: 283. (2) Grupen CG, *et al.* (2002) *Mol. Reprod. Dev.* **62**(3): 387–396.

10.1071/SRB03Ab95

95. COHESINS: LINKING CANCER AND MEIOSIS

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Improper transmission of genetic information from a cell to its daughters results in aneuploidy, a hall marker of cancer. Genes involved in the maintenance of genome stability are thus of great interest, and are potential targets for future therapies. The recently discovered multi-protein complex, called 'cohesin' serves as a chromosomal glue which plays an important role in chromosome segregation and DNA repair, guarding the cell against malignant transformation (2). In mitotic cells, cohesin complex comprises at least four proteins: RAD21 (also known as SCC1), SCC3, SMC1 and SMC3. All these cohesin subunits except RAD21 are required for meiosis in yeast (1). The RAD21 subunit is replaced by its meiosis-specific paralogue, REC8. Abundant evidence suggests that cohesin defects may have detrimental consequences for genome stability, such as aneuploidy as seen in the cells of malignant tumors and chromosomal birth disorders (e.g. Down's syndrome). To investigate the role of cohesins in chromosome stability and segregation in mammals, our laboratory cloned human and mouse homologs of yeast *Rad21* and *Rec8* genes. We recently produced the first cohesin mutant mice by targeted deletion of the *Rec8* gene. Abrogation of *Rec8* gene function results in sterility in both males and females, confirming that the essential role of *Rec8* in meiosis is conserved in mammals. Analysis of chromosome spreads of spermatocytes revealed that *Rec8* mutant cells display aberrant meiotic chromosomal structures. These studies may provide insight into chromosome segregation defects contributing to human disorders such as infertility and pathogenesis of cancer.

(1) Nasmyth, K. (2001) Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. *Annu. Rev. Genet.* **35**, 673–745. (2) Michaelis, C., Ciosk, R. & Nasmyth, K. (1997) Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell* **91**, 35–45.

10.1071/SRB03Ab96

96. THE PSA-RELATED KALLIKREIN ENZYMES AND HORMONE-DEPENDENT CANCERS

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The human tissue kallikreins are a multigene family of 15 serine proteases, which are expressed in hormone-dependent cancers. Prostate-specific antigen or PSA, a member of this family, is the current serum biomarker for detection and monitoring of prostate cancer. Other kallikreins are being investigated as potential diagnostic/prognostic markers, particularly for prostate (KLK2) and ovarian cancer (KLKs 4-11 & 14, 15), the latter specifically as biomarkers for serous epithelial ovarian carcinomas. Interestingly, some ovarian KLKs (8, 9, 14 & 15) appeared to be useful indicators of good prognosis whereas others (KLKs 4, 5, 6, 7, 10 & 11) indicated a poor outcome. One novel aspect of the human KLKs is the number of differentially-spliced transcripts that could encode truncated proteins devoid of protease activity. We have shown that some variant transcripts (for PSA, KLK2, KLK4, KLK5 & KLK7) are more highly expressed than full-length transcripts in malignant compared to benign tissues suggesting they may have potential as more cancer-specific biomarkers. Intriguingly, some KLK4 variants are localized to the nucleus, which is not a typical intracellular site for a protease that is normally secreted, emphasizing possible non-proteolytic functions of these variants. From biochemical studies, it appears that kallikreins are involved in a range of functional activities via the degradation of polypeptides (extracellular matrix proteins, IGFBPs) or polypeptide activation via hydrolysis of the pro-peptide (uPA, TGFβ). Although not yet proven, these observations suggest that kallikreins play a role in events associated with tumour progression. We have recently developed prostate cancer PC3 cell lines stably-transfected with prepro-PSA, -KLK2 and -KLK4. We have shown that PSA and KLK4 over-expression, but not KLK2, elicits a morphological change, increased migration to various chemo-attractants and increased attachment to extracellular matrix components (KLK4 only). The mechanisms underlying these changes are currently under investigation.

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97. STEROID REGULATION OF BREAST CANCER CELL PROLIFERATION

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Estrogens are potent mitogens in a number of target tissues including the mammary gland where they play a pivotal role in the development and progression of mammary carcinoma. The demonstration that estrogen-induced mitogenesis is associated with an increased rate of progression through G_1 phase of the cell cycle has focussed attention on the estrogen regulation of molecules in the cyclin/CDK/pRb pathway that controls G_1 to S phase progression. Steroid-responsive breast cancer cells pretreated with a pure estrogen antagonist arrest in quiescence, i.e. G_0, and respond to estrogen treatment with synchronous progression into S phase. Entry into S phase is preceded by increased expression of c-Myc and cyclin D1, activation of cyclin D1–Cdk4 and cyclin E–Cdk2 complexes and phosphorylation of the retinoblastoma gene product, pRb. Activation of cyclin D–Cdk4 is due predominantly to estrogen-induced transcriptional activation of cyclin D1. In contrast, cyclin E–Cdk2 activation does not involve major changes in cyclin E expression but rather redistribution of the p21 CDK inhibitor away from cyclin E–Cdk2 complexes. This is mediated by two distinct mechanisms: sequestration into newly formed cyclin D1–Cdk4–p21 complexes and transcriptional inhibition of p21 gene expression. In the same model, progestins are growth inhibitory and arrest cells in G_1 phase. Growth arrest is accompanied by decreased expression of both cyclin D1 and cyclin E and induction of the CDK inhibitor $p18^{INK4C}$. These changes lead to reassortment of cyclin–CDK–CDK inhibitor complexes and increasing availability of p27 to form inhibitory cyclin E–Cdk2–p27 complexes. Thus, both cyclin D–Cdk4 and cyclin E–Cdk2 activities are inhibited, resulting in decreased pRb phosphorylation and arrest in G_1 phase. These data indicate that steroid hormones stimulate or inhibit cell cycle progression through effects on multiple targets in the pRb pathway. The aberrant expression of several of these targets in breast cancer, i.e. overexpression of c-Myc, cyclin D1 and cyclin E and loss of expression of p27, potentially contributes to the loss of steroid sensitivity and endocrine resistance associated with the progression of breast cancer.

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98. HORMONAL INFLUENCES IN HEREDITARY BREAST CANCER

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Approximately 5 to 10% of all breast cancer (BC) cases are hereditary. Germline mutations in the tumour suppressor genes, BRCA1 and BRCA2, account for up to 40% of hereditary cases. Carrying a mutation in either of these genes confers a high lifetime risk for female BC. Hormonal factors may modify BC risk in BRCA1 and BRCA2 carriers. Recent studies of BRCA1 and BRCA2 carriers show that (premenopausal) prophylactic oophorectomy decreases BC risk by about 50%. Conversely, use of the oral contraceptive pill (particularly for greater than 5 years or if started before age 30) may be associated with a significantly increased risk for BC in BRCA1 carriers. Also, BRCA1 or BRCA2 mutation carriers who have had a full term pregnancy may be more likely to develop BC by age 40 than nulliparous carriers. Unfortunately, data on the role of the anti-oestrogen Tamoxifen for BC prevention in mutation carriers is limited and conflicting. Importantly, the results of most studies to date of BC risk modifiers (including hormonal factors) in BRCA1 and BRCA2 carriers must be interpreted with caution. Most have been retrospective prevalent case-control studies using living cases of BC drawn from families with multiple cases of the disease. Data collection has thus usually been restricted to survivors and their living relatives, and data on exposures to potential risk modifiers have been based on recall of life events many decades earlier. These have been opportunistic, rather than designed, research studies and thus there is a high likelihood of systematic biases. Randomised controlled trials of most potential modifiers (e.g. prophylactic surgery, use of the oral contraceptive pill, parity) are not feasible. Thus it is recognized that long-term prospective, systematic follow-up of large BC family cohorts, will provide better information. Such a study is currently underway in Australasia as part of the Kathleen Cuningham Foundation for Research into Familial Breast Cancer (kConFab). Such studies are essential in order to optimise the clinical risk management strategies of individuals attending Family Cancer Centres, and hence reduce the morbidity and mortality of hereditary BC.

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99. THE EFFECT OF TESTOSTERONE AND SEASON ON PREPROENKEPHALIN mRNA EXPRESSION IN THE PREOPTIC AREA–HYPOTHALAMUS OF THE RAM

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Enkephalin appears to exert an inhibitory action on LH secretion (1), but whether testosterone regulates enkephalin gene expression is unknown. Testosterone regulates expression of proopiomelanocortin mRNA in the hypothalamus in a season-dependant manner (2). This study tested the hypothesis that testosterone and/or season modulate preproenkephalin mRNA expression in specific areas of the hypothalamus in the ram. Adult Romney Marsh rams were castrated (wethers) either during the 'breeding' season or 'non-breeding' season and 1 week later received intramuscular injections of either peanut oil (vehicle) or testosterone propionate (8 mg/12 h for 7 days) (5/group). Blood samples taken every 10 min for 12 h were assayed for plasma LH and testosterone. Preproenkephalin mRNA expression was quantified in hypothalamic sections by *in situ* hybridisation using an ^{35}S-labelled riboprobe and computer-aided image analysis. Plasma testosterone levels were higher in testosterone propionate-treated than oil-treated sheep. Mean plasma LH concentrations were reduced and the interpulse interval for LH pulses was greater in testosterone propionate-treated wethers compared to oil-treated wethers, with no change in LH pulse amplitude. Testosterone propionate treatment reduced proenkephalin mRNA expression in the diagonal band of Broca, the caudal preoptic area and the bed nucleus of the stria terminalis. Proenkephalin mRNA expression was higher in the 'breeding' season in the bed nucleus of the stria terminalis, lateral septum and periventricular nucleus than in the 'non-breeding' season but was higher in the 'non-breeding' season than 'breeding' season in the paraventricular nucleus. No differences were observed between treatments in seven other regions of the hypothalamus. We conclude that testosterone and season regulate preproenkephalin mRNA levels in the preoptic area/hypothalamus in the ram in a region-specific manner.

(1) Armstrong & Spears (1991) *J. Anim. Sci.* **69**: 774–781. (2) Hileman *et al*. (1998) *J. Neuroendocrinol*. **10**: 587–592.

10.1071/SRB03Ab100

100. CHARACTERISATION AND EXPRESSION OF A NOVEL MOUSE GENE ENCODING A PREGNANCY-RELATED SERINE PROTEASE

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This study characterised a novel gene, previously identified as uniquely regulated at implantation in mouse uterus. We cloned its full mRNA sequence encoding a serine protease possessing an IGF-binding domain and named it pregnancy-related serine protease (PRSP). PRSP is structurally similar to mammalian HtrA1 (56% amino acid similarity); thus it represents an additional member of the mammalian HtrA protein family. Northern analysis revealed that the uterine expression of PRSP mRNA was low before pregnancy, but it was increased at implantation and dramatically up-regulated post-implantation. Both in situ hybridisation and immunohistochemistry localised PRSP expression at a low level in the epithelium and stroma during very early pregnancy, but at a high level in the decidual cells on day 8.5, primarily at the mesometrial pole where the placenta was forming. By day 10.5, a high level of PRSP was localized in the decidual compartment of the newly formed placenta. We also cloned an alternatively spliced PRSP mRNA that is expressed at a very low level in the mouse uterus. We determined the genomic structure of PRSP and revealed how the two mRNA variants are produced through alternative splicing. Based on its protein domain structure and unique expression during pregnancy, we propose that PRSP plays an important role in the formation/function of the placenta during pregnancy.

10.1071/SRB03Ab101

101. NUCLEAR IMPORT PROTEINS DISPLAY DISTINCT EXPRESSION PATTERNS DURING SPERMATOGENESIS

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Spermatogenesis requires a precisely ordered sequence of gene expression changes within the male germ cell. Transcription factors (TFs) and nuclear proteins transit between the nucleus and cytoplasm to mediate these functional changes and thereby direct differentiation. We hypothesised that changes in TF function would be accompanied by changes in the proteins that bring them into the nucleus. We investigated the expression of two nuclear import proteins, importins β1 and β 3, during fetal and postnatal development of mouse male germ cells by *in situ* hybridisation and immunohistochemistry. Importin β1 mRNA and protein were observed in mitotic germ cells from 12.5 dpc to adulthood. Importin β3 signals were detected in mitotic and quiescent gonocytes in fetal and neonatal testis, and in the mitotic spermatogonia of the neonate. However, in contrast to importin β1, β3 was synthesised exclusively in post-mitotic germ cells of adult testis. Examination of male and female fetal gonads revealed that these importins move between the nucleus and cytoplasm in gender and age-specific patterns. These data suggest that the mitotic and meiotic germ cells employ distinct sets of nuclear transport proteins, and this is likely to reflect developmentally regulated shifts in the requirement for transport of proteins into the nucleus.

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102. CLONING AND CHARACTERISATION OF TAMMAR *ATRX* AND *ATRY*

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ATRX (on the human and mouse X chromosome) is responsible for X-linked α-thalassemia and mental retardation. Male patients with mutations in *ATRX* display gonadal dysgenesis, suggesting that *ATRX* has a fundamental role in the testis development pathway. In the tammar wallaby (*Macropus eugenii*), there are two distinct homologous ATRX loci, one on the X chromosome (*ATRX*) and one on the Y (*ATRY*). *ATRX* and *ATRY* were cloned from the tammar by a combination of RT-PCR, 3' and 5' RACE walking and genomic library screening. Tammar *ATRX* is highly homologous to human and mouse *ATRX*, whereas tammar *ATRY* is homologous in most regions to tammar *ATRX*. The full sequence of *ATRX* is about 10 kb in length, whereas *ATRY* appears to be only about 7.5 kb. Using RT-PCR, marsupial *ATRX* is detected in a wide range of tissues, but is absent from the developing testis, whereas *ATRY* is expressed exclusively in the developing and adult testis (1). Antibody to ATRY is not yet available, but immunolocalisation of ATRX with a mouse antibody that will recognise both the ATRX and Y showed ATRX protein present in the testis and ovary from day 23 of pregnancy to 3-4 days before birth (well before gonadal sex differentiation) to the adult. During testis differentiation, ATRX immunostaining was localised in the Sertoli cells, the Leydig cells and in the germ cells suggesting that ATRY may play a role in the differentiation of the testis.

(1) Pask, A., Renfree, M.B. and Graves, J.A.M. (2000). *Proc. Natl Acad. Sci.* **97**, 13198–13202.

10.1071/SRB03Ab103

103. CLONING AND EXPRESSION OF THE OVARIAN PROLACTIN RECEPTOR (PRLR) IN THE TAMMAR WALLABY (*MACROPUS EUGENII)*

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Prolactin, a lactogenic hormone primarily synthesized by the anterior pituitary, has an inhibitory effect on luteal function during embryonic diapause in the tammar wallaby. Hypophysectomy during lactational and seasonal quiescence in the tammar results in reactivation of the corpus luteum (CL), but prolactin treatment in these animals delays reactivation (1). Early binding studies demonstrated prolactin receptors (PRLRs) in the CL, mammary gland and adrenal gland (2, 3). The concentration of PRLRs in the CL was higher 7 days post coitum (blastocyst in diapause) compared with mid pregnancy, which suggests that reactivation of the CL involves a reduction in luteal PRLRs (3). However, it is not known at which stage of early pregnancy this decrease occurs. Therefore, the aim of this study was to first obtain the cDNA sequence of the tammar wallaby PRLR and determine the expression of PRLRs in a variety of tissues by RT-PCR. Subsequently, the temporal expression of PRLRs in the CL was examined on days 0, 3, 5, 7 and 10 after removal of the pouch young (RPY) using quantitative real time PCR. Partial nucleotide and derived amino acid sequences of the tammar PRLR cDNA molecule were first obtained from RNA extracted from the mammary gland, using RT-PCR strategies with different combinations of oligonucleotide primers based on the brushtail possum PRLR sequence (4). Two PCR clones of 270 and 750 bp were sequenced and shown to encode a 321 amino acid fragment, with 96% homology compared with possum PRLR cDNA and between 79–83% homology compared with PRLR genes of eutherian species. Using tammar specific primers in RT-PCR, PRLR gene transcripts were detected in all tissues examined, with the highest expression observed in the CL, adrenal gland, uterine tissue, cervix, mammary gland and nipple. Luteal PRLR mRNA concentrations were significantly higher in CLs taken at days 0 and 3 RPY, and decreased on days 5, 7 and 10 RPY. These data support the hypothesis that reactivation of the quiescent CL is associated with a rapid reduction in PRLR expression in the ovary.

(1) Hearn (1973) *Nature* **241**: 207–208. (2) Sernia, Tyndale-Biscoe (1979) *J. Endocrinol*. **83**: 79–89. (3) Stewart, Tyndale-Biscoe (1982) *J. Endocrinol*. **92**: 63–72. (4) Demmer (1999) *Mol. Cell. Endocrinol*. **148**: 119–27.

10.1071/SRB03Ab104

104. MOLECULAR EVOLUTION OF THE PROLACTIN/GH PROTEIN FAMILY IN MONOTREMES

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Prolactin and growth hormone (GH) belong to a family of polypeptide hormones that are thought to have evolved from a common ancestral gene by gene duplication approximately 4–500 $\times$ 10^6 years ago, followed by sequence divergence. Molecular evolution of this hormone family in mammals is characterised by a slow underlying rate of evolution with, in certain species, occasional bursts of rapid change (1). Because monotremes diverged from marsupials and eutherian mammals more than 100 $\times$ 10^6 years ago, and show major differences in reproduction and lactation from the other mammals, we decided to determine the nucleotide sequence for prolactin and GH from the platypus and echidna. Sequences were determined by 3'- and 5'-rapid amplification of cDNA ends (RACE) as previously described (2) and verified by a separate round of RT-PCR with species specific primers to the 5'- and 3'-UTRs. Analyses were performed on deduced amino acid sequences for the hormone coding region by the method of Fitch and Margoliash as determined by the programs Protdist and Fitch in the PHYLIP package as described in (1, 2). Echidna and platypus GHs were very similar to each other (99.0 % sequence identity) and more similar to mammalian GHs than those of reptiles or birds (e.g. echidna GH had 89.0, 88.5, 83.2 and 79.6 % sequence identity with brushtail possum, pig, sea turtle and chicken GHs). Further analysis confirmed a slow rate of molecular evolution (<0.2 substitutions/amino acid site/year $\times 10^9$), similar to that seen for GH in the majority of mammalian species. In contrast, echidna and platypus prolactins were only 89.9 % identical and the difference was due mostly to a higher rate of evolution for echidna prolactin (2.5 substitutions/aa site/year $\times 10^9$) since divergence from the platypus. As for GH, monotreme prolactins were more similar to those of mammals than other vertebrates (e.g. echidna prolactin showed 78.9, 75.4 and 70.9 % sequence identity with possum, pig and chicken prolactins). The functional significance, if any, of these observations remains to be determined, but the prolactin results suggest that some selective pressure associated with speciation of echidnas, and presumably associated with the biological actions of prolactin, has occurred.

(1) Wallis M. (2000) *Mol. Evol*. **50**: 465–473. (2) Curlewis *et al*. (1998) *Gen. Comp. Endocrinol*. **111**: 61–67.

10.1071/SRB03Ab105

105. CYTOSENSOR MONITORING OF MICROVASCULAR ENDOTHELIAL CELLS

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The cytosensor is a silicon-based device capable of measuring minute alterations in extracellular pH by monitoring the changes in μvoltage of a low-buffered medium. It is used to measure the metabolic response of cells to a variety of ligands specific for membrane receptors. Microvascular endothelial cells (MEC) from the human uterus have been implicated in a number of clinical problems including contraception-associated break-through bleeding. The aim of this study was to investigate the direct and indirect effect of estrogens and progestins on primary cultures of MEC, including modification of an inflammatory response by progestins. Myometrial MEC (MMEC) were isolated from six hysterectomies from premenopausal women not taking any hormones. MMEC were purified using *ulex*-coated Dynabeads™ and cultured for 3 passages. MMEC culture purity was monitored using flow cytometry for the expression of CD31. Eighteen hours prior to the experiment, cells were plated at 5×10^6 or 8×10^6 cells per well and kept under low serum media conditions. In the cytosensor the cells were exposed to varying concentrations of histamine (for 120 s) or TNFα (for 360 s). Pilot work has been conducted exposing MMEC to physiological concentrations of estrogen (10 nM), progesterone (100 nM) and levenorgestral (1 nM) for 60 min. The MMEC culture purity was 98 ± 2% (mean ± SD). The optimal cell number per well was 8×10^6 providing a basal voltage of ≥60 μV. Histamine induced an immediate spiked response in acidification rate and produced a non-continuous dose response due to receptor saturation. Maximal stimulation occurred at 10 ± 2 nM with an EC_{50} of 1 ± 0.1 nM. TNFα produced an unusual acidification response, with an initial small receptor response, followed by a second larger response peaking 90 min after the TNFα solution was removed. Furthermore, TNFα had its greatest effect at 10 ng/mL as determined from the initial response. However, 20 ng/mL produced the largest second phase acidification increase. Preliminary data showed that MMEC exposed to estrogen and the progestins did not significantly alter the acidification rate compared the vehicle controls. The interactions of progestins and inflammatory cytokines are still under investigation.

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106. ANTIPHOSPHOLIPID ANTIBODIES BIND TO ACTIVATED, BUT NOT RESTING ENDOTHELIAL CELLS

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Antiphospholipid antibodies (aPL) are autoantibodies that cause pregnancy failure, but the mechanism remains unknown. During human pregnancy, maternal blood is supplied to the placental via the uterine. It has been suggested that aPL bind to and activate endothelial cells in the spiral arteries resulting in the formation of procoagolant surfaces which lead to clot formation and pregnancy failure. In this study, we examined the ability of aPL in patient sera and monoclonal aPL to bind to and activate endothelial cells in culture. Our data show that aPL in patient sera did not bind to either resting or PMA-activated endothelial cells. One monoclonal aPL, IIC5, did bind to activated and apoptotic endothelial cells, but not to resting endothelial cells. In addition, our data demonstrate that monoclonal aPL did not activate resting endothelial cells as indicated by the expression of ICAM-1 and E-selectin or NF-kappa B activation. All these data suggest aPL do not activate resting endothelial cells. We conclude that aPL do not induce pregnancy loss by activating vascular endothelial cells with subsequent clot formation.

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107. MOLECULAR PROFILE OF HUMAN ENDOMETRIUM DURING THE MENSTRUAL CYCLE

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Human endometrium undergoes a series of cyclic changes each month associated with cellular events such as proliferation, differentiation and apoptosis. The aim of this study is to investigate the gene expression profile of human endometrium during the menstrual cycle using cDNA microarray technology. Curettings of endometrium (n = 39) were snap frozen at the time of surgery. Menstrual cycle stage was determined by histological evaluation. Total RNA extracted from a mixture of endometrial samples was pooled to make a common reference RNA. Total RNA of the reference and individual samples were processed for reverse transcription with Cy3 and Cy5 fluorescent-labeled dCTPs respectively, and hybridized on a 10.5 K cDNA glass slide microarray. Expressed genes were identified using Scanarray 5000 UV laser scanner. Quantarray software was used to calculate the gene expression ratio between individual endometrial samples and the reference. Normalization and visualization of the gene expression changes were performed using GeneSpring software. Gene expression ratios between 9 different stages of the cycle were analysed by nonparametric ANOVA with Benjamini-Hochberg false discovery rate correction at the $P<0.05$ significance level. The analysis identified just over 1000 genes with significant changes across the cycle. Five of those genes are being validated by real-time RT-PCR. A CSIRO algorithm called GeneRave was used to determine whether histopathological evaluation correlated with molecular profile of the samples. The groups were treated as both ordered and disordered categorical data. Preliminary studies show that based on the expression profiles of 7 key genes, 23 samples correlated exactly with the stage of the cycle determined by the pathologist and 12 of the remaining 16 samples were only misclassified by 1 cycle stage. Thus 35 out of 39 endometrial biopsies show a high degree of correlation between histopathology and molecular profile. A bioinformatics algorithm that allows cycle stage prediction based on gene expression will allow identification of genes with expression changes associated with different endometrial physiology and pathology.

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108. EXPRESSION OF PROPROTEIN CONVERTASE(PC) 6 IN HUMAN AND RHESUS MONKEY ENDOMETRIUM

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Proprotein convertase (PC) 6 is a member of the serine protease family processing molecules from precursors to bioactive forms. PC6 expression has been identified in mouse endometrium (1). It is up-regulated during decidualization at the site of implantation. We hypothesized that the expression of PC6 in decidualizing cells is critical for implantation through activating regulatory molecules including cytokines important for the event. The present work aimed to establish the spatial and temporal cellular expression of both PC6 mRNA and protein in human and rhesus monkey endometria across the menstrual/oestrous cycles respectively and at the implantation sites during early pregnancy. In situ hybridisation was performed using a digoxygenin (DIG)-labelled cDNA probe against human PC6. For immunohistochemistry, a polyclonal rabbit anti-mouse PC6 antibody (Alexis US) was applied to sections and binding was visualized using the Strept ABC HRP kit (Dako Denmark). Both PC6 mRNA and protein were localized in the same cells predominantly in luminal and glandular epithelia during the proliferative phase of the cycle. During the secretory phase and in early pregnancy, PC6 expression was predominantly localized in decidualizing cells or in the decidua adjacent to implantation sites. The staining patterns were consistent between human and monkey tissues. It is proposed that PC6 plays a role in decidualization and implantation in human and rhesus monkey as in the mouse. It will be important to establish the functional role of PC6 in the uterus. Its manipulation may provide a mechanism for regulating fertility in infertile women or, in post-coital contraception.

(1) Nie *et al*. (2003) *Biol. Reprod*. **68**: 439–447.

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109. EFFECT OF LONG-TERM PROGESTIN TREATMENT ON ENDOMETRIAL VASCULATURE IN NORMAL CYCLING MICE

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Although studies have shown that progestin-only contraception causes structural changes in endometrial vasculature, the precise mechanism leading to breakthrough bleeding has not been elucidated. The aim of this study was to develop a mouse model to investigate the effects of long-term progestin-only exposure on endometrial vascular structure. A silastic implant containing either medroxy progesterone acetate (MPA) or norgestrel (LNG) was inserted subcutaneously into normal cycling mice. Mice were dissected after 1, 2, 3 or 6 w. Endometrial vascular profiles were identified using a monoclonal antibody against mouse CD31. Endometrial vascular density was significantly increased after 1 w of MPA (482 ± 40.2 vessels/mm^2) or LNG (440 ± 26.5 vessels/mm^2) treatment in comparison to normal cycling mice (293 ± 10.5 vessels/mm^2); the increased density was sustained over 2, 3 and 6 w of treatment. Both MPA and LNG increased stromal cell density after 1 w of treatment (MPA: 13813 ± 1450 cells/mm^2, LNG: 11727 ± 851 cells/mm^2) in comparison to normal cycling mice (8256 ± 928 cells/mm^2); however, only MPA maintained this increased density over 2, 3 and 6 w of treatment. There was no significant change in the ratio of vascular density to stromal cell density between treated and normal cycling mice. Vascular endothelial growth factor (VEGF) immunostaining in luminal epithelium was significantly increased after 1, 2, 3 and 6 w of MPA or LNG treatment in comparison to normal cycling mice. VEGF immunostaining in stroma was only significantly increased from normal cycling mice after 6 of progestin treatment. There was no significant difference in VEGF immunostaining in glandular epithelium. These results demonstrate that one week of progestin-only treatment is sufficient to cause significant changes in the endometrium of normal cycling mice, including changes in endometrial vasculature. This mouse model may facilitate further investigations into breakthrough bleeding due to long-term progestin use.

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110. MATRIX METALLOPROTEINASE-2 (MMP-2) IN MURINE UTERINE TISSUE DURING EARLY EMBRYO IMPLANTATION

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While the exact factors regulating attachment and invasion of a blastocyst into the maternal endometrium are yet to be determined, it is known that the invasion process involves the secretion of proteases, including matrix metalloproteinases (MMPs), from both the endometrium and the trophoblast. The development of a new mating protocol, which allows the detection of the specific time of implantation (99–101 h post insemination) has permitted further investigation into the expression and function of MMPs. Previous studies have involved the collection of uterine flushes and tissue extracts at hourly intervals around the time of implantation (45, 95–104 h post insemination), from QS mice mated using the new protocol. Gelatin zymography has shown that MMP-2 increases just prior to implantation in uterine flushes. Immunohistochemistry completed on implantation sites have shown that the MMP-2 can be localised to the endometrial epithelia and the trophoblast cells of the blastocyst. Blastocysts were also collected and cultured on fibronectin to look at the expression of MMP-2 from the trophoblast i*n vitro*. Culture media analysed using gelatin zymography showed no expression of MMP-2 under any of these conditions. In order to clarify the relationship between the peak in MMP-2 and the presence of a blastocyst, MMP-2 presence was analysed in pseudopregnant mice. These analyses showed no peak in expression of MMP-2. These experiments suggest that the MMP-2 expressed in the lumen just prior to invasion may require the interaction of the blastocyst with endometrial tissues. Further investigation into the role of the MMP-2 expression is currently being completed. These experiments include the culture of blastocysts on epithelial monolayers, the injection of MMP inhibitors into pregnant uterine lumens and the completion of *in situ* zymography on implantation sites. It is anticipated that these data in conjunction with the data presented within will offer further clarification into the presence and function of MMP-2 present in the uterine lumen during the apposition phase of embryo implantation.