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Held at the Department of Physiology, University of Melbourne
Edited on behalf of the Society by
J. R. GODING and I. A. CUMMING

The acting Chairman, Dr Alan Grant, opened the meeting. Mr I. A. Cumming welcomed members to the Conference.

SESSION 1
THE CONCEPTUS AND THE FEMALE TRACT. I
Chairman: Professor C. WOOD

The development of an artificial Fallopian tube. R. TAYLOR, Department of Obstetrics and Gynaecology, Monash University, Queen Victoria Memorial Hospital, Melbourne, Victoria.

The results of surgery on the human Fallopian tubes to restore fertility are uniformly poor and the pregnancy rate is probably no better than about 15%. Where very extensive tubal damage is present, the success rate is virtually nil. In an attempt to rectify this, we have developed a tubal prosthesis which effectively places the ovary in contact with the uterine cavity and allows, using a syringing technique, the flushing of oocytes into the uterus where fertilization can take place. This procedure has also been used to provide a repeatable source of oocytes for in vitro culture procedure. The first human tubal prosthesis has been inserted and appears to be a technical success.

The acoustic component of the foetal environment. D. WALKER, J. GRIMW Ade and C. WOOD, Department of Obstetrics and Gynaecology, Monash University, Queen Victoria Memorial Hospital, Melbourne, Victoria.

A sensitive microphone inserted into the uterus of a pregnant patient at term will measure a sound pressure level of 85 dB with a range of frequencies of 20 to 250 Hz. Periodic peaks in the noise level follow the maternal ECG by 300 msec. The sound detected in the pregnant uterus was of greater magnitude in the upper frequencies when compared to the non-pregnant. This difference may be due to greater turbulence of uterine blood flow in pregnancy which provides extra vibratory energy in the environment immediate to the foetus. Measurement shows that there is considerable loss of external sounds in transmission through the maternal tissues and it is unlikely that, in ordinary circumstances, the foetus would receive any such energy. It remains to be established whether
the internally generated sound and vibration is detected by the foetus and, if so, by which sensory pathway, and whether it influences foetal development and the subsequent behaviour of the newborn.

Decomposition of sodium pyruvate in culture media stored at 5° C and its effects on the development of the preimplantation mouse embryo. R. G. Wales and D. G. Whittingham, Department of Veterinary Physiology, University of Sydney, Sydney, N.S.W.

Storage of sterile culture media at 5° C for 1 month resulted in a decrease in the percentage of two-cell mouse embryos developing into blastocysts. This depression in development appeared to be related to a failure in the intracellular accumulation of pyruvate carbon. Using medium containing [1-14C] pyruvate (0.5 mm), accumulation of C-1 of pyruvate over 30 min fell from 0.23 pg atoms/embryo from fresh medium to 0.03 pg atoms/embryo from medium stored for 3 months. Storage for a further 3 months caused an additional sevenfold decrease in intracellular accumulation.

During storage under these conditions, [1-14C]pyruvate underwent slow spontaneous decarboxylation with the loss of 5 to 10% of the radioactivity over 3 months. No loss of radioactivity occurred from medium containing [2-14C]pyruvate. Chromatography on a silicic acid column indicated that the loss of radioactivity was due to the formation of acetate from pyruvate. This acetate formation did not explain the fall in the uptake of the remaining pyruvate, as the addition of similar amounts of acetate to fresh pyruvate did not decrease the uptake of pyruvate by two-cell embryos. It is suggested that parapyruvate, a major product formed during storage of pyruvate in aqueous solution at -40° C, is formed in small amounts during storage at +5° C and, although undetected in these experiments, may be present in sufficient quantities to interfere with pyruvate metabolism.

If radioactive pyruvate was stored at -40° C as small dried aliquots, there was no evidence of decomposition even after 2 years and intracellular accumulation of pyruvate carbon by embryos from medium containing pyruvate stored under these conditions was similar to that from fresh pyruvate. In view of these findings, the preparation of minimal quantities of incubation media containing this substance is advocated. Moreover, when radioactive pyruvate is to be used, it should be stored as small dried aliquots at sub-zero temperatures and minimum quantities reconstituted as required.

The in vitro metabolism of [U-14C]glucose by the preimplantation rabbit embryo. P. Quinn and R. G. Wales, Department of Veterinary Physiology, University of Sydney, Sydney, N.S.W.

To study the metabolism of rabbit embryos during preimplantation development, various stages from the zygote to the morula were cultured for 24 hr in medium containing 1 mg/ml [U-14C]glucose (3 μCi/μmole); later preimplantation stages were incubated for 2 to 4 hr in similar medium with glucose of lower specific activity (1 μCi/μmole).

The total incorporation of glucose carbon in the embryos showed substantial
increases through all developmental stages and ranged from 1.8 pg atom/embryo/hr in developing zygotes to 20,800 pg atom/embryo/hr for 6-day blastocysts. Incorporation in the acid soluble fraction of the embryos represented 55 to 65% of the total glucose carbon incorporated up to blastocyst formation but, thereafter, the proportion of this fraction increased to 77 to 97%, a large part of the increase being in the neutral portion of this fraction. Even before blastocyst formation, neutral compounds accounted for about half of the acid-soluble fraction of the embryos, indicating that the accumulation of glucose is greater than its metabolism. The total accumulation of label in the protein fraction of the embryos also increased at all subsequent developmental stages but the proportion of the label in this fraction fell from 35 to 43% of the total uptake in the preblastocyst stages to less than 3% in the 6-day blastocyst. The incorporation of label into the lipid fraction represented less than 1% of the total glucose carbon incorporated at all stages. Considerable amounts of labelled carboxylic acids accumulated in the medium during the culture period, lactate accounting for 60 to 90% and pyruvate for 8 to 38% of these compounds. Acetate made up the remainder of the carboxylic acids present.

These studies have emphasized the considerable quantitative and qualitative changes in the in vitro metabolism of glucose by the preimplantation rabbit embryo. These changes in metabolism may explain the differences in the culture requirements of the rabbit embryo before and after blastocyst formation.

SESSION 2

THE SPERMATOZOO

Chairman: Dr P. E. MATTNER

Spermagglutinins in human sera. B. BOETTCHER and D. J. KAY, School of Biological Sciences, The Flinders University of South Australia, Bedford Park, S.A.

Spermagglutinins in human sera have been proposed as a cause of some cases of infertility by several workers. Such agglutinins generally have been assumed to be anti-sperm antibodies. Fractionation of spermagglutinating sera in our laboratory has shown basically two classes of agglutinins, immunoglobulins (IgG) and non-immunoglobulins. Whereas sera containing the former are active in the Kibrick (macroagglutination) technique (Kibrick, Belding & Merrill, 1952), but not in the microagglutination technique (Franklin & Dukes, 1964), this pattern is reversed with sera containing the latter. One serum with IgM spermagglutinins is active in both tests.

Human sera with spermagglutinins which are true antibodies (either IgG or IgM) are not active in a passive haemagglutination test, using glutaraldehyde-treated sheep red cells coated with human seminal plasma. However, sera with non-immunoglobulin agglutinins are active in this test.

In our studies, the highest proportion of sera active in the Franklin and
Dukes test have been found among pregnant women (sixty-seven of eighty-six women), and women taking oral contraceptives (eighteen of twenty-five women), though active sera have been found among infertile couples and among blood donors.

Since there are two classes of spermagglutinins in human sera, immunoglobulins and non-immunoglobulins, which react differently in in vitro tests, it is considered likely that they are involved in different physiological processes in vivo.

These results offer an explanation of apparently contradictory results obtained by different workers using different spermagglutination techniques.

REFERENCES


Fractionation of human spermagglutinating sera. D. J. Kay and B. Boettcher, School of Biological Sciences, The Flinders University of South Australia, Bedford Park, S.A.

Sera found to be spermagglutinating by the microagglutination method of Franklin & Dukes (1964), but which are not active in a macroagglutination test (Kibrick, Belding & Merrill, 1952), have been fractionated by chromatography on DEAE cellulose, by gel filtration using Sephadex G-200 and by Pevikon block preparative electrophoresis. The proteins present in active fractions have been identified by immuno-electrophoresis and immunodiffusion. Results indicate that the agglutinins are not immunoglobulins, but are macro-molecules with β-mobility. Some Kibrick-positive sera have been fractionated and their agglutinins identified as IgG. Another serum, active in both the microagglutination and the Kibrick test, has agglutinins which have been shown to be IgM.

Activity has been lost during fractionation of sera positive in the microagglutination test, and dialysis was suspected as the cause of this loss. Prolonged dialysis was found to remove all of the spermagglutinating activity from one serum, though the haemagglutinating activity was unaffected. Non-agglutinating serum, dialysed against a spermagglutinating serum, developed spermagglutinating activity. Phosphate-buffered saline, dialysed against a spermagglutinating serum, did not become spermagglutinating but, when added to the serum which had lost its activity by dialysis, caused it to regain activity.

Fractionation of sera with immunoglobulin, and with non-immunoglobulin, spermagglutinins on Sepharose 6B provided a further example of dissimilarity. The former provided fractions, some of which were active, whereas no activity was found among any of the fractions from the latter, nor from a pool composed of all the fractions obtained.

REFERENCES

Iron-binding proteins in the reproductive tract. T. K. Roberts and B. Boettcher, School of Biological Sciences, The Flinders University of South Australia, Bedford Park, S.A.

As part of a general study of the antigens of spermatozoa, the iron-binding proteins present in the male and female reproductive tracts are being examined. It has been found that, in certain species, seminal spermatozoa are coated by iron-binding proteins (IBP). In an attempt to define the significance of these coating proteins, the following investigation has been undertaken in six species.

Freshly ejaculated semen was separated into seminal plasma and spermatozoa fractions by centrifugation. The spermatozoa were washed six times with phosphate-buffered saline and made up to a concentration of $2 \times 10^7$ spermatozoa/ml. Antisera were then prepared to seminal plasma and to washed spermatozoa by immunizing heterologous animals with the antigen combined with Freund's Adjuvant. Genital secretions were then labelled using radioactive iron, $^{59}$Fe and the IBPs were examined immunologically and electrophoretically.

Semen has been examined from the following species: cattle, sheep, chicken, possum, rabbit and man. Anti-seminal plasma antisera reacted with IBP in five: human, bull, possum, rabbit and chicken. Anti-sperm antisera precipitated IBP from seminal plasma of bull, possum and human. Cross-reactions of antisera with homologous epididymal spermatozoa or with heterologous seminal plasma were not shown. Cellulose acetate autoradiographs indicated the presence of at least two IBP in the seminal plasma of cattle, man and possum, and one in chicken and rabbit (sheep not tested). In man and cattle, the IBP of spermatozoa has been shown to cross-react with antisera to lactoferrin, the IBP of human and cow milk.

The physiological rôle of IBP in the reproductive tract is not yet established. However, recently it has been shown that an anti-complementary agent in human serum appears to be transferrin. Consequently it seems possible that, by being able to inactivate complement, IBP in semen and on spermatozoa might interfere with the immunological reaction normally used against an invading organism and, hence, render seminal spermatozoa non-antigenic within the same species.

Cross-reacting antigens on spermatozoa and erythrocytes. B. A. Baldo and B. Boettcher, School of Biological Sciences, The Flinders University of South Australia, Bedford Park, S.A.

A thorough understanding of the chemical composition of the surface components of spermatozoa would aid the understanding of certain physiological and pathological mechanisms in reproduction.

Immunoochemical methods provide one of the most useful means of studying such surface components since the isolation of many of the predominant vertebrate cell surface antigens is difficult. An immunoochemical approach has been used in our laboratory to study one such antigen which is found on the surface of human spermatozoa. Antisera to human seminal plasma and antisera to human spermatozoa have been found to agglutinate human erythrocytes.
(including 'Bombay' erythrocytes), as well as human spermatozoa. This provides a convenient and highly sensitive method for detecting the antigenic component at each stage of its isolation from human seminal plasma. Work so far has revealed the sperm-erythrocyte common antigen to be a glycoprotein of high mol. wt. with N-acetyl neuraminic acid important in its antigenicity.

The origin of this glycoprotein antigen is uncertain. It may:

1. originate on the spermatozoa and be released by degenerating spermatozoa into the surrounding seminal plasma.
2. originate in the seminal plasma and be absorbed to the spermatozoa.
3. be common to both seminal plasma and spermatozoa.

It is suggested that a possible rôle for this 'species' antigenic component on human spermatozoa may be to mask the tissue-specific sperm antigens and thus prevent these antigens from stimulating an immune response in the female.

**Some factors affecting the intracellular accumulation of metabolites by spermatozoa of the ram.** T. O'Shea, Department of Agricultural Biology, University of New England, Armidale, N.S.W.

The purpose of this study was to examine the interactions of the effects of sperm concentration, addition of potassium and magnesium ions, and gas phase on the accumulation of metabolites.

Washed cells were incubated with [U-14C]fructose, separated from the medium by centrifuging through a lactose solution, and the intracellular and extracellular reaction products separated by silicic acid and paper chromatography.

The main metabolites found were carbon dioxide, lactate and acetate. The effects of the addition of potassium plus magnesium were greater with $1 \times 10^8$ spermatozoa/ml than with $4 \times 10^8$ cells/ml.

There was as much lactate in the lactose rinse solution as in the cells, but most was found in the incubation medium.

Acetate was mainly intracellular with aerobic conditions but was mostly extracellular (77 to 84%) after anaerobic incubation. Addition of potassium and magnesium during aerobic incubation increased the proportion of acetate found within the cell from 62 to 95%. Under anaerobic conditions these ions increased the amount of extracellular acetate in the dilute suspensions by 50%.

The specific radioactivity of the acetate was $23 \pm 2$ nCi/µmol whereas the specific activity of acetate arising from fructose would have been 63 nCi/µmol.

It was concluded that dilute suspensions of washed spermatozoa must be used to examine the effects of potassium and magnesium on accumulation of acetate.

Some two-thirds of the acetate did not arise from the added fructose, suggesting continued metabolism of endogenous material in the presence of fructose. Acetate is normally retained within the cell under aerobic conditions but in the absence of potassium and magnesium it is lost from the cell. The acetate accumulated under anaerobic conditions is possibly formed by a different mechanism.
In contrast, lactate is freely lost from the cells both during incubation and washing.

SESSION 3

THE CONCEPTUS AND THE FEMALE TRACT. II

Chairman: Professor R. G. WALES

The insemination of sheep ova. N. W. Moore, Department of Animal Husbandry, University of Sydney, Sydney, N.S.W., and McGauhey Memorial Institute, Jerilderie, N.S.W.

Observations on the permeability properties of the tertiary egg membranes of the marsupial Trichosurus vulpecula. R. L. Hughes and C. D. Shorey, School of Zoology, University of New South Wales, Kensington, N.S.W., and Electron Microscope Unit, University of Sydney, Sydney, N.S.W.

The early uterine egg of Trichosurus is surrounded by two tertiary membranes, an inner strongly acidic mucoprotein coat of 50 to 80 µ in thickness and an outer keratinous shell of 7 to 10 µ. As the blastocyst expands the mucoid coat disappears and the shell thins but persists as a barrier between the foetal membranes and the maternal uterine epithelium until the terminal stages of gestation.

The permeability properties of thirteen eggs were investigated by incubating them in a mixture of the animal’s own blood plasma and one of the following substances: vital toluidine blue (mol. wt. 306); horse radish peroxidase (mol. wt. 40,000); ferritin (mol. wt. 460,000, diameter of particles 110 Å). The degree of penetration of these tracers was assessed microscopically at a magnification of ×100 for toluidine blue and peroxidase and by electron microscopy for peroxidase and ferritin. The visualization of peroxidase penetration was assessed on the location of a brown reaction product that forms after treatment in a saturated solution of 3-3' diamino-benzidine hydrochloride.

Toluidine blue penetrated the shell within 5 min and entered the vitellus within 30 min. In an egg without a shell, toluidine blue entered the vitellus within 5 min. Peroxidase penetrated the shell and mucoid coat to enter the vitellus within 5 min. No reaction product developed in a control. Ferritin particles penetrated the superficial portion of the shell in low concentrations. The deeper regions of the shell contained a few scattered particles, while ferritin particles were extremely rare in the mucoid coat. No penetration of the vitellus occurred.

The passage of large molecules such as peroxidase and ferritin, across the shell and mucoid coat suggests that these tertiary membranes are not involved in regulating the transfer of nutrients or wastes and would not act as a barrier to quite large macromolecules.
Ovum transport in the ewe. P. J. Holst, C.S.I.R.O., Division of Animal Physiology, Prospect, N.S.W.

Time of ovulation in a flock of sixty mature, cyclic Border Leicester × Merino ewes was determined by means of endoscopy. At 6, 8, 36 and 60 hr after ovulation, laparotomies were performed under general anaesthesia (pentobarbitone sodium), the oviducts and surrounding mesosalpinx sprayed with 2% lignocaine (without adrenaline), and then excised. The ampulla–isthmus junction was identified on the stage of a dissection microscope and the ampulla divided into three sections and the isthmus into two. Ova were flushed from the segments using 1-5 ml of normal saline.

Average length of oestrus was 24-8 hr with ovulation occurring some 2-5 hr after cessation of oestrus. Ova quickly moved into the middle segment of the ampulla and most were here at 6 hr. By 8 hr after ovulation the majority of the ova had reached the ampulla–isthmus junction. It was not until 60 hr that the ova were found in the isthmus.

Ewe fertility—production of cervical mucus. J. F. Smith, Institute of Agriculture, University of Western Australia, Nedlands, W.A.

The volumes and patterns of production of cervical mucus produced by infertile and fertile ewes were studied in a series of tests.

Two groups of fifteen ewes were selected from a flock on the basis of their individual lambing records over a period of 3 years. The infertile group consisted of ewes which had lambed at least once before 1968, but did not lamb in 1968 and 1969. The fertile group consisted of ewes from the same flock which had lambed in 1967, 1968 and 1969. Mucus production was measured by weight after absorption onto cotton-wool swabs.

In the first test, oestrus was synchronized by treating the ewes with progesterone for 16 days. Following cessation of treatment, the mucus produced in 72 hr by the infertile group was significantly greater than that produced by the fertile group (34-6 g versus 23-3 g, \( P < 0.05 \)).

In the second test, mucus was collected at 6-hr intervals from 30 hr before to 30 hr after the onset of natural oestrus. The infertile group produced significantly more mucus over this period than the fertile group (37-3 g versus 25-7 g, \( P < 0.05 \)). There was a quadratic pattern of mucus production over the period of collection with maximum values being produced about the time of onset of oestrus. The duration of oestrus (13-2 hr) was similar for both groups.

The excess mucus produced by infertile ewes may be responsible for a disturbance in the pattern of sperm transport and subsequent failure of fertilization in these animals. This excess production is considered to be due to an increased sensitivity of the reproductive tract to oestrogen and/or an increased production of oestrogen by the ovary.

Protein composition of uterine fluids during early gestation of the tammar wallaby. M. B. Renfree, Zoology Department, Australian National University, Canberra, A.C.T.

The embryo of the wallaby lies free and unattached in the uterus for almost two-thirds of the gestation period. During this time its only source of nutrition is the
uterine fluid which bathes it. This paper reports preliminary analyses of the protein composition of uterine fluids and endometrial homogenates in early pregnancy to determine whether they are a true secretion rather than a simple transudate of maternal serum.

Uterine fluid was collected in buffered saline by reverse flushing through the cervix, thus avoiding contamination by blood, and yolk-sac fluid was collected directly from the embryo. Protein composition of uterine flushings, yolk-sac fluids and maternal sera were compared by acrylamide gel and immunoelectrophoresis, while endometrial homogenates were analysed by gradient acrylamide gel electrophoresis.

The amount of protein flushed from each uterus was about 150 µg and it consisted of two major fractions, an albumen-like and a pre-albumen-like component. Dilute endometrial homogenates consisted predominantly of these two components, but more concentrated samples showed the presence of many more proteins, some of which were absent from serum. Gradient gel electrophoresis of the homogenates showed a large number of proteins in the pre-albumen region. Most of these were absent from serum.

Electrophoretic patterns of early stage yolk-sac fluid show a striking similarity to those of uterine flushings, and when the two were mixed and run together to test for homogeneity the same pattern was obtained. This indicates that the proteins in the vesicle are probably the same as the uterine fluid proteins.

These results favour the view that the uterine fluids are a true secretion, perhaps supplemented by maternal serum but largely due to an independent secretion of the uterine glands. Furthermore, it is likely that the secretions are transferred across the yolk-sac membranes and provide a source of nutrients for the embryo, and that the small proteins and peptides which are found in endometrial homogenates may be important in the initiation and/or growth of the embryo during early gestation.

**Session 4**

*Combined session with the Australian Endocrine Society*

**Reproduction in the Female**

*Chairman: Professor C. W. EMMENS*

**Luteinizing hormone releasing activity of an ultrafiltrate of rat hypophysial portal plasma.** G. Fink, V. W. K. Lee and H. G. Burger, *Department of Anatomy, Monash University, Clayton, Victoria, and Medical Research Centre, Prince Henry's Hospital, Melbourne, Victoria.*

**Ovarian secretion of oestriadiol during the oestrous cycle in the ewe.** R. I. Cox, P. E. Mattner, D. A. Shutt and G. D. Thorburn, *C.S.I.R.O., Division of Animal Physiology, Prospect, N.S.W.*

During the normal 17-day oestrous cycle in the ewe, oestriadiol-17β has been shown to be secreted by the ovary for a short period of 1 to 2 days in late pro-
oestrus with little oestradiol secretion on Day 1 or in the luteal phase (Moore, Barrett, Brown, Schindler, Smith & Smyth, 1969). The present work reports on the secretion of ovarian oestradiol during the oestrous cycle in conscious ewes and on the finding of significant post-ovulatory changes in oestradiol levels.

A preparation in which the utero-ovarian vein is anastomosed to the mammary vein (Thorburn & Mattner, 1969) has permitted the timed collection of utero-ovarian vein blood during the oestrous cycle of the conscious ewe. Oestradiol-17β was measured in 5-ml aliquots of plasma by a modification of the method of Shutt (1969) in which alumina column chromatography was used.

Oestradiol secretion was found to rise rapidly on the day before oestrus (Day 1) and usually remained high until early oestrus. These results were similar to the data of Moore et al. (1969). With serial samples from six ewes, the peak of oestradiol secretion was shown to be of short duration, occurring over about 24 hr with an average value of 326 ng/hr. Unconjugated oestradiol levels did not rise above 0.5 ng/100 ml in jugular vein plasma. The secretion of oestradiol declined to 10 to 12 ng/hr on Days 1 and 2. A further major rise in oestradiol was found to occur after ovulation on Days 3 and 4 with secretion rates reaching similar values to those found in pro-oestrus. Oestradiol secretion declined sharply on Days 5 and 6. The magnitude of this post-ovulatory oestradiol secretion suggests that it is likely to be of physiological significance. It occurs at a time when it may influence ovum transport or uterine endometrial development.

REFERENCES


Constancy of interval between luteinizing hormone release and ovulation in the ewe. I. A. Cumming, J. M. Brown, M. A. de B. Blockey, C. G. Winfield, R. Baxter and J. R. Goding, Department of Physiology, University of Melbourne, Parkville, Victoria, and Department of Agriculture, S. S. Cameron Laboratories, Werribee, Victoria.

The time relationship between the pre-ovulatory luteinizing hormone release (LH release) and ovulation was investigated in naturally cycling ewes, in young and old ewes, and in ewes experiencing their first or second synchronized oestrus after withdrawal of intravaginal sponges impregnated with 9α-fluoro-11β-hydroxy-17α-acetoxy-progesterone (Gronolone).

In order to bring this experiment within the bounds of practicality, experiments were undertaken to shorten the time required for the solid-phase radioimmunoassay for LH in current use in this laboratory (Goding, Catt, Brown, Kaltenbach, Cumming & Mole, 1969). It was determined that a minimum incubation period of 4 hr was needed before the assay acquired the degree of precision adequate to categorize plasma LH concentrations into the following ranges 0 to 10, 10 to 20, 20 to 50 and > 50 ng/ml. The commencement of
the LH release was taken to be that time at which the LH concentration rose to 10 ng/ml and remained high. The validity of the shortened assay was checked both by showing that the LH release lasted for the usual 10-hr period and by checking selected samples in the conventional solid-phase assay. The short assay permitted determinations of LH concentration within 6 hr from sampling. Blood samples were collected hourly from Day 16 of the cycle until LH release occurred. The time for determination of ovulation by single laparotomy for each sheep was then nominated. All ewes were run with vasectomized rams and the time of onset of oestrus recorded.

The results were clear cut. There was no constant time interval between onset of oestrus and ovulation. No ovulations had occurred by 21 hr after LH release, but all sheep of all groups had ovulated by 26 hr (Text-fig. 1). Fifty per cent of

![Text-fig. 1. Percentage of ewes ovulated at varying times between LH release and laparotomy. Number of ewes in each hour-group shown above blocks.](Image)

the flock had ovulated by 23.9 hr. Three out of twenty-one Cronolone-treated sheep ovulated without any sign of overt oestrus; this finding may account for some of the infertility associated with the use of such treatment (Cumming, Blockey, Brown, Catt, Goding & Kaltenbach, 1970).

Young ewe fertility is often low. As the time between LH release and ovulation is normal in these animals, it would be possible, using the short form of LH assay, to investigate this phenomenon by artificial insemination or hand mating at known times before ovulation.

In any event, the constancy of interval between LH release and ovulation in these experimental situations confirms the rôle of LH as 'the' ovulatory hormone in sheep.

REFERENCES


Last year, we reported the results of experiments in which prostaglandins (PG) were infused into the ovarian arterial circulation of sheep with ovarian autotransplants. PGF<sub>1α</sub>, in particular, caused a 3- to 5-fold drop in the secretion rate of progesterone (P). This paper reports similar experiments with infusions of PGF<sub>2α</sub> at 40 µg/hr for 180 min.

![Text-fig. 1. The effects of an infusion of PGF<sub>2α</sub> on the secretion of progesterone (●), oestradiol (○) and LH (□). Onset of oestrus shown by M.](https://www.bioscientifica.com/content/doi/10.1016/S0032-3894(05)66879-7/fig/fg1.png)

In Exp. 1, four sheep with ovarian autotransplants were synchronized using implants of P; they were also given PMSG (1000 i.u.) on withdrawal of the implants. PGF<sub>2α</sub> infusions commenced 7 days later. In all animals secretion rates of P fell abruptly. Within 100 to 120 min of beginning infusions, P dropped to 20 to 46% of the initial secretion rate. P continued to fall over the ensuing 6 hr. All sheep mated within 3 days of the infusions.

In Exp. 2, PGF<sub>2α</sub> was infused as before to a ewe with a persistent CL (no pretreatment with P or PMSG). Luteinizing hormone (LH) was measured by a solid-phase tube assay (Godin, Catt, Brown, Kaltenbach, Cumming & Mole, 1969). P was determined by a modification of the method of Murphy (1967) and oestradiol-17β (E<sub>2</sub>) was measured by the method of Obst, Seamark & Brown (1971).

As in Exp. 1, PGF<sub>2α</sub> caused a sustained fall in the secretion of P. This was followed by a rise in E<sub>2</sub>, and, 16 hr after the first peak of E<sub>2</sub>, LH began to rise. At this time, there was a shorter second peak of E<sub>2</sub> secretion, presumably due to stimulation by LH (Text-fig. 1).
These experiments show that PGF$_{2\alpha}$ is capable of initiating a new cycle in sheep with persisting corpora lutea. It affords a new and powerful tool for the study of the events leading to ovulation. It remains to be shown whether PGF$_{2\alpha}$ is ‘the’ uterine luteolytic factor. This work confirms the finding of McCracken, Glew & Scaramuzzi (1970).

An experiment was conducted in which a fold of peritoneum was interposed between the ovarian artery and the uterine vein in four ewes, with three sham-operated controls (Days 2 to 3 of the cycle). In the three ewes in which the separation of ovarian artery from uterine vein was complete to above the entry of the ovarian veins, the CL were found to have persisted until slaughter (Day 25). In the fourth ewe, the separation was incomplete and, at autopsy, the marked CL had retrogressed. None of the sham-operated ewes showed persisting CL.

It therefore appears likely that a uterine luteolysin could reach the ovary by way of a counter-current mechanism in the ovarian pedicle.

REFERENCES


SESSION 5

**Combined session with the Australian Endocrine Society**

**REPRODUCTION IN THE MALE**

*Chairman: Professor B. HUDSON*

The effect of oestrogen on hydrolytic enzymes and spermatogenesis in the rat. J. S. H. Elkington and A. W. Blackshaw, Department of Physiology, University of Queensland, Brisbane, Queensland.

The effect of steroid hormones on the metabolism of ram spermatozoa. I. G. White, R. N. Murdoch, J. K. Voglmayr and R. F. Seamark, Department of Veterinary Physiology, University of Sydney, Sydney, N.S.W., and Department of Obstetrics and Gynaecology, University of Adelaide, S.A.

Since steroid hormones occur in testicular fluid, it was of interest to study their effect on the metabolism of the ejaculated and testicular spermatozoa of the ram.

Semen was collected by electro-ejaculation and testicular spermatozoa were obtained through a cannula inserted into the rete testis. The washed sperm suspensions were incubated in Warburg flasks for estimation of oxygen uptake and changes in composition of the medium.

Progesterone, 20α-OHP, 20β-OHP, androstenedione and testosterone depressed the respiration of washed ejaculated ram spermatozoa and enhanced
aerobic glycolysis. 17β-Oestradiol, oestriol, androstenedione, 5β-androstenedione, pregnanediol and pregnanolone reduced respiration without stimulating aerobic glycolysis. The steroids appeared to act principally on the oxidative phase of spermatozoal metabolism since the oxidation of isotopically labelled glucose, acetate and citric acid cycle intermediates were all depressed when spermatozoa were incubated in the presence of these steroids.

When freshly collected testicular spermatozoa were incubated with glucose under aerobic conditions, only a small proportion of the utilized glucose could be accounted for as lactate. The addition of a number of steroids, including testosterone, androstenedione, 5β-androstenedione, androsterone, epi-androsterone and 5β-androsterone, greatly increased aerobic glycolysis, the oxidation of the substrate and the proportion of the utilized substrate converted to lactic acid.

After 3 days’ storage at 3°C testicular spermatozoa respired at a greater rate than spermatozoa freshly collected from the testes. Although the stimulating effect of steroids on aerobic glycolysis increased after storage, they depressed rather than stimulated the oxidation of glucose by stored testicular spermatozoa. The results suggest that the effects of testosterone and related steroids in vivo may depend on the age of the spermatozoa after their release from the Sertoli cells: the steroid may be of importance in relation to maturation.

Hypogonadotrophic hypogonadism: the effects of gonadotrophin replacement therapy. H. G. Burger, B. Hudson, D. M. de Kretzer, I. Ekkel and A. Mirovics, Medical Research Centre, Prince Henry’s Hospital, Melbourne, Victoria, Departments of Medicine and Anatomy, Monash University, Clayton Victoria, and Ewen Downie Metabolic Unit, Alfred Hospital, Prahran, Victoria.

Seasonal changes in testicular function of the viviparous lizard Tiliqua rugosa (Gray). A. R. Bourne, J. A. Kirkland and R. F. Seamark, Department of Animal Physiology, and Department of Obstetrics and Gynaecology, University of Adelaide, S.A.

Seasonal variation in androgen production by the testis of Tiliqua rugosa (Gray), a viviparous lizard common to South Australia, was investigated using in vitro techniques.

Each month, testicular tissue (200 mg) from four individual animals was incubated with [4-14C]progesterone (0·14 μCi; 2·4 mμM) and [3H]pregnenolone (1·4 μCi; 2·8 mμM) in 10 ml of Krebs-Henseleit Ringer (pH 7·4) containing 200 mg% glucose. Incubations were for 60 min at 37°C in air.

Radioactive metabolites and their derivatives were isolated by chromatography and identified by crystallization with authentic steroid to radiochemical homogeneity.

Epitestosterone, testosterone and androstenedione were the principal androgens formed. 17α-Hydroxyprogesterone, a known precursor of androgens, was also isolated. During the period investigated (November to June) epitestosterone accounted for 35 to 57% of recovered radioactivity, testosterone 2 to 11% and androstenedione 2 to 15%. The maximum conversion to epitestosterone...
sterone occurred in February (56.5 ± 1.8 S.E.M.) but conversions to testo-
sterone and androstenedione were maximal in December and January,
respectively. ³H:¹⁴C ratios of androgens isolated in individual incubations
showed no significant differences.

Mating activities of T. rugosa have been observed in the spring, and hibernation
occurs in the winter months (June to August). Mean testis weight fell between
November (990 mg ± 223 S.E.M.) and December (162 mg ± 13 S.E.M.) and
remained low during summer.

Although the maximum percentage conversion of C₂₁ steroid to androgens
occurs in the summer, this may not reflect maximum androgen production by
the testis. Studies investigating the relationship between the biochemical and
histological changes are in progress.

SESSION 6
STEROIDS AND REPRODUCTION. I

Chairman: Dr R. I. COX

Ovarian steroid secretion rates in the marsupial Trichosurus vul-
pecula. G. D. THORBURN, R. I. Cox and C. D. SHOREY, C.S.I.R.O. Division of
Animal Physiology, Prospect, N.S.W., and the Electron Microscopy Unit, University of
Sydney, Sydney, N.S.W.

Ovarian secretion rates and arterial concentration of progesterone (P) and 17α-
hydroxyprogesterone (17-OH P) have been measured in the Australian mar-
supial, the brush-tailed possum. The day of oestrus (Day 0) was determined by
vaginal smears. The twenty-one possums (2.0 to 2.5 kg body weight) were
anaesthetized with Inactin (80 mg/kg) on specified days of the 26-day oestrous
cycle. After collection of an arterial sample, timed collections of utero-ovarian
vein blood were made. n-Hexane extracts of plasma were chromatographed on
alumina and the P and 17-OH P fractions were assayed by a protein-binding
method.

The concentration of P in the peripheral plasma was low (0.5 ng/ml) during
anoestrus and from Days 0 to 3 of the cycle. The concentration increased to 0.8
ng/ml between Days 5 and 7, and then increased further between Days 8 and 11 to
reach a level of 3.8 to 5.0 ng/ml between Days 11 and 15. The concentration had
decreased markedly by Day 18 (1.6 ng/ml) and by Day 22 reached low levels
(0.3 ng/ml) which were maintained until the following oestrus (Day 26). The
P secretion rate was low (<1 ng/min) on Days 0 and 1, but increased to 10 to 12
ng/min on Days 3 to 5. Between Days 7 and 9 a further increase in secretion rate
was found, reaching a value of 40 ng/min between Days 9 and 15. The P secretion
had decreased to 7 ng/min by Day 18 and was less than 0.5 ng/min by Day 22.
The changes in secretion rate of 17-OH P followed those of P; however, the
secretion rates were much smaller (maximum 0.7 ng/min). The values during
pregnancy (gestation length 17.5 days) were not significantly different from
those during the cycle. The secretion rate of an oestradiol-like substance was
high (about 12 ng/hr) just before oestrus but fell to low values on Days 0 and 1.

The competitive protein binding assay of Korenman (1968) has been adapted to provide a measure of oestrogens in the peripheral plasma in sheep. Plasma (4 ml) was extracted once with 2 vol. ethyl acetate, the extract concentrated at 45°C under a stream of air and the residue assayed as described by Korenman. Standard curves were constructed by taking known amounts of 17β-oestradiol (17β E2) through the extraction and assay procedure. Recoveries of 6,7-3H-17β E2 (4000 c.p.m.) added as internal recovery standard to each sample ranged from 76 to 90%. As little as 10 pg 17βE2/4 ml plasma was reliably detected. Water blanks invariably gave zero values.

Oestrogen concentrations determined in oophorectomized ewe plasma was 2.5 ± 0.48 pg/ml (n = 20). Oestrone, oestradiol-17α and oestriol determined in the assay gave values 20 to 40% of similar amounts of 17βE2. Progesterone, 17α-hydroxyprogesterone, cortisol and dehydroepiandrosterone did not react.

Mean oestrogen concentrations determined thrice daily (expressed as 17βE2) in two normal cycling and two conceiving ewes showed a midcycle peak of 36 pg/ml (Day 6 to 7) falling to 7.0 pg/ml by Day 9 to 10.

Oestrus peaks varied from 30 to 140 pg/ml. Evidence was obtained to suggest a diurnal variation in oestrogen levels during the luteal phase as plasma oestrogen concentrations at 24.00 > 16.00 > 08.00 hours.

In one experiment, plasma oestrogens, progesterone and luteinizing hormone (LH) were determined thrice daily in cycling ewes treated with progesterone (25 mg/day, Day 1 to 6 cycle). The fall in progesterone occurring on Day 12 to 13 of the cycle was followed by a rise in plasma oestrogen on Day 15. A peak in plasma LH occurred within 16 hr of the oestrogen peak (Day 16), supporting the view that oestrogen triggers LH release.

It was concluded that the oestrogen assay described is sufficiently sensitive, specific and reliable to be of use in studies of ovarian function in sheep and has shown circadian cyclicity and two peaks in plasma oestrogen in cycling ewes.

REFERENCE


The contribution of the placenta and the corpus luteum (CL) of pregnancy to the secretion of progesterone into the maternal circulation was investigated in conscious ewes. Utero-ovarian and jugular venous blood was collected during the last 2 months of pregnancy in twelve hemi-spayed Merino ewes in which
one utero-ovarian vein was anastomosed to the midline mammary vein at
Days 66 to 76 of pregnancy. The ovary left in situ contained either one cl or no
cL.

In single- and twin-bearing ewes lacking a cl, the mean concentration of
progesterone in utero-ovarian venous plasma rose from 18 ng/ml and 32 ng/ml,
respectively, at Day 100 of pregnancy to plateau at 45 to 60 ng/ml and 68 to 80
ng/ml, respectively, between Days 120 and 140. In single-bearing ewes with a
cL, the concentration fell from 115 to 145 ng/ml at Days 100 to 110 to 53 ng/ml
at Days 135 to 140. A corresponding fall occurred in twin-bearing ewes with a
cL: from 160 to 175 ng/ml at Days 100 to 110 to 72 ng/ml at about Day 130.

Estimates indicated that the daily output of progesterone by the placenta in
single-bearing and twin-bearing ewes was about 4 mg and 8 mg, respectively, at
Day 100 of pregnancy and rose to about 33 mg and 56 mg, respectively, at 5 days
before parturition.

After the 2nd month of pregnancy the ovine placenta can, in the absence of a
cL, secrete sufficient progesterone to maintain pregnancy. During the last
month of pregnancy the contribution of the cl to progesterone secretion is small
relative to that of the placenta.

The biosynthesis of oestrogens in the ovine foeto-placental unit.
J. K. Findlay and R. F. Seamark, Waite Agricultural Research Institute and
Department of Obstetrics and Gynaecology, University of Adelaide, Adelaide, S.A.

Ovine foetal blood has been shown to contain high levels of oestradiol-17α
(17αE₂), oestrone (E₁) and oestradiol-17β (17βE₂) mainly as sulphoconjugates
(Findlay & Cox, 1970). Evidence is now presented for the biogenesis of these
oestrogens from dehydroepiandrosterone (DHEA) and certain other C₁₉-
steroids. DHEA is present (10 to 20 mg/ml) in ovine foetal plasma in a
conjugate form (unpublished observations).

A mixture of [7α-3H]DHEA and [4-14C]androstenedione was infused into
a 114-day male foetus in utero through a cannula placed in the umbilical vein.
Blood samples for analysis were obtained during and after infusion from the
umbilical artery and maternal uterine and jugular veins. Maternal urine,
collected from a Foley catheter, and tissue samples were also examined.

Both [14C]- and [3H]oestrogens identified as 17αE₂, 17βE₂, E₁ and 2-
methoxy derivatives were formed in utero. In all cases, including maternal blood
and urine, the 3H:14C ratio of the isolated oestrogens (36-15 to 43-31) was less
than that of the administered radioactivity (48-38).

To determine their sites of formation, individual foetal and placental tissues
were incubated in vitro with labelled C₁₉-steroid precursors. Of the tissues
examined, only the placenta showed significant activity, forming sulpho-
conjugates of E₁, 17αE₂ and 17βE₂ from DHEA, androstenedione and testo-
sterone: oestriol formation was not detected.

It was concluded that oestrogen biosynthesis in the ovine foeto-placental unit
of the sheep differs from that in the human in that the placenta is capable of
synthesizing the range of oestrogens so far isolated from the conceptus without
apparent necessity for foetal participation. Enzymatic activities found in foetal
blood and tissues (Findlay & Seamark, 1968, 1969) would probably be involved in regulation of the levels of individual oestrogen metabolites observed.

REFERENCES


‘Free’ and conjugated oestrogens in plasma have been measured throughout four menstrual cycles to investigate which were, quantitatively, the chief forms of circulating oestrogen.

Peripheral blood was collected on most days throughout each cycle, and 5- to 10-ml samples of plasma were assayed. The oestrogens were separated by solvent partition into ‘free’ sulphate and glucuronide fractions. Oestrone (E1), oestradiol (E2) and oestriol (E3) were measured in each of these extracts by fluorimetry (Brown, Macnaughtan, Smith & Smyth, 1968), yielding a total of nine different oestrogens estimated in each sample.

Twenty-four hour urinary ‘total’ oestrogens (Brown, MacLeod, Macnaughtan, Smith & Smyth, 1968) and pregnanediol (Barrett & Brown, 1970) were measured and all cycles showed a pattern of steroid excretion consistent with ovulation.

The results showed E1-sulphate to be the major component of the circulating oestrogens measured. ‘Free’ E2, at roughly one-tenth the concentration, was the next most abundant oestrogen. These two oestrogens, together with ‘free’ E1 and E1-glucuronide, showed a diphasic fluctuation throughout each cycle, with an ‘ovulatory’ peak corresponding to the urinary peak, and with a subsequent rise and fall during the luteal phase.

For E1-sulphate, the mean of the lowest value encountered in each cycle was 29-9 ng/100 ml of plasma (range 15-5 to 43-1 ng/100 ml). The mean of the highest value encountered, which was at the ovulatory peak, was 334 ng/100 ml (range 142 to 488 ng/100 ml). The corresponding means and ranges for ‘free’ E2 were 5-6 ng/100 ml (range 1-6 to 8-2 ng/100 ml) and 36-2 ng/100 ml (range 27-1 to 45-1 ng/100 ml), respectively.

E2-sulphate, E2-glucuronide, ‘free’ E3, E3-sulphate and E3-glucuronide levels were generally too low to be accurately measured and no diphasic pattern could be clearly discerned in these fractions.

The rôles of the different oestrogens in plasma are not yet understood. E1-sulphate levels showed the greatest fluctuation throughout the cycle.

REFERENCES

Prolonged anti-oestrogenic and anti-fertility actions of some triarylalkenes. C. W. Emmens, Department of Veterinary Physiology, University of Sydney, Sydney, N.S.W.

Following the observation that certain triarylalkene derivatives reduce or abolish the vaginal smear response in the spayed mouse or rat for weeks after injection, their course of action was studied. Since these compounds are also potent anti-fertility agents, the duration of this action was also studied.

ICI-46474 and various similar compounds were injected in doses of 0.1 to 2.7 mg to groups of spayed mice, exerting a weak oestrogenic action at the time of injection and not reducing the response to oestradiol if given with it. Subsequent vaginal smear tests with the same animals showed a reduction of up to 100% in response to oestradiol which reached a maximum 2 to 4 weeks later. Oral administration did not cause the same effect.

With the most potent compounds, groups of normal female mice receiving 1 mg in a single injection on Day 1 and allowed to mate from Day 3 onwards did so over a period of up to 38 days, but produced few litters. In one test, fifty-eight out of fifty-nine mice mated, thirty-one within 10 days of injection, but no litters were produced except in those mating after the initial 10-day period. Of the remaining twenty-seven mice which mated, eight littered. In controls, nineteen of twenty mice mated within 10 days and eighteen produced litters. Similar but less pronounced effects were seen with oral dosage.

It is concluded that a prolonged anti-fertility action occurs, which affects postcoital events without abolishing coitus. Whether the accompanying anti-oestrogenic action is implicated remains to be seen.

Duration of the oestrous cycle and pseudopregnancy following uterine transection in rats. J. D. O'Shea, Department of Veterinary Preclinical Sciences, University of Melbourne, Parkville, Victoria.

Crushing and transection of the posterior ends of the uterine horns and their vessels, in rats, led to accumulation of fluid in the uterine lumen, and an increase in the duration of pseudo-pregnancy (O'Shea, 1970). Interference with uterine luteolysis was suggested as a possible mechanism. Further experiments have been performed to determine the extent to which the effects of uterine transection parallel those of hysterectomy.

Effect on the oestrous cycle. The mean duration of forty-seven oestrous cycles (4.57 ± 0.12—S.E.M.—days) in twelve rats following bilateral uterine transection performed as described by O'Shea (1970) was not significantly different from the mean of thirty-six pre-operative cycles in the same rats (4.61 ± 0.10 days).

Uterine transection in subsequent experiments was performed without section of the uterine vessels.
Quantitative effect on pseudopregnancy. The mean duration of pseudopregnancy in three groups was:

(a) Control—13.0 ± 0.20 days (sixteen rats).
(b) Bilateral uterine transection at the junction of the anterior and middle thirds of the uterine horns—13.7 ± 0.39 days (nineteen rats).
(c) Bilateral transection of posterior ends of the uterine horns—14.7 ± 0.29 days (twenty-three rats).

Local effect on pseudopregnancy. The mean duration of pseudopregnancy following left ovariectomy in each of three groups was:

(a) Control—12.8 ± 0.25 days (ten rats).
(b) Left posterior uterine transection—13.7 ± 0.20 days (nineteen rats).
(c) Right posterior uterine transection—14.5 ± 0.28 days (twenty-one rats).

The observed effects parallel those of hysterectomy and support the suggestion that the effects of uterine transection on pseudopregnancy are mediated by way of a uterine luteolytic mechanism. In no case was the effect as great as that reported for hysterectomy.

REFERENCE


Prolactin in the dairy cow during suckling and machine milking. L. R. Fell, C. Beck, M. A. de B. Blockey, J. M. Brown, K. J. Catt, I. A. Cumming and J. R. Goding, Department of Agriculture, State Research Farm, Werribee, Victoria, Department of Physiology, University of Melbourne, Parkville, Victoria, and Department of Medicine, Monash University, Prince Henry's Hospital, Melbourne, Victoria.

A solid-phase radioimmunoassay method was developed for the measurement of bovine and ovine prolactin in plasma. This procedure is similar to that described for luteinizing hormone (Goding, Catt, Brown, Kaltenbach, Cumming & Mole, 1969). The sensitivity and specificity of the method are acceptable. However, although the within-assay variability is <10%, the between-assay variability may be as high as 50%. This variability is associated with a relatively high non-specific binding of prolactin to the polypropylene tubes.

Concentrations of prolactin were determined in the external jugular venous blood of trained dairy cows during machine milking (Text-fig. 1), also during manual stimulation of the mammary gland and before and after suckling by new born calves.

A three- to ten-fold increase in plasma prolactin concentration occurred after stimulation of the udder. A peak was reached after 5 to 15 min and control levels were regained after a further 30 to 60 min. The response appeared to be related to the stage of lactation of the cows. Suckling caused a similar response.

REFERENCE

The effect of lamb removal on reproductive activity in Dorset Horn × Merino ewes after lambing. B. J. Restall, New South Wales Department of Agriculture, Sydney, N.S.W.

An experiment was conducted to determine the effect of lamb removal on the occurrence of oestrus and ovulation in Dorset Horn × Merino ewes following an autumn lambing.

Forty Dorset Horn × Merino ewes were randomized into two groups, one having the lambs removed at birth, the other being allowed to suckle lambs. The occurrence of oestrus and ovulation was recorded during the first 40 days following parturition. Daily peripheral blood samples were collected for hormone analyses.

Lamb removal at birth resulted in:
A. Ninety per cent of the ewes showing behavioural oestrus within 48 hr of lambing. This oestrus was not accompanied by ovulation.
B. Subsequent behavioural oestrus occurred at a mean time of 17 and 34 days. Ovulation accompanied these oestrous periods.
C. A silent ovulation occurred in 70% of the ewes at a mean time of 10 days after parturition. The corpus luteum resulting from this ovulation had only half the normal life-span and did not result in elevated levels of progesterone in peripheral plasma.

In contrast, ewes with lambs at foot gave the following results:
A. Twenty-five per cent of ewes had a behavioural oestrus within 48 hr of lambing. This oestrus was unaccompanied by ovulation.
B. A further 40% of ewes exhibited oestrus randomly from Day 11 onward.
C. A silent ovulation occurred at a mean time of 15 days after lambing in 70% of the ewes.

It was concluded that the presence of the lamb appeared to block the expression of a regular oestrus pattern. However, pituitary activity was evident whether lambs were present with ewes or not, although the mean time of first

Text-fig. 1. The prolactin concentration in a cow over a 48-hr period, during which she was milked four times by machine (M).
ovulation was later in suckling ewes. Further, the first silent ovulation following parturition resulted in a corpus luteum with an abnormal life-span, and did not appear to secrete progesterone in significant quantities.

SESSION 8
STEROIDS AND REPRODUCTION. II

Chairman: Dr R. F. SEAMARK

Rôle of progesterone in implantation in the sheep. B. M. BINDON, Department of Veterinary Physiology, University of Sydney, N.S.W.

Merino ewes were ovariectomized on Days 4, 8 or 12 of pregnancy and injected daily from that time with 1, 4 or 16 mg progesterone in oil. The ewes were killed on Day 20 and examined for embryos. Sections of uteri were examined histologically. There was a clear effect of dose of progesterone on the number and viability of embryos. Of the ewes receiving 1, 4 and 16 mg progesterone, there were 0/12, 2/11 and 11/14 with viable embryos on Day 20. The effect of increasing progesterone was also seen in significantly increased glandular and luminal epithelial cell heights in ewes receiving 16 mg/day.

Plasma progesterone was measured daily during Days 1 to 5 and twice daily during Days 6 to 15 of pregnancy or of the oestrous cycle. The number of corpora lutea and embryos in these animals was verified at laparotomy on Days 16 to 18. Mean plasma progesterone in pregnant and non-pregnant ewes did not differ significantly until Day 16. The individual pattern of progesterone levels was characterized by a large (up to 16 ng/ml) and transient peak in the period from Days 11 to 14. Different ewes had peak values at different times so that the mean progesterone values took the form of a variable plateau of 2 to 3 ng/ml lasting several days.

Peak levels of progesterone appear in the plasma at times consistent with the idea that this hormone is responsible for the rapid preimplantation growth phase of the embryo during Days 11 to 15.


Undernutrition has been shown to be an important factor contributing to embryonic loss in the ewe. As progesterone is essential for the maintenance of pregnancy, the effect of nutrition on plasma progesterone was studied in ewes during early pregnancy. Three planes of nutrition were imposed, 25%, 100% and 200% of a maintenance ration (1/2M, M and 2M).

The flock contained 250 Merinos and 250 Border, Leicester × Merino ewes. Treatments were imposed from the 2nd to the 16th day after identification of mating. Fertilization rates were determined by egg recovery in a sample of the
flock 72 to 96 hr after mating; 99 ova were recovered, of which 95% were fertilized. The percentage of ova surviving in ewes on 4M, M and 2M were 73, 79 and 72%, respectively.

In some ways, the results were as expected. Where embryonic loss occurred, plasma progesterone fell; but the fall did not become significant ($P<0.01$) until Day 15. Also, in ewes with no embryonic loss, those with two cl.s had higher plasma progesterones after Day 12 than those with one cl. However, the difference only reached significance ($P<0.05$) on Days 15 and 16.

The unexpected finding was that progesterone levels were greatly elevated in ewes on 25% maintenance.

Throughout the experimental bleeding period, while animals were on differential feeding, the progesterone levels on 25% maintenance were significantly higher ($P<0.01$) than those of the 100% maintenance group. The 100% maintenance group in turn had progesterone levels which were usually higher than the 200% maintenance group, but this only reached significance ($P<0.05$) on Days 7 and 8.

These results appear to eliminate low plasma progesterone as a cause of embryonic loss due to undernutrition in the ewe.

The increase in plasma progesterone may be due to decreased metabolic clearance rate, mobilization of stores of progesterone, or increased rate of secretion. The last contingency was investigated in part by estimating peripheral levels of luteinizing hormone (LH) and ACTH. LH was measured by radio-immunoassay and ACTH was inferred from plasma cortisol concentrations (double isotope method performed by Dr B. A. Scoggins). There was no increase in LH. Occasional values of plasma cortisol were high, but these were not consistently related to the plane of nutrition. The cause of the rise in plasma progesterone during undernutrition in the ewe is, therefore, as yet unexplained.


The injection or infusion of a physiological dose of oestrogen into the anoestrous ewe induces luteinizing hormone (LH) release, and in some instances, ovulation (Goding, Catt, Brown, Kaltenbach, Cumming & Mole, 1969; Radford, Wallace & Wheatley, 1970). These responses have been further examined, with particular regard to the effect of varying the dose of oestrogen. Anoestrous Border Leicester ewes were used, each being injected intramuscularly with 25, 100, 400 or 1600 µg oestradiol-17β di-benzoate (ODB). There were eight ewes on each treatment. Jugular plasma samples were collected at 3-hr intervals for 36 hr after injection and were later assayed for LH content.

Invariably, and irrespective of dose, there was a massive release of LH into the circulation within 24 hr of the ODB injection. While the pattern of changes in circulatory LH was similar for all doses, the interval between injection of ODB and peak secretion of LH decreased as the dose of ODB increased. Oestrus occurred in five of the eight ewes in each group given 400 or 1600 µg ODB, and up to half
the ewes on the various treatments ovulated. The ewes which did ovulate were
given a second injection of 100 \( \mu \text{g} \) ODB 17 days after the first injection. All
mated with an entire ram, but none became pregnant.

The relatively low frequency of induced ovulation and the infertility from
entire mating, where it occurred, argue against the use of oestrogen in attempts
to induce anoestrous breeding.

REFERENCES

Endocrinology, 85, 133.

Regulation of the oestrous cycle in the ewe. I. A. CUMMING, J. M. BROWN,
M. A. DE B. BLOCKEY and J. R. GODING, Department of Physiology, University of
Melbourne, Parkville, Victoria, and Victorian Department of Agriculture, S. S. Cameron
Laboratories, Werribee, Victoria.

Collaborative studies have adduced the following evidence for some of the
proposed steps. (1) Decreasing progesterone (P) leads to a surge of oestradiol-
17\( \beta \) (E\(_2\)). Time delay 24 to 36 hr (Barrett, Blockey, Brown, Cumming, Goding,
Mole & Obst (1971). (2) Decreasing P leads to pre-ovulatory release of luteinizing
hormone (LH release). Delay approximately 2 days (Barrett et al., 1971;
Cumming, Blockey, Brown, Catt, Goding & Kaltenbach, 1970). (3) Follicle-
stimulating hormone (FSH-PMSG) causes increased secretion of E\(_2\). Delay : 2+
days (Baird, Goding, Ichikawa & McCracken, 1968). (4) E\(_2\) leads to release of
LH. Delay: 11 to 16 hr (Goding, Catt, Brown, Kaltenbach, Cumming & Mole,
1969; Obst, Seamark & Brown, 1971). (5) LH stimulates E\(_2\) secretion. Delay:
<3 hr (McCracken, Uno, Goding, Ichikawa & Baird, 1969; Barrett et al.,

This paper presents further evidence on the characterization of the control
system for LH.

1. Intramuscular injections of PMSG or horse pituitary extract led to LH
release (delay: 41 to 46 hr).

2. Intravenous infusions of P may suppress LH surge due to E\(_2\). (a) Suppression
is complete if P starts 20 hr before E\(_2\). (b) Partial suppression may occur if
P starts 0 to 5 hr before E\(_2\).

3. Intravenous infusion of E\(_2\) does not evoke LH surge at Day 10 of cycle
(presumably due to suppression by endogenous P).

4. LH release can be evoked by stilboestrol (20 mg i.m.) and intermittent
release of LH occurs after feeding the oestrogenic clover, T. subterraneum L.,
cv. Yarloop.

5. No combination of i.v. infusions of E\(_2\), oestrone and P was found which
caused sustained reduction in LH concentration in the spayed ewe (15 to 20
ng/ml) to the baseline levels in the cycling ewe (3 to 5 ng/ml).

6. Minor episodes of LH release occur spontaneously. When blood was taken
at 5-min intervals, LH concentration was found to rise abruptly from 2 to 7 ng/ml
and there was approximately an exponential decay to 2 ng/ml over the ensuing
90 min.
In conclusion, the evidence for the model (Text-fig. 1) seems convincing but final verification awaits the measurement of follicle-stimulating hormone in plasma. The mechanism of control of baseline levels of LH is still uncertain.

REFERENCES


SESSION 9

THE MALE

Chairman: Dr I. G. WHITE


The importance of lack of libido in young rams at their first joining period was investigated.
A total of seventy-five 1-year-old Merino rams from four different stud flocks were used.

_Preliminary tests._ On each of three separate occasions, each ram was placed in a pen with five oestrous ewes for 20 min and the mating behaviour was studied. Subsequently, the rams wearing marking harnesses and crayons were joined singly with forty cyclic ewes for 2 days and the ewes were inspected daily for markings. At the completion of the series, the proportion of ‘non-worker’ rams (no mounts nor services recorded) in the four groups was 0/10, 1/10, 6/30 and 11/25, respectively.

_Flock mating._ Twelve pairs of rams (twelve workers and twelve non-workers) were each joined with sixty-six ewes for 5 weeks. The rams in each pair bore marking crayons of different colours so that markings of ewes by individual rams could be distinguished.

Three of the twelve non-worker rams failed to mount or serve during the 5-week joining period. In the remaining nine the mean time lapse before mounting or service commenced was 13·3 (S.E. 4·3) days. The percentage of ewes marked by worker and non-worker rams was 95 (S.E. 1·4) % and 37 (S.E. 10·4) %, respectively.

The results suggest that lack of libido in young rams may contribute significantly to lowered marking percentages in commercial flocks. The absence of normal sexual behaviour in a proportion of young rams may be related to the practice of rearing rams in large monosexual groups.

_Early changes in the histology and cytochemistry of the heated ram testis._ J. I. Samisoni and A. W. Blackshaw, Department of Physiology, University of Queensland, Brisbane, Queensland.

Pachytene primary spermatocytes are known to disappear from certain stages of the spermatogenic cycle of the ram testis within 24 hr of immersion of the scrotum in warm water (40·5° C) for 120 to 150 min (Waites & Ortavant, 1968). Samples from similarly treated rams castrated at 6, 12 and 24 hr after heating and also from untreated animals were histologically and cytochemically examined to localize any earlier changes in the tubular epithelium.

At 6 and 12 hr after heating, pachytene cells showed an increased intensity of cytoplasmic staining when eosin or alcian blue was used. Progressive pyknotic changes followed in these cells which eventually disappeared within 24 hr of the heat treatment. Marked increases in the histochemical activities of acid phosphatase and amino-peptidase occurred in these cells also, and estimation of the ‘free’ enzymes as percentages of the total enzyme activities gave values of 69 %, 59 % and 43 % for acid phosphatase and 64 %, 56 % and 41 % for acid proteinase, respectively, at 6, 12 and 24 hr after the heat treatment, compared with values of 32 % and 21 % for the two enzymes in the normal testis.

These results suggest that within a few hours of heating, changes occur in the cellular membranes of certain heat sensitive cells of the ram testis. These changes are seen as an increase in dye uptake by the cytoplasm, together with the formation of autophagic vacuoles and an increase in lysosomal enzyme activity. The
latter are probably responsible for the nuclear pyknosis and eventual disappearance of the cells as in the heated rat testis (Blackshaw & Hamilton, 1971).

REFERENCES


Early histological and histochemical changes in the heated and cryptorchid rat testis. A. W. Blackshaw and D. Hamilton, Department of Physiology, University of Queensland, St. Lucia, Queensland.

The scrotal testis of several species reacts to heating in a characteristic way. The first response of the rat testis to 30-min heating at 42°C is cystoplasmic chromophilia (1 to 2 hr) and major changes in the pattern of lysosomal acid phosphates and amino-peptidase (2 to 4 hr) in pachytene primary spermatocytes. Stabilizers of lysosomes (cortisol, phenergan, chlorpromazine and chloroquine) caused only minor reduction in heat damage.

The effect of a smaller, sustained rise in temperature on testicular histology was tested by unilateral cryptorchidism. After 6 to 12 hr there was increasing disruption of the normal acid phosphatase pattern with secondary lysosome formation in stages I to IV of spermatogenesis (Roosen-Runge & Giesel, 1950). Chromophilia was not seen until 48 hr after the operation and then in a relatively small number of cells. Sudan black staining of lipid began within 24 hr and at 96 and 192 hr it was a prominent feature of degeneration. Round and long spermatids did not appear to be lost by maturation depletion, being absent at 192 hr.

The pachytene primary spermatocyte approaching meiosis is most susceptible to damage by heat (Chowdhury & Steinberger, 1964). In these cells, it is possible that the rise in metabolic rate produced by heating alters cell membrane function and initiates lysosomal activation and cell destruction.

REFERENCES


Effects of site of insemination, sperm motility and contractions of the genital tract on the transport of spermatozoa in the ewe. R. J. Lightfoot* and B. J. Restall†, Department of Animal Husbandry and Department of Veterinary Physiology, University of Sydney, Sydney, N.S.W.

Three experiments were conducted.

In the first experiment, both the proportion of ewes from which spermatozoa were recovered from the Fallopian tubes 2 hr after insemination and the mean number of spermatozoa recovered tended to be higher in control than in oxytocin injected (1-0 or 10-0 i.u., i.m.) ewes. The proportion of ewes from which

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spermatozoa were recovered was also higher for those ewes in which the inseminating pipette was inserted more than 3 cm into the cervix during insemination.

The second experiment examined the effects of sperm motility (live versus dead spermatozoa), inhibition of genital tract contractions (halothane anaesthesia; −, +) and stimulation of genital tract contractions (oxytocin injections; −, +) on the distribution of spermatozoa throughout the genital tract 30 min after insemination. Few spermatozoa were recovered from the mid- and cranial segments of the cervix after insemination with dead, as compared with live spermatozoa. Oxytocin appeared to have little effect, but inhibition of contractions by halothane anaesthesia reduced sperm transport. Following insemination with dead cells, the mean numbers of spermatozoa recovered from the cranial cervix and Fallopian tubes were 1000 and 0 in anaesthetized ewes, compared with means of 16,000 and 230, respectively, in conscious ewes.

The effects of site of insemination (external versus internal cervical os) and sperm motility (live versus dead spermatozoa) on the distribution of spermatozoa throughout the genital tract 2 hr after insemination were examined in the third experiment. Following insemination at either the internal or external cervical os, few spermatozoa were found between the mucosal folds of the cervix when dead spermatozoa were used. Large numbers of spermatozoa were recovered from both the cervix and Fallopian tubes after insemination at the internal cervical os, particularly following the use of motile spermatozoa.

The results demonstrate the importance of sperm motility, particularly in relation to transport through the cervix.