The contribution of degenerating spermatozoa to the epididymal plasma after castration. R. Jones and T. D. Glover, Unit of Reproductive Biology, University of Liverpool, Life Sciences Building, P.O. Box 147, Liverpool L69 3BX.


The ultrastructure of the prostate gland in the cat. Elizabeth Aughey, Department of Veterinary Histology and Embryology, University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow.

The cat compact prostate is a compound tubular gland with tubules of varying diameter lined by a secretory epithelium (Aitken & Aughey, 1964). There is a strong acid phosphatase reaction in the majority of the cells. Ribonucleoprotein is present in the basal zone, together with some glycogen, and there is non-specific protein in the luminal cytosome. Three cell types can be identified on the basis of their ultrastructural characteristics. The Type 1 cell is simple columnar with a rounded basal nucleus, a prominent Golgi body and rough endoplasmic reticulum (RER) in the infranuclear zone. The electron-dense secretion granules observed in the supranuclear cytosome are identical to the secretion granules in the mucus-secreting disseminate prostate cell. The Type 2 cell is columnar, again with a rounded basal nucleus, infranuclear RER, an extensive Golgi apparatus and a number of secretory vesicles in the cytosome. The vesicles are filled with a fine flocculent material with condensed electron-dense inclusions. The vesicles of the luminal cytosome are often more concentrated, and the individual granules are apparent in the luminal contents and free between the microvillous border of these cells. The Type 3 cell is tall columnar with a dark, granular, uneven nucleus and an electron-dense cytosome filled with vesicles. The secretion appears to consist of the secretory vesicles, superficial parts of the cell and even whole cells. The secretory vesicles of the Type 2 and 3 cells are membrane-bound, and some at least react positively following the non-specific acid phosphatase technique (Holt & Hicks, 1961). The ultrastructural findings,
therefore, tend to suggest the presence of two cell types, one secreting a simple mucopolysaccharide and the second secreting a protein fraction, including acid phosphatase. The Type 2 and 3 cells are thought to be different stages in the secretory process. In a study of zinc in the cat prostate (Aughey, 1970), it was suggested that zinc is an integral part of the secretion of this gland and is associated with the production of metalloenzymes. A study of the distribution of \(^{65}\)Zn at fine structural level appears to indicate the presence of the isotope in the secretory vesicle and in the cell nucleus within 18 hr of subcutaneous injection.

REFERENCES


Follicular development in ovarian transplants in the domestic fowl. 
Helen A. Scott and A. B. Gilbert, *ARC Poultry Research Centre, Edinburgh.*

The successful low-temperature preservation of mouse and cow embryos. I. Wilmut and L. E. A. Rowson, *ARC Unit of Reproductive Physiology and Biochemistry, Animal Research Station, 307 Huntingdon Road, Cambridge CB3 0JQ.*

The technique of embryo transfer is being used extensively as a research tool but is used relatively little in animal breeding. Although the potential value of the technique in animal breeding is well known (Hammond, 1950; Rowson, 1971) its utilization on a large scale depends upon the development of a number of other techniques; for example, techniques for *in-vitro* maturation and fertilization of oocytes and for the culture and storage of embryos. This paper is concerned with the low-temperature preservation of embryos.

A study has been made of the effect of varying cooling and warming rates and cryoprotective agents on the survival of mouse embryos during freezing and thawing (Wilmut, 1972). No mouse blastocysts survived after being frozen and thawed in the presence of the extracellular cryoprotective agents, sucrose and polyvinylpyrrolidone. In contrast, a proportion survived in the presence of the penetrating compound, dimethylsulphoxide (DMSO). Maximum survival was obtained when the blastocysts were cooled at 0·22° C/min and warmed at 12° C/min in the presence of 1·5 m-DMSO. After being frozen and thawed in this way, sixty-six out of seventy-seven (85·7%) early blastocysts developed to fully expanded blastocysts during 24-hr culture. Similar survival was obtained when 8-cell embryos were frozen and thawed by this method.

Eight-cell cow embryos were recovered on Day 3 of pregnancy. The concentration of DMSO was increased to 1·0 m or 1·5 m in 0·5-m steps and the embryos were then cooled at 0·22° C/min. Groups of six or seven embryos were warmed at 1·1, 4, 12 or 60° C/min before the DMSO was removed in 0·5-m steps. The embryos were then transferred to the ligated oviducts of pseudopregnant rab-
bits which were killed 48 hr later so that the embryos could be recovered (Lawson, Rowson & Adams, 1972). No embryos survived after being frozen and thawed in the presence of 1-0 m-DMSO or after being warmed at 1-1, 4 or 60° C/min in the presence of 1-5 m-DMSO. In contrast, two of seven which had been cooled in the presence of 1-5 m-DMSO and warmed at 12° C/min developed normally in the rabbit.

It is most unusual for the optimum warming rate for animal cells to be as slow as 12° C/min although in some circumstances, slow warming of frozen plant cells has been shown to be optimal (Levitt, 1966). It seems probable that it is the presence of the zona pellucida which determines that the warming rate has to be slow (Wilmut, 1972).

Experiments have also been carried out with cow blastocysts recovered on Days 10 to 13 of pregnancy. Blastocysts recovered at this stage have shed the zona pellucida. After being cooled at 0-22° C/min and warmed at 360° C/min in the presence of 2-0 m-DMSO, some blastocysts were able to re-establish a normal appearance during culture for 24 hr in a medium composed of TCM 199 and fetal calf serum (3:1). Nine blastocysts frozen and thawed in this way have been transferred to five non-pregnant recipient cows. Four recipients did not become pregnant, but one recipient did not return to heat and rectal palpation on Day 42 showed that she carried two implantations.

Live mouse young have been born after the transfer of embryos which had been frozen and thawed by a method (Whittingham, Leibo & Mazur, 1972) which is similar to the present one and which was developed independently. Long-term storage of mouse embryos is now possible, and the results presented here provide encouragement that similar techniques can be developed for cow embryos.

We wish to acknowledge assistance given by a number of colleagues during the operations, and in particular to thank Dr R. Tervit. I.W. gratefully acknowledges the award of a Milk Marketing Board Fellowship.

REFERENCES


Embryotoxic effects of flushes from rat and mouse uteri with or without intrauterine sutures on mouse eggs in culture. W. G. Breed, Department of Anatomy, University of Birmingham, The Medical School, Birmingham B15 2TJ.

It now appears to be established that the IUD exerts its contraceptive effect in rodents by inducing a hostile uterine environment to fertilized ova and/or by preventing uterine receptivity. This adverse environment may well be due to
cellular decomposition within the uterus and it has been invariably shown in rodents that most of the cells present within the uterine lumen are polymorphonuclear leucocytes when an IUD is present.

Parr (1969) first showed that rat morulae cultured in vitro with extracts of polymorphonuclear leucocytes did not develop, but although blastulation occurred in his control embryos, they were probably dead the following morning. The other more recent in-vitro studies to elucidate IUD action have always used mouse eggs. Smith, El Sahwi, Wilson & Moyer (1971) tested the effects of different concentrations of rabbit polymorphonuclear leucocytes on two-cell embryos in culture, whereas Joshi & Kraemer (1970) investigated the effects of rat and baboon uterine flushes on mouse ova.

In the present study, a comparison is made between rat and mouse uterine flushes, obtained with Brinster's medium, at different physiological states, on mouse morulae in culture. Morulae were used since this is the stage that normally enters the uterus.

Unilateral IUDs used were 3-0 silk thread for rats and 6-0 for mice. After resting the animals for 3 weeks or more, 0.1 ml Brinster's medium was passed through the uterus and the resulting flush spun at 3000 rev/min for 5 min. Drops, each of about 20 µl, of supernatant and/or cellular deposit were then placed under oil. Undiluted pro-oestrous uterine fluid from rats was also aspirated directly and treated likewise. Numbers of morulae developing into expanded blastocysts (EB) and subsequently hatching (HB) were recorded.

In flush supernatants obtained from IUD horns of dioestrous rats (six replicates), there was no reduction in EB development from morulae (78/98, HB: 42), compared to controls (61/117, HB: 36). Similar results were obtained with supernatants obtained from rats on Day 4 post coitum (six replicates)—IUD-horn flushes (50/104, HB: 40) versus controls (61/96, HB: 30). Results for cellular deposits were similar.

Flushes from oestrous rats suggested that the IUD horn was slightly toxic to mouse morulae whereas in undiluted uterine-fluid supernatants from the IUD horns of pro-oestrous rats, there was a significant reduction (χ², 2×2 test, P<0.05) in EB development in three out of six replicates. The number of EB developing in IUD-horn fluid was 41/120, HB: 16, whilst that in control-horn fluid was 83/121, HB: 42.

In mice, control-horn flushes were obtained from normal females as the IUD has a partial bilateral effect (Mackay & Breed, 1972). Flushes from dioestrous mice did not reduce EB development—IUD horn (58/88, HB: 38) versus control horn (54/95, HB: 37). Results were also noted with flushes obtained from mice on Day 4 post coitum—IUD horn (80/124, H.B: 35) versus control horn (89/130, H.B: 63), but flushes through oestrous mouse uteri (8 replicates) demonstrated potent toxicity: IUD horn (3/79, HB: 0) versus controls (66/104, HB: 54).

It appears, therefore, that mouse IUD-horn flushes are markedly more toxic than rat flushes to mouse ova in culture. Toxicity cannot be demonstrated in flushes from rats or mice in dioestrous or on Day 4 post coitum, possibly due to a greater dilution of uterine fluid with the culture medium.

From these results, therefore, and those of Mackay & Breed (1972), the IUD appears to be embryotoxic.
I should especially like to thank Mrs C. Jefferies for technical assistance.

REFERENCES


SESSION 2

Chairman: Professor I. D. COOKE

Results of treatments of infertile women with defective luteal phases by human menopausal gonadotrophin and human chorionic gonadotrophin. I. D. Cooke*, M. A. Pearce, K. Davies and H. Campbell, *Department of Obstetrics and Gynaecology, The University, Jessop Hospital for Women, Sheffield S3 7RE, and Department of Medical Statistics, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XX.

Further studies on infertile women with defective luteal phases have been completed. Human menopausal gonadotrophin (HMG) treatment (Pearce and his colleagues, 1972) in up to thirty-nine treatment cycles showed significantly greater preovulatory urinary oestrogen excretion \( P<0.05 \) but no change in the luteal-phase excretion compared to control cycles. Human chorionic gonadotrophin (HCG) treatment alone resulted in a decreased luteal phase oestrogen but HMG followed by HCG therapy resulted in a significant increase in luteal-phase oestrogen excretion: HMG alone caused a significant reduction in plasma progesterone concentration but HMG followed by HCG resulted in a significantly greater plasma progesterone elevation than HCG alone.

Urinary pregnanediol excretion also showed a significant increase in the early part of the luteal phase after HMG/HCG therapy, but the increased excretion occurred earlier than the increases in plasma progesterone levels.

These data show that, in infertile patients with poor follicular oestrogen excretion and variable progestational effects in the luteal phase, sequential HMG/HCG therapy produces an optimum hormonal response, whereas HMG or HCG alone do not. This sequential therapy, previously used only in amenorrhoeic patients, clearly has a place in the treatment of regularly cycling infertile patients with signs of poor follicular development and variable luteal-phase sufficiency.

REFERENCE

Plasma oestradiol and plasma progesterone levels in the normal menstrual cycle and during induction of ovulation by menopausal gonadotrophin and chorionic gonadotrophin. A. Klopper, Department of Obstetrics and Gynaecology, University of Aberdeen, Aberdeen.

Effect of oral cyproterone acetate on urinary FSH and LH levels in adult males being treated for hypersexuality. Janet Brotherton and A. W. Harcus, Schering Chemicals Ltd, Burgess Hill, Sussex.

Cyproterone, which is a ‘pure’ anti-androgen, is known to produce an increase in plasma testosterone, FSH and LH levels and to be without effect in the treatment of a variety of androgen-dependent conditions (Brotherton, 1972). Cyproterone acetate, which is also a powerful progestagen, has been experimentally used for the treatment of hypersexuality since 1967 and is very effective in the reduction of libido and potency together with spermatogenesis. By about 6 weeks of treatment, there is an absence of ejaculate without significant pathological changes in the testes. Hormone assays have consistently shown that plasma testosterone levels are significantly reduced to about a fifth of the normal mean. The effect on gonadotrophins is less clear.

Members of a group of eleven male hypersexual patients from three clinical centres each received 50 mg cyproterone acetate twice a day. All the patients had normal secondary sexual characteristics and were adults aged 22 to 54. Some were residents in homes for the mentally retarded while others had drawn the attention of the police to their behaviour. Gonadotrophins were estimated after different periods of treatment in 24-hr urine specimens after storage at −20° C. Radioimmunoassays of FSH and LH were carried out by Dr W. R. Butt (Butt & Lynch, 1971; Raiti, Light & Blizzard, 1969; Wikramanayake, Keenan, Spathis, Nabarro, Leonard & Gallagher, 1972).

Values of LH and FSH before and during treatment fluctuated widely and there was a wide spread of results. The six pretreatment values for FSH had a mean of 7·32 i.u. ± 5·10 (S.D.) and all fell within the quoted normal range of 2 to 17 i.u. 2nd IRP-HMG/24 hr and quoted mean of 8·5 i.u. ± 3·6 (S.D.). Similarly the six paired pretreatment values for LH had a mean of 26·5 i.u. ± 35·2 although one was significantly higher than the quoted normal range of 4 to 45 i.u. 2nd IRP-HMG/24 hr, being 104 i.u. The quoted mean was 23·9 i.u. ± 15·5 (S.D.). For the twenty-eight values obtained during treatment there was no evidence of a trend towards lower or higher values as treatment progressed. The mean of the FSH values was 9·16 i.u. ± 4·40 (S.D.) There were two individual values slightly above 17 i.u. The mean of the LH values was 23·4 i.u. ± 24·1 (S.D.). Treated as independent groups, the limited number of results showed that there was no statistically significant difference between the pretreatment and during-treatment values.

This lack of change in LH agrees with the serum radioimmunoassay results of Sorcini, Sciarra, di Silverio & Fraioli (1971) after only 3 days treatment with 300 mg/day in three doses, and of Schoonées, Schalch & Murphy (1971) who found no change during 16 weeks’ treatment with 100 mg twice daily. In contrast, Vosbeck & Keller (1971), using urinary bioassay, showed a slight decrease in LH with 200 mg daily. Similarly, the lack of change in FSH agrees with the
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\textit{pure} anti-androgen, i.e. cyproterone, and from those of a progestagen without significant anti-androgenic effects, e.g. medroxyprogesterone acetate. The latter appears to cause a reduction of plasma testosterone and LH but not FSH (Kirschner & Schneider, 1972). It seems that the anti-androgenic component in cyproterone and its acetate is responsible for inhibition of the specific suppression of LH by androgen, so allowing the continued production of LH and hence testosterone. The progestagenic component of cyproterone acetate appears to allow a partial suppression to normal values of the excess LH produced under anti-androgen alone, while the progestagenic potency of medroxyprogesterone acetate allows the direct suppression of LH to values below normal, possibly by synergism with androgen. It now seems that the suppressive effects of cyproterone acetate on libido and potency and on spermatogenesis can be achieved in the presence of normal LH and/or FSH levels.

REFERENCES

Brotherton, J. (1972) \textit{Biblphy Reprod.} 20, 913.
Kirschner, M. A. & Schneider, G. (1972) \textit{Acta endocr.}, Copenhagen, 69, 385.

Estimation of fertilization times from birth weight distribution. J. K. Burns, Department of Physiology, University College, Galway, Eire.

Session 3

Chairman: Dr W. P. Collins

Problems with a radioimmunoassay for testosterone. J. P. P. Tyler, Department of Obstetrics and Gynaecology, King's College Hospital, Denmark Hill, London, S.E.5.

The measurement of testosterone (T) in peripheral venous plasma by radioimmunoassay presents certain difficulties, many of which have been solved satisfactorily (Collins, Mansfield, Alladina & Sommerville, 1972). A major problem remaining is that 5α-dihydrotestosterone (DHT), a structurally similar and biologically active androgen, cross-reacts to varying degrees with antisera raised to testosterone.
In view of this limitation, the characteristics of antisera raised to six different antigens have been compared. If T is linked to bovine serum albumin (BSA) at carbon 3 through the carboxymethyl oxime derivative, the cross-reaction with DHT, using tritiated T in the assay system, is of the order of 60%. If the linkage is at carbon 6 through the 6β-carboxymethyl derivative, then the cross-reaction with DHT increases to around 80% (Riley, Smith, Robertson & Kellie, 1972). This result probably reflects the fact that the relatively large molecule of BSA is in close proximity to that portion of the molecule which characterizes DHT. Another approach has been to apply the linkage at carbon 7, through the 7α-carboxyethyl thioether derivative. This structure effectively increases the length of the linkage, exposes slightly more of rings A and B and decreases the cross-reaction with DHT to about 40% (H. R. Lindner, personal communication). By studying the stereochemistry of T and DHT it would appear desirable to apply the linkage to the β surface of the molecule at carbon atoms 9 or 11. These antigens are more difficult and expensive to produce but antisera to T-19-succinyl-BSA (A. A. A. Ismail, personal communication) and T-11α-succinyl-BSA cross-react with DHT to the extent of 30% and 20%, respectively. Antisera raised to T-17β-succinyl-BSA have the lowest cross-reaction with DHT (<10%), but cross-react (80 to 100%) with other Δ4-3-ketosteroids including progesterone and androstenedione (A). Consequently, if this antiserum is used, adequate purification must be introduced into the procedure.

These studies suggested that it would be of value to devise a chromatographic system, which would separate T from DHT. This would enable greater specificity to be introduced when analysing unusual samples and enable both compounds to be determined. A column of Sephadex LH20 (30 x 1 cm) will readily separate A from T using a variety of solvent systems. However, even
under ideal conditions with authentic standards, DHT is incompletely separated from T. The recent introduction of hydroxy-alkoxypropyl-Sephadex containing approximately 54% (w/w) of C11 to C14 alkyl side chains enables the separation of A, DHT and T in 80 ml of a suitable solvent. The separation, using a 14-cm gel bed and the solvent system, petroleum ether (80 to 100° C)/cyclohexane (95:5, v/v), at a flow rate of 40 ml/hr, is shown in Text-fig. 1. The collection of large volumes of eluate—10 ml A, 16 ml DHT and 20 ml T—does not add significantly to the blank values for the assay.

The results suggest that T may be determined without a chromatographic step using an antiserum to T-11α-succinyl-BSA. However, this will give a mean over-estimate in the region of 10%. If a more specific determination is required on samples from patients with various pathologies, or from species where the steroid profile is not known, then a chromatographic step on alkoxypropyl-Sephadex should be included.

REFERENCES


The production of testosterone by normal, cryptorchid and castrated male horses. J. E. Cox, J. H. Williams and P. H. Howe, Department of Veterinary Clinical Studies, University of Liverpool, 'Leahurst', Neston, Wirral, Cheshire L64 7TE, and *Unit of Reproductive Biology, University of Liverpool, Life Sciences Building, P.O. Box 147, Liverpool L69 3BX.

Contrasting effects of testosterone propionate and 5α-dihydrotestosterone on sexual behaviour in the male golden hamster. Julia S. Brayshaw, A. P. Payne and Heidi H. Swanson, Department of Anatomy, University of Birmingham, Birmingham B15 2TJ.

Interrelationships between hibernation and reproduction in noctule bats, Nyctalus noctula. P. A. Racey, Unit of Reproductive Biology, University of Liverpool, Life Sciences Building, P.O. Box 147, Liverpool L69 3BX.


The purpose of this study was to determine the pattern of steroid hormone changes during late pregnancy, parturition, lactation and after weaning. Six pregnant Large White sows, of approximately 200 kg body weight, were prepared with indwelling ear-vein catheters 7 to 12 days before parturition (Ash, Banks, Broad & Heap, 1972). Blood samples were taken each morning, usually at 09.00 hours, before feeding, and more frequently about the time of parturi-
Plasma progesterone concentrations were measured by competitive protein-binding and radioimmunoassay, total unconjugated oestrogens by radioimmunoassay, and corticoids by competitive protein-binding.

During late pregnancy, plasma progesterone concentrations of about 8 to 10 ng/ml showed a gradual decline over the last 7 days of gestation in three animals, but decreased sharply within 48 hr before delivery in three other sows. Plasma oestrogens, in contrast, rose steadily in all sows, reaching concentrations of 1 to 4 ng/ml during the last week of gestation. The peripheral oestrogen concentrations tended to be greater in multiparous animals and in sows carrying a large number of fetuses. Plasma oestrogens in late pregnancy consisted mainly of oestrone and small quantities of oestradiol-17β, as reported previously for urinary oestrogens in pregnant sows (Fèvre, Léglise & Rombauts, 1968). Plasma corticoid concentrations (mainly cortisol) in late pregnancy were on average 33 ± 1 ng/ml but they varied appreciably from day to day.

In two parturient sows, plasma progesterone concentrations were low at the time of delivery of the first piglet. In one sow, however, values remained high (about 10 ng/ml) until within 6 hr before parturition, whereas in another they were low for about 24 hr before delivery. Plasma oestrogens were still high (about 3 ng/ml) when the first piglet was born, but they then declined gradually during delivery reaching undetectable concentrations after the placentae had been expelled. Plasma corticoid concentrations showed no consistent pattern of change. Analyses of blood samples from the maternal jugular and umbilical veins and from the right side of the heart of newborn piglets indicated that, in late pregnancy, the placenta secreted a small quantity of progesterone, the fetal adrenals produced an appreciable amount of corticosteroids (as in the fetal lamb, Bassett & Thorburn, 1969), and that the high oestrogen concentrations in late pregnancy are of uterine origin (as reported by Fèvre et al., 1968).

During lactation, there was no evidence of cyclical changes in progesterone, oestrogen or corticoid concentrations. Progesterone and oestrogen values remained low or undetectable, while plasma corticoids were on average slightly lower than in pregnancy. After weaning at 4 to 5 weeks, plasma oestrogen concentrations rose to peak values of 10 to 62 pg/ml immediately before or on the day of behavioural oestrus. Within 3 weeks of a fertile mating, plasma progesterone concentrations had reached 20 to 35 ng/ml, typical of the values found in early pregnancy by other workers (Guthrie, Henricks & Handlin, 1972). After early weaning (within 24 hr of birth), changes in peripheral progesterone concentrations indicated the cyclic formation of corpora lutea. However, only small and transient changes were found in plasma oestrogen concentration at oestrus, and pregnancy was not established until about 7 weeks after parturition.

REFERENCES

Studies on the endocrinology of parturition in the guinea-pig. Doreen V. Illingworth, N. Ackland, A. M. Burton,* J. R. G. Challis, R. B. Heap and J. S. Perry, ARC Institute of Animal Physiology, Babraham, Cambridge, and *Department of Chemical Pathology Research, St Bartholomew’s Hospital Medical School, London, E.C.1

We have reported elsewhere that guinea-pigs hypophysectomized within 4 days of a fertile mating will maintain pregnancy for the normal duration when treated with cortisone acetate (12.5 mg subcutaneously, twice weekly) (Ash and his colleagues, 1973). Parturition, however, did not occur and, in this respect, the guinea-pig appeared to differ from the sheep (Denamur & Martinet, 1961). In subsequent work, when the hypophysectomized guinea-pigs were given the same dose of cortisone acetate more frequently, daily from Day 55 onwards, spontaneous parturition occurred at the expected time. Pelvic relaxation was normal and viable fetuses were born, though the duration of delivery was greatly protracted (several hours instead of about 1 hr). A similar treatment given to intact guinea-pigs did not advance parturition, so that the cortisone acetate appears to have a general effect on maternal well-being rather than a specific effect on the induction of parturition.

Concurrent with this work, measurements of various hormones associated with the onset of parturition have been made in conscious animals with indwelling carotid catheters. There were no striking changes in the arterial concentrations of progesterone (which remained at >50 ng/ml), total unconjugated oestrogens (less than 20 pg/ml), corticoids (about 3 µg/ml), progesterone-binding globulin (about 10 to 15 µM), and corticosteroid-binding globulin before the delivery of the first fetus. Maternal oxytocin could not be detected at this time but, thereafter, a rapid increase in arterial concentrations was found. High circulating levels (about 500 pg/ml) were sustained during the expulsion of the other fetuses (Burton, Challis, Illingworth & McNeilly, 1972). No oxytocin could be detected in maternal plasma of hypophysectomized guinea-pigs during parturition, though small amounts were detected in fetal plasma. Thus, the rôle of oxytocin in the guinea-pig appears to be confined to facilitating the expulsion of fetuses after the initiation of parturition.

Other studies on the possible mechanisms involved in the initiation of parturition indicate that the fetal adrenal weight shows no marked increase relative to body weight. Attempts to advance parturition experimentally have included the fetal and maternal administration of dexamethasone, the maternal implantation of oestrogens, the maternal injection of 17β-oestradiol antiserum and of prostaglandins (PG), and the maternal infusion of corticotrophin (ACTH) (Synacthen, Ciba Laboratories), PG and a PG analogue. Parturition was prevented by stilboestrol and oestradiol, but advanced by ACTH infusion, PGF2α injection and the infusion of PGF2α, PGE2 and a synthetic analogue of the former.

We gratefully acknowledge support from the Lalor Foundation (D.V.I. and J.R.G.C.) and the SRC (A.M.B.) and we thank Dr A. L. Walpole of ICI Pharmaceuticals for prostaglandins.
The effects of endogenous compounds on ovine myometrial contractility and adenyl cyclase system. Gadija Rossier, C. G. Pierrepont and K. Griffiths, Tenovus Institute for Cancer Research, The Welsh National School of Medicine, Heath Park, Cardiff CF4 4XX.

The contractility of ovine myometrium in vitro at various stages of the oestrous cycle and pregnancy has been investigated using isometric and isotonic recording techniques.

Prostaglandins PGF$_{2\alpha}$ and PGE$_2$ (0·6 µg/ml) were found to cause tetanic contractions of the myometrium and, in the early pregnant myometrium in particular, these effects were very similar to the response elicited by oxytocin (570 µi.u./ml) and vasopressin (570 µi.u./ml).

Adrenaline (0·6 µg/ml) greatly depressed myometrial contractility and, furthermore, antagonized the actions of the prostaglandins. Following a specific lag phase, a reversal of this inhibition was usually observed and strong contractions recommenced. This secondary activity could be abolished by the addition of indomethacin (2 µg/ml) to the bath medium. Indomethacin is a specific inhibitor of prostaglandin synthesis and its effect on this reversal phenomenon is in accord with the conclusions of Tothill, Rathbone & Willman (1971), working with rat tissue, that adrenaline induces prostaglandin synthesis in the myometrium.

Cyclic AMP (0·5 mM) increased myometrial contractility but to a much lesser extent than did the prostaglandins or oxytocin. Furthermore, NaF (10 mM) greatly enhanced myometrial tone.

The contractile activity and intrauterine pressure changes of the non-pregnant sheep's uterus were measured in vivo by means of an open-ended intrauterine catheter connected to an extraterine radio-pill and radio-receiver system. Infusion of PGE$_2$ (50 or 100 µg) through a second intrauterine cannula initially caused an increase in myometrial tone which was quickly followed by a profound relaxation. These results were demonstrated in two ewes in which the ovaries on the side of the experimental horn bore follicles whilst the contralateral ovaries carried corpora lutea.
This dual effect of PGE\textsubscript{2} on the myometrium was not demonstrated in the in-vitro experiments. Infusion of PGF\textsubscript{2\alpha} (40 \mu g) in a similar manner increased the frequency of myometrial contractions but not the intrauterine pressure. Adrenaline caused an inhibition of uterine contractility but the adrenaline reversal observed in vitro was not shown in vivo. Cyclic AMP (500 \mu g) increased the amplitude of contractions in vivo.

Myometrial adenyl cyclase activity was investigated by measuring the conversion of [8-\textsuperscript{14}C]ATP to [8-\textsuperscript{14}C]cyclic-AMP.

Our preliminary investigations indicate that PGE\textsubscript{2} and PGF\textsubscript{2\alpha} (10\textsuperscript{-4} to 10\textsuperscript{-6} M) stimulate the activity of this enzyme present both in early-pregnant and in non-pregnant ovine myometrium.

Adrenaline (10\textsuperscript{-6} or 5 \times 10\textsuperscript{-7} mol), NaF (10 mM) and vasopressin (50 \mu i.u.) significantly reduced adenyl cyclase activity whilst oxytocin (50 \mu i.u.) had no effect on this enzyme from early-pregnancy sheep myometrium.

Our investigations of myometrial activity have shown that cyclic AMP (0.5 mM) stimulates tone and contractility. It does not seem, therefore, that there is a correlation between the ability of all the above compounds investigated to stimulate uterine tone and motility and their ability to raise or depress intracellular levels of cyclic AMP through the adenyl cyclase system present in this target tissue.

It is possible that the prostaglandins, PGE\textsubscript{2} and PGF\textsubscript{2\alpha}, and the catecholamine, adrenaline, could influence the functional activity of ovine myometrium through stimulating and inhibiting adenyl cyclase activity, respectively. The results obtained for NaF, vasopressin and oxytocin suggest that these compounds could produce their effects on the ovine myometrium through an alternative mechanism(s).

REFERENCE


Luteolytic effect of oestrogen injected directly into corpora lutea of ewes. F. J. Karsch, B. Cook, D. L. Foster and A. V. Nalbandov, Animal Genetics Laboratory, University of Illinois at Urbana, Champaign, Illinois, and \*Department of Steroid Biochemistry, University of Glasgow, Glasgow.

Induction and synchronization of oestrus in mares with prostaglandins. W. R. Allen and L. E. A. Rowson, ARC Unit of Reproductive Physiology and Biochemistry, Animal Research Station, 307 Huntingdon Road, Cambridge CB3 07Q.

Lengthening of the oestrous cycle of the guinea-pig by indomethacin in slow-release form within the uterine lumen. P. B. Marley, Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX.

Daily rhythms of LH release in quail when gonadal development is initiated by long daylengths. T. J. Nicholls and B. K. Follett, Department
Sexually mature Japanese quail, *Coturnix coturnix japónica*, of both sexes were caused to undergo gonadal regression by subjection to short daily photoperiods (8 hr light/day) for 14 to 21 days. It was established by radioimmunoassay that a single long photoperiod (20 hr light, 4 hr darkness) was then sufficient to initiate a significant increase in plasma immunoreactive luteinizing hormone (IRLH). The temporal pattern of secretion of this gonadotrophin was investigated by killing groups of birds at intervals throughout the first and second long photoperiods experienced after the gonadal regression phase.

During the 1st day of subjection to long photoperiods, plasma IRLH concentrations remained at constant low values (about 1 ng/ml) until approximately 18 hr from ‘dawn’ when levels rose significantly to about 2 ng/ml. This elevated titre remained constant throughout the ‘night’ and first 8 hr of the 2nd day, falling somewhat thereafter. A subsequent highly significant increase in circulating levels was noted approximately 18 hr from ‘dawn’ of the 2nd day. Precise measurement of the rise in levels during the 1st day of the long-photoperiod regime indicated that the increase in plasma concentrations, presumably due to increased pituitary secretion, occurred between 18½ and 19½ hr from ‘dawn’.

Since 12 hr of light/day induces gonadal development in *Coturnix*, it is assumed that a photoinducible phase predicted by the Bunning model of photoresponsivity must be positioned in this species at or somewhat before 12 hr from ‘dawn’ in an entraining light cycle. It would appear, therefore, that a delay of at least 6½ hr occurs between coincidence of light with the photoinducible phase and response of the pituitary by IRLH secretion. The reason for this is not known.

**A progressive decrease in pituitary responsiveness to gonadotrophin-releasing hormone during gestation in the sheep.** W. A. Chamley, J. K. Findlay, I. A. Cumming, J. M. Buckmaster and J. R. Goding, Department of Physiology, University of Melbourne, S.S. Cameron Laboratory, State Research Farm, Werribee, Victoria 3030, Australia.
PROCEEDINGS OF A JOINT MEETING OF THE BLAIR BELL RESEARCH SOCIETY AND THE SOCIETY FOR THE STUDY OF FERTILITY

 Held at the Zoological Society of London, Regent’s Park, London, N.W.1 on 15th December 1972

MALE FERTILITY

SESSION 1

Chairman: PROFESSOR T. R. R. MANN

HORMONAL CONTROL OF TESTICULAR ACTIVITY

Cyclical and seasonal changes in testicular activity of animals. R. V. Short, MRC Unit of Reproductive Biology, Edinburgh.

Cyclical and seasonal changes in human testicular activity. G. A. Lincoln, Department of Veterinary Medicine, Liverpool.

Hormonal control of spermatogenesis. E. Steinberger, University of Texas Medical School, Houston, Texas, U.S.A.

The rôle of inhibin. B. P. Setchell, ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT.

SESSION 2

Chairman: DR R. V. SHORT

INVESTIGATION OF MALE INFERTILITY

Gonadotrophin levels in infertile patients. P. Franchimont, University of Liège, Belgium.

The place of testicular biopsy in male infertility. D. Ellis, Churchill Hospital, Oxford.

Testicular antibodies. J. V. T. H. Hamerlynck, MRC Unit of Reproductive Biology, Edinburgh.

SESSION 3

Chairman: DR J. NEWTON

TREATMENT OF OLGOSPERMIA AND ASPERMIA

Treatment of oligospermia with hormonal therapy. R. Schoysman, University Hospital, Brugmann, Brussels.

Artificial insemination with donor semen. E. Steinberger, University of Texas Medical School, Houston, Texas, U.S.A.

Deep freezing of human semen. R. Schoysman, University Hospital, Brugmann, Belgium.
VASECTOMY

The surgical techniques of vasectomy. J. Newton, King’s College Hospital, Denmark Hill, London.


Vasectomy counselling. J. McEwan, Department of Family Planning, King’s College Hospital, Denmark Hill, London.