

Human Reproduction, Department of Obstetrics and Gynaecology, King's College Hospital Medical School, Denmark Hill, London SE5 8RX, England

Ovaries from pubertal mice were dissected to yield fragments containing 1–4 antral follicles. These were cultured on the surface of a chemically defined, modified Eagles Minimum Essential Medium (MEM). After 17–20 h oocytes were released from the cultured follicles and their meiotic status recorded. Preliminary experiments evaluating this simple, short-term technique for whole follicle culture demonstrated that oocytes behaved meiotically as they would *in vivo*. Follicles were then cultured in MEM containing various concentrations of different steroids. When progesterone (10 μM), testosterone (100 μM), or androstenedione (100 μM) were added to the medium significantly more oocytes remained at the dictyate stage than when follicles were cultured in control medium ($P < 0.001$). The meiotic status of oocytes from follicles cultured in lower concentrations of the above steroids, or in any of the concentrations tested of oestradiol or pregnenolone (1–100 μM), was not significantly different from that of oocytes from follicles cultured in steroid-free medium. Of the dictyate oocytes released from all these steroid-treated follicles the majority (61%) were capable of resuming and/or completing the first meiotic division when cultured further in steroid-free medium. However, after follicles were cultured in 100 μM progesterone, most follicle cells were pyknotic, and 99% of the oocytes were necrotic. The results of these experiments suggest that some steroids may be involved directly or indirectly in the control of oocyte maturation and atresia.

TESTES AND STEROIDS

25. A simple method using R 1881 to differentiate between androgen binding protein and androgen receptor in the rat epididymis. J. P. RAYNAUD, J. SECCHI and M. M. BOUTON, Centre de Recherches, Roussel Uclaf, 93230 Romainville, France

Sperm maturation is androgen-dependent and occurs in the epididymis which contains two androgen binding proteins: androgen binding protein (ABP) present in the epididymal fluid and androgen receptor (Rc) present in the epididymal epithelium. An accurate estimation of their individual binding capacity has been difficult so far since the hormone currently used for their measurement, dihydrotestosterone (DHT), binds both ABP and Rc with high affinity (5×10^{-9} M and 5×10^{-10} M respectively at 4°C). ABP measurement with DHT is relatively precise since its concentration is about 50 times higher than that of Rc, but Rc levels may be markedly underestimated. The steroid R 1881 (17 β -hydroxy-17 α -methyl-estra-4,9,11-trien-3-one) has a higher affinity for Rc and a lower affinity for ABP than DHT and can be used to evaluate Rc, whatever the ABP level, on condition that the Dextran-coated charcoal concentrations chosen to measure bound steroid take into account the different affinities of R 1881 for these two proteins. To validate the use of R 1881, rats were hemi-castrated for 4 weeks and bilaterally castrated 24 h before sacrifice. In the epididymis corresponding to 4-weeks castration, the ABP level was very low, but the Rc level was maintained by testosterone secretion from the other testis. Rc could be detected with DHT (1.7 pmol/g tissue) and accurately measured with R 1881 (3 pmol/g). In the epididymis corresponding to 24 h-castration, the ABP level was high; no Rc was detected with DHT but concentrations of 2.3 pmol/g tissue were found with R 1881. The similarity in the Rc values obtained with R 1881 24 h and 4 weeks after castration in the presence of very different ABP concentrations validates this methodology for epididymal Rc measurement.

26. Dihydrotestosterone and testosterone concentrations in spermatic venous blood and seminal plasma from patients affected by azoospermia due to germinal cell arrest and Sertoli cell only syndrome. M. SERIO,* D. BORRELLI,† G. FORTI,* M. PAZZAGLI,* C. F. SCARSELLI,‡ R. GUAZZELLI,§ G. FIORELLI,* P. CICCHI† and G. GIUSTI,* *Endocrinology Unit, University of Florence, †Department of Surgery, University of Florence, ‡Department of Obstetrics and Gynaecology, University of Florence, and §Medical Genetic Unit, University of Florence, Italy

In order to study the effects of tubular lesions on DHT secretion by the human testis we have measured T and DHT concentrations in spermatic and peripheral venous plasma and in seminal plasma of patients affected by azoospermia due to primitive tubular damage.

The patients examined were classified in 3 groups on the basis of testicular biopsy and FSH levels. As DHT concentrations in seminal plasma of normal controls showed a linear relationship with the days of sexual abstinence, each sample of ejaculate was obtained after the same period of sexual abstinence.

The mean values of T and DHT concentrations in spermatic venous blood of the group of patients with germinal cell arrest and normal FSH, with germinal cell arrest and high FSH and with Sertoli cell only syndrome were in normal range (for T = 22–77 $\mu\text{g}/100$ ml; for DHT = 0.5–0.71 $\mu\text{g}/100$ ml).

DHT concentrations in seminal plasma of normal controls (332 ± 130 pg/ml) were higher than T concentrations (190 ± 120 pg/ml) while T/DHT ratio in peripheral plasma is about 10. T and DHT concentrations in seminal plasma of all groups of patients examined were not significantly different from normal controls.

From our results it seems likely that germinal cells are not involved in DHT production *in vivo* because in Sertoli cell only syndrome DHT concentration was normal in spermatic venous blood as well as in seminal plasma.

27A.—see p. xxii.

27. Androgen binding protein (ABP) in the genital tract of the ram. B. JEGOU*, J. L. DACHEUX*, M. TERQUI, D. H. GARNIER and M. COUROT, *Laboratoire de Physiologie Comparée, Faculté des Sciences, 37000 Tours, and Laboratoire de Physiologie de la Reproduction, I.N.R.A., 37380 Nouzilly, France

An ABP was demonstrated in the genital tract of the ram. Its properties (R_f in SS PAGE; k_a ; specificity, thermolability) were similar in the rete testis fluid (RTF), the cauda epididymal plasma (CEP) and the seminal plasma (SP). The concentrations of ABP, determined by SS PAGE and/or Dextran coated charcoal methods were 2.6 ± 0.62 nM ($n = 7$) in the RTF; $57^* \pm 9$ nM ($n = 6$) in the CEP and $63^* \pm 11$ nM ($n = 4$) in the SP (*: NS), during the non breeding season. The concentration of ABP in the RTF was significantly lower during the non-breeding season (2.6 ± 0.62 nM) than in the breeding season: 4.4 ± 0.98 nM ($n = 6$; $P < 0.037$), whereas the affinity constant was independent of the season (2.45 vs 2.66×10^9 M $^{-1}$; NS). In addition, ABP was positively correlated with 5 α DHT ($r = 0.51$; $P < 0.009$), testosterone ($r = 0.45$; $P < 0.003$), total proteins ($r = 0.33$; $P < 0.02$) spermatozoa ($r = 0.41$; $P < 0.006$) in the RTF and with blood plasma testosterone ($r = 0.58$; $P < 0.0001$). These results suggest a seasonal activity of the Sertoli cells in the ram in relation with seasonal hormonal variations.

28. Steroid and prostaglandin (PG) interrelations in epididymis and vas deferens of immature (35 day old) rats. K. GEROZISSIS and F. DRAY, Unité de Radioim-