Hormone production by the intact human corpus luteum *in vitro*

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Summary. Human CL removed during the menstrual cycle were cultured for up to 5 days. Production of progesterone, measured by radioimmunoassay of the culture fluid, continued throughout each experiment. Addition of hCG to the culture medium stimulated progesterone production in a biphasic manner.

Introduction

Information on steroid hormone production by the corpus luteum (CL) has mostly been obtained indirectly by the measurement of urinary or blood levels of hormones which characterize the luteal phase of the ovarian cycle. Studies have also been carried out in which slices (LeMaire, Rice & Savard, 1968; LeMaire *et al.*, 1971; Marsh, Savard & LeMaire, 1976) or homogenates (Ryan, 1963) of the CL have been incubated for short periods and hormone production measured by assay of the culture fluid. In all *in-vitro* studies there is loss of the normal blood supply to the tissues, and slicing or homogenization may add cell damage and disruption of normal cellular interrelationships to the problems faced in culture experiments. A method by which tissue disruption may be minimized is culture of the whole gland and this is possible for a discrete tissue such as the corpus luteum. The present paper describes organ culture studies of the intact human CL for up to 5 days.

Materials and Methods

Tissue collection and culture

Ovarian tissue was obtained from women undergoing oophorectomy for reasons unrelated to the present study (e.g. non-invasive cervical cancer). Menstrual cycles were regular in each case and the dates provided by the patient were verified by histological examination of endometrial biopsies obtained at the time of surgery.

The ovarian biopsies were placed in Eagle's medium (see below) at 4°C and were dissected within 2 h. Each CL was carefully trimmed of other ovarian tissues and photographed against a graduated background to obtain a record of its appearance and size. It was then placed on lens tissue supported on a stainless steel platform in a 5-cm plastic Petri dish. More lens tissue was draped over the CL so that the edges touched the culture fluid on each side of the stainless steel grid. In this way the CL received nutrients (and seemingly released hormones) by capillary action in the lens tissue, but was not immersed in the fluid (Baker, Hunter & Neal, 1975).

The culture fluid comprised Eagle's minimum essential medium with Hepes buffer and Earle's salts, to which were added newborn calf serum (20%), Kanamycin (40 µg/ml), and Amphotericin B (2 µg/ml) (all from Flow Laboratories, Irvine, U.K.). The tissue was cultured at 37°C in an atmosphere of 5% CO₂, 57% O₂ and 38% N₂ at 0.703 kg/cm² above atmospheric pressure in modified 'Kilner' glass preserving jars (Baker & Neal, 1969).

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After first ensuring thorough mixing in the culture dish, the culture medium was removed for hormone assay. Sometimes the fluid was completely replaced with fresh medium as above, with or without gonadotrophins: the CL was washed by playing a jet of 4 ml medium over the gland before adding the replacement medium. At other times 0.1 ml of the fluid was removed every hour for assay and replaced by 0.1 ml fresh medium. The used medium was stored at -18°C until assayed.

Cultures were treated with hCG (Pregnyl: Organon) at a dose of 5 or 7 i.u./ml culture medium, or with human pituitary gonadotrophin (hPG: Commonwealth Serum Laboratories, Melbourne) at a dose of 0.4 i.u./ml. The potency of the hPG was determined against the 2nd IRP hMG and the ratio of FSH:LH in the material was 1.6:1.0 (B. M. Hobson, personal communication).

Steroid assays

Oestradiol in the culture medium was measured using a radioimmunoassay developed by Fraser, Leask, Drife, Bacon & Michie (1976). The antiserum was raised in sheep to oestradiol-17 β -17-hemisuccinate-bovine serum albumin (provided by Dr G. Abraham), and cross-reacted with oestrone (35%) and oestriol (2.3%). An internal standard of tritiated oestradiol was added to the samples to measure extraction and column recovery (see Fraser *et al.*, 1976). Mean recoveries ($\pm$ s.d.) in two assay runs were $76.5 \pm 7.5\%$ ($n = 78$) and $74.3 \pm 6.7\%$ ($n = 34$). With 1 ml culture medium a lower limit of sensitivity of 150 pg/ml was obtained. The coefficient of variation was 10.4% ($n = 10$) between assays and 7.9% ($n = 25$) within assays. Unused culture medium (i.e. control blank) always gave readings below the lowest point on the standard curve. The addition of oestradiol-17 β to the culture medium at concentrations of 3.0 and 6.0 ng/ml gave assay means ($\pm$ s.d.) of 3.08 ± 0.37 ng/ml ($n = 12$) and 6.27 ± 0.66 ng/ml ($n = 12$).

Progesterone was measured by the radioimmunoassay method of Neal, Baker, McNatty & Scaramuzzi (1975), which was a modification of that of Scaramuzzi, Corker, Young & Baird (1975), for measurement in culture medium rather than serum. The antiserum was raised in rabbits to progesterone-11 α -bovine serum albumin (see Scaramuzzi *et al.*, 1975); significant cross-reactions occurred with 11 α - and 11 β -hydroxyprogesterone (121 and 77% respectively; progesterone 100%), and with 11-keto-progesterone (82%). The specificity of the antibody has been discussed by Dighe & Hunter (1974). The coefficient of variation between assays was 14.8% ($n = 11$), and within assays was 5% ($n = 64$). Progesterone was not detectable in blank (unused) culture medium and the lowest detectable amount was 200 pg progesterone/ml. The addition of 780, 3120 or 6250 pg progesterone/ml to fresh culture medium gave assay values (mean $\pm$ s.d., $n = 6$) of 785 ± 37.8 , 3216.7 ± 222.9 and 6566.7 ± 463.2 pg/ml respectively.

Histology

After culture the CL were fixed in Bouin's fluid and processed for histology. Serial sections cut at 5 μm were stained with haematoxylin and eosin and their appearance in the light microscope was compared with that of other CL from the same stage of the menstrual cycle, processed and stained in the same way but fixed immediately after surgical recovery.

Results

Histology

Apart from a slight reduction in the affinity of the cells for haematoxylin and eosin, the histology of the cultured CL was similar to that of non-cultured controls. However, the luteal cells in cultured CL appeared to have shrunk slightly causing an increase in the extent of intercellular spaces, but there were no signs of pyknosis in any region of the tissue (Plate 1).

Production of steroid hormones

As shown in Table 1, progesterone was produced throughout the period of culture (up to 126 h). It was relatively unaffected by the duration of culture. The addition of hPG to the medium of CL No. 29 gave an increase (to 176%) in production of progesterone while oestradiol levels declined (to 58%) compared with the initial production rates (see Table 2).

Human corpora lutea, stained with haematoxylin and eosin, $\times 100$.

Fig. 1. Outer region of a CL cultured for 126 h. The luteal cells are smaller than those in non-cultured controls (Fig. 3).

Fig. 2. Central region of the same CL shown in Fig. 1. The cells have been maintained in culture although some cytological changes can be seen.

Fig. 3. Outer region of a control (non-cultured) corpus luteum fixed immediately after surgery. Compare with Fig. 1.

Fig. 4. Central region of the same CL as shown in Fig. 3. Compare with Fig. 2.

Table 1. Hormone production by whole human corpora lutea maintained throughout the entire period of culture, including the time of stimulation with gonadotrophins

CL No.	Day of cycle†	Diam. (mm)	Calculated wt‡ (g)	Total time cultured (h)	Total production (µg)		Production rate (µg/g·24 h ⁻¹)	
					Progesterone	Oestradiol	Progesterone	Oestradiol
H26	16–18	14	1.4	29	24.5*	2.3	16.8*	1.4
H29	22–23	13	1.2	76	32.4	0.8	8.5	0.2
H33	20	10	0.5	46	14.7	4.2	15.3	4.4
H35a	25	10	0.5	126	27.2	7.4	10.4	2.8
H35c	25	10	0.5	126	44.8	10.9	17.1	4.2

* Measured over 25 h.

† Day 1 = first day of menstrual bleeding.

‡ Calculated from mean diameter, assuming density of 1.

Table 2. Mean hormone production (ng/h) by whole corpora lutea treated with gonadotrophins

CL No.	Initial hormone production			Treatment (i.u./ml)	Subsequent hormone production		
	Time (h)	Progesterone	Oestradiol		Time (h)	Progesterone	Oestradiol
H29	53	347	12	hPG	23	609	7
H33	21	199	90	hCG (7)	7	538	14
				Wash	18	222	93
				hCG (5)	7	276	205
H35a	23	105	79	Wash	24	278	108
				Wash	65	138	10
				hCG (5)	7	488	9
				hCG (5)	7	869	823
H35c	23	112	78	Wash	24	374	85
				Wash	65	196	5
				hCG (5)	7	1058	11
				hCG (5)	7	1058	11

The addition of hCG to the culture fluid also enhanced the production of progesterone when first added and after 96 h (Table 2). The changes in oestradiol production were variable. For example, the oestradiol output of CL No. 33 fell to 16% of the pretreatment value whereas that of CL Nos 35a and 35c increased to 259 and 1055% of the initial rates.

A close examination of the results for CL Nos 35a and 35c showed that after hCG additions progesterone was produced in a biphasic fashion: it increased by a mean factor of 5.7 (1.8–11.0, $n = 4$) during the first 1–2 h of treatment, decreased during the next hour, and then increased again between 3 and 7 h after addition of hCG by a mean factor of 9.4 (4.0–22.0, $n = 4$). The pattern of increased production of oestradiol also appeared to be biphasic. Output rose in the first hour after hCG addition by a factor of 3, and at 5–7 h by a factor of 8 in CL No. 35a and of 33 in No. 35c.

Discussion

There have been few quantitative studies of hormone production by the human CL *in vitro*. LeMaire *et al.* (1968), studying CL slices in culture, showed a mean production of progesterone in the luteal phase of the menstrual cycle of 55 µg/g tissue over 3 h for 9 glands when corrections were made for initial hormone concentrations. Information is also available from studies in which steroids were extracted from corpora lutea removed at surgery; e.g. Zander, Forbes, Von Münstermann

& Neher (1958) reported a mean progesterone concentration of 14.65 µg/g tissue in 29 human CL and Stutt *et al.* (1976) found a mean progesterone and oestradiol concentration of 10.8 and 0.11 µg/g respectively in 24 recent human CL.

The results of the present study show, firstly, that the whole human CL can produce hormones throughout a prolonged culture period. During periods of up to 5 days the mean daily production of progesterone was similar to the mean static concentrations reported by Zander *et al.* (1958) and Shutt *et al.* (1976) and the mean daily oestradiol production exceeded the static concentrations found by Shutt *et al.* (1976) in 4 out of 5 cases. The rates of progesterone production were within a fairly narrow range (8.5–17.1 µg/g per day; mean value 13.6 ± 3.9 (s.d.) µg/g per day) but were much smaller than those reported (see above) by LeMaire *et al.* (1968). The difference in progesterone production may be due to poorer diffusion of metabolites into (and of steroids out of) the corpus luteum in our studies. It is also possible that the short-term incubations of LeMaire *et al.* (1968) are measuring different aspects of in-vitro hormone production.

Additions of gonadotrophins to the culture medium resulted in stimulation of progesterone production by all 5 CL studied. Similar effects were described by LeMaire *et al.* (1968) for the culture of CL slices. The variation in the oestradiol response may be related to the stage of the menstrual cycle at which the CL were removed and more studies of this possibility are needed. Alternatively, the shorter-lived production of oestrogen may be due to its origin from more vulnerable cells in the cultured CL, or to steps in the oestrogenic pathways being more susceptible to the in-vitro conditions employed.

In the present study, the CL responded to hCG by a biphasic secretion of progesterone with a peak in the first hour and at 3–7 h. Watson & Wrigglesworth (1975) have described a biphasic response to LH in studies with slices of pig CL and suggested that the initial response was due to increased release of preformed hormone while the second peak was the result of accelerated synthesis of new hormone. Savard (1973) indicates that the initial steroidogenic response of luteal cells to LH *in vitro* occurs within 10–20 min.

The ability of the intact human CL to respond to stimulation after 119 h in culture, and the normal histological appearance of the tissues after culture, suggests that at least some of the functions of the gland have been maintained and the culture system may be of further value in studies on the corpus luteum. However, parallel studies of porcine corpora lutea with the same culture system have failed to reveal any stimulatory or inhibitory effects with added LH, hCG, prostaglandin E-2 or prostaglandin F-2α (K. Kucheria, M. G. Hunter & T. G. Baker, unpublished results).

This work was assisted by grants to T.G.B. from the Population Council and the Ford Foundation. The hPG used in the study was provided by the Australian Human Pituitary Advisory Committee. The technical assistance of Mrs Gail Graham, Miss Rose Leask, Mrs Anne Rae and Mr H. P. Boyle is gratefully acknowledged.

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Received 24 October 1977