Concentrations of steroid hormones, and of prolactin, in washings of the human uterus during the menstrual cycle

B. A. Stone*, O. M. Petrucco, R. F. Seamark and B. M. Godfrey

Department of Obstetrics and Gynaecology, University of Adelaide, The Queen Elizabeth Hospital, Woodville, South Australia 5011, Australia

Summary. Levels of steroid hormones, prolactin and protein were determined in trans-cervical flushings of uteri of 73 consenting women presenting for reversal of sterilization. Median total levels of steroids (pmol), prolactin (μi.u.) and protein (mg) in the washings were: pregnenolone, 4.22; pregnenolone sulphate, 15.1; progesterone, 1.01; dehydroepiandrosterone (DHEA), 8.92; DHEA sulphate, 368; androstenedione, 2.23; testosterone, 1.04; oestrone, <0.7; oestrone sulphate, 0.49; oestradiol, 0.08; prolactin, 23.8; and protein, 5.75. Levels of these components of uterine flushings did not vary significantly between Days 6–10, 11–14, 15–20 and 21–28 after the onset of the previous menstrual period (P > 0.05). Uniform levels of free steroids in uterine washings throughout the menstrual cycle, and low free steroid/total protein ratios (all <3 pmol/mg), support other evidence for a paucity of steroid-binding proteins in human histotroph. The predominance of DHEA sulphate and of pregnenolone sulphate in human uterine washings is in accord with their abundance in plasma, and may provide an important precursor pool for de-novo steroidogenesis by human embryos before implantation. Our results support the view that human histotroph is a filtrate of plasma.

Introduction

Preimplantation growth and development of mammalian embryos is dependent upon provision of nutrients in the uterine cavity. Deficiencies in quantitative and/or qualitative properties of this nutrient pool may therefore impair embryonic growth and account for a component of the high level of embryonic mortality which occurs during the peri-implantation period of pregnancy in women (Edmonds, Lindsay, Miller, Williamson & Wood, 1982). Furthermore, as preimplantation embryos are nurtured by the uterine secretions in which they float, and elements of these secretions will appear in uterine fluids, chemical analysis of uterine washings can identify those substrates that are provided for preimplantation embryonic growth in vivo.

The present study investigates the occurrence of steroid hormones in human uterine washings at different stages of the menstrual cycle, particularly during that period which coincides with the preimplantation phase of cycles of conception. Measurements of sex steroids in uterine luminal fluids of animals (Fowler, Johnson, Walters & Eager, 1977; Stone & Seamark, 1985) have shown selective concentration of particular steroids in histotroph relative to plasma. These hormones can effect changes in endometrial secretions (Aitken, 1979), which nurture preimplantation conceptuses, and can participate in the establishment of pregnancy (Heap et al., 1977).

In view of recent evidence that prolactin is released by human endometrial tissues during the menstrual cycle, and suggestions that this hormone, also, may be involved in implantation (Maslar & Riddick, 1979), the occurrence of prolactin in uterine washings was investigated.

Total levels of protein in washings were determined to establish the effectiveness of the uterine washing technique, and to provide a basis for comparison of hormone concentrations in uterine washings with those in plasma.

**Materials and Methods**

**Uterine washing.** Flushings were recovered (by O.M.P.) from the uteri of 73 anaesthetized, consenting, women (between Days 6 and 28 after the onset of the last menstrual period; LMP) presenting for reversal of sterilization. There was no history of endocrine disorder in any of the patients. Uteri were flushed transcervically with 5 ml sterile saline (0.9% w/v NaCl), using a glass-barrelled syringe attached to a stainless-steel side-arm catheter (o.d. 3.2 mm, i.d. 2.1 mm). To avoid contamination of uterine washings with cervical secretions, the perforated tip of the catheter was inserted into the uterine lumen before infusion of saline. Washings recovered from uterine cavities were centrifuged immediately (2011 g, 20 min, 4°C), and the supernatants were stored at −15°C.

**Steroid determination.** Concentrations of pregnenolone, pregnenolone sulphate, progesterone, dehydroepiandrosterone (DHEA), DHEA sulphate, androstenedione, testosterone, oestrone, oestrone sulphate and oestradiol were measured in aliquants of uterine flushings by validated radioimmunoassays. Unconjugated steroid standards were purchased from Steraloids (Wilton, NH, U.S.A.). Pregnenolone-3-sulphate (sodium salt) and oestrone-3-sulphate (potassium salt) were obtained from Sigma Chemical Company (St Louis, MO, U.S.A.). Standard steroids were not recrystallized further.

Solvents for extraction were of analytical reagent grade and were redistilled before use. The solvent:sample ratio (v/v) exceeded 10:1 in all assays. Solvent was evaporated from sample extracts at 37°C under air.

To separate unconjugated steroids before radioimmunoassay, solvent extracts of flushings were fractionated by column chromatography (hydroxyalkoxypropyl Sephadex; Lipidex, Packard Instrument Company, IL, U.S.A.). Characterization of antisera, and detailed descriptions of the appropriate chromatography and steroid radioimmunoassay methods, have been documented from this laboratory recently (Stone & Seamark, 1985). All samples were assayed for each component synchronously.

**Prolactin determination.** Aliquants of uterine washings (2 ml) were concentrated by freeze-drying and reconstituted in distilled water (to a total volume of 250 µl). Concentrations of prolactin in aliquants of the concentrates were determined using a TANDEM-R prolactin immunoradiometric assay (Hybritech Incorporated, San Diego, CA, U.S.A.), the limit of sensitivity of the assay being 0.3 ng prolactin/ml. Assay results were corrected for saline blank values, and for cryoconcentration.

**Total protein determination.** Total levels of protein in aliquants of washings were determined using a micromethod based upon coprecipitation of protein and Ponceau S dye by trichloroacetic acid, dissolution of the precipitate in dilute alkali, and spectrophotometric determination of the dye in alkaline solution (Pesce & Strande, 1973). The method is sensitive to 20 µg protein/ml sample.

**Statistics.** Total concentrations of steroids, prolactin, and of protein in washings were calculated (concentration × flush volume). As the data were not normally distributed, differences in these values between phases of the menstrual cycle (Days 6–10 after LMP, N = 20; Days 11–14 after LMP, N = 20; Days 15–20 after LMP, N = 15; Days 21–28 after LMP, N = 18) were analysed using non-parametric methods (Kruskal–Wallis, Mann–Whitney; Snedecor & Cochran, 1980).
Results

The median (95% confidence interval; C.I.) total protein content of uterine washings in this study (5.75, 3.60–7.03 mg) was comparable to average values reported by others (e.g. 7.29 mg; Sylvan, MacLaughlin, Richardson, Scully & Nikrui, 1981), confirming the effectiveness of our technique for quantitative recovery of free uterine fluids from women.

Median (95% C.I.) total levels of steroids (pmol) in the washings (2 ml) were: pregnenolone, 4.22 (3.12–5.38); pregnenolone sulphate, 15.1 (9.3–21.4); progesterone, 1.01 (0.78–1.60); DHEA, 8.92 (5.99–16.62); DHEA sulphate, 36.8 (30.0–50.8); androstenedione, 2.23 (1.48–2.86); testosterone, 1.04 (0.41–1.19); oestrone sulphate, 0.49 (0.30–0.64); and oestradiol, 0.08 (0.00–0.13). Total concentrations of oestrone in all washings were <0.7 pmol. The median (95% C.I.) total level of prolactin in flushings was 23.8 (13.8–45.4) µu.

Total contents of the measured components in uterine flushings did not vary significantly between Days 6–10, 11–14, 15–20 and 21–28 after LMP (P > 0.05, Kruskal–Wallis). However, median levels of DHEA sulphate, oestradiol and prolactin in flushings of uteri during the period coincident with the preimplantation phase of cycles of conception (Days 15–20 after LMP) were lower (P < 0.05, Mann–Whitney) than respective median values for combined data from the remainder of the cycle (Table 1).

<table>
<thead>
<tr>
<th>Steroid/Protein</th>
<th>Days 15–20 after LMP</th>
<th>Days 6–14, 21–28 after LMP (combined)</th>
<th>Significance (Mann–Whitney)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnenolone (pmol)</td>
<td>0.66 (0.00–9.70)</td>
<td>4.39 (3.12–5.38)</td>
<td>P = 0.10</td>
</tr>
<tr>
<td>Pregnenolone sulphate (pmol)</td>
<td>5.2 (2.3–21.1)</td>
<td>16.6 (11.6–22.5)</td>
<td>P = 0.06</td>
</tr>
<tr>
<td>Progesterone (pmol)</td>
<td>1.05 (0.74–3.58)</td>
<td>1.00 (0.69–1.60)</td>
<td>P = 0.69</td>
</tr>
<tr>
<td>DHEA (pmol)</td>
<td>5.4 (1.9–24.4)</td>
<td>10.1 (6.2–17.9)</td>
<td>P = 0.10</td>
</tr>
<tr>
<td>DHEA sulphate (pmol)</td>
<td>202 (110–474)</td>
<td>436 (316–578)</td>
<td>P = 0.01</td>
</tr>
<tr>
<td>Androstenedione (pmol)</td>
<td>2.61 (0.58–4.53)</td>
<td>2.17 (1.48–2.64)</td>
<td>P = 0.62</td>
</tr>
<tr>
<td>Testosterone (pmol)</td>
<td>1.00 (0.06–1.19)</td>
<td>1.02 (0.32–1.19)</td>
<td>P = 0.83</td>
</tr>
<tr>
<td>Oestrone sulphate (pmol)</td>
<td>0.42 (0.25–0.73)</td>
<td>0.49 (0.26–0.66)</td>
<td>P = 0.82</td>
</tr>
<tr>
<td>Oestradiol (pmol)</td>
<td>0.01 (0.00–0.13)</td>
<td>0.09 (0.04–0.15)</td>
<td>P = 0.02</td>
</tr>
<tr>
<td>Prolactin (µu.)</td>
<td>8.8 (2.5–46.3)</td>
<td>35.8 (18.8–62.5)</td>
<td>P = 0.01</td>
</tr>
<tr>
<td>Protein (mg)</td>
<td>3.15 (2.01–6.37)</td>
<td>5.85 (3.61–8.54)</td>
<td>P = 0.22</td>
</tr>
</tbody>
</table>

Median (95% C.I.) ratios of total steroid/protein content of flushings (pmol/mg) were: pregnenolone, 1.17 (0.08–1.84); pregnenolone sulphate, 1.22 (0.89–2.45); progesterone, 0.13 (0.04–0.37); DHEA, 2.83 (1.14–3.88); DHEA sulphate, 63 (46–102); androstenedione, 0.59 (0.21–0.66); testosterone, 0.18 (0.05–0.35); oestrone, <0.28; oestrone sulphate, 0.11 (0.04–0.18); and oestradiol, 0.01 (0.00–0.03).

Discussion

Changes in total levels of steroid hormones in uterine washings from animals are associated with changes in uterine fluid protein content (Fowler et al., 1977), including those uterine-specific proteins that bind steroid hormones with high affinity and specificity (Beato, 1976). In these species, uteroglobin-like proteins in histotroph provide for specific concentration of steroid hormones
within the uterine lumen. By contrast, the predominant proteins in human endometrial secretions derive from serum (Hirsch, Fergusson & King, 1977) and contributions of uterine-specific proteins to this milieu appear to be insignificant (Voss & Beato, 1977). Furthermore, the ability of human histotroph proteins to bind steroids is similar to that of serum proteins (Voss & Beato, 1977), in accord with the low total uterine content of un conjugated steroids relative to protein in uterine washings in the present study. The average ratio of total progesterone/total protein in rabbit uterine flushings (near 67 pmol/mg; Fowler et al., 1977), in which uteroglobin constitutes about half of the total protein content (Krishnan & Daniel, 1967), exceeds our derived median value for human flushings by >500-fold. Our evidence for consistent steroid content of human uterine luminal fluids during the menstrual cycle is in accord with the earlier evidence for uniform and low levels of steroid-binding proteins in human uterine fluids (Maathuis & Aitken, 1978).

In the present study, DHEA sulphate and pregnenolone sulphate were the predominant steroids in washings of human uter i throughout the menstrual cycle, constituting a potentially important precursor pool for de-novo steroidogenesis by embryonic tissues before implantation. In apparent discord with this proposal, the median content of DHEA sulphate in uterine fluids between Days 15 and 20 after LMP (coincident with the preimplantation phase of cycles of conception) was lower ($P<0.05$) than the value for the remainder of the menstrual cycle, and no other measured component of uterine washings was more abundant during this 'preimplantation' period (Table 1). However, studies with domestic animals have established a capacity for preimplantation conceptus tissues to stimulate endometri al secretion (e.g. Geisert, Renegar, Thatcher, Roberts & Bazer, 1982). Human conceptus tissues may similarly effect accumulation, in histotroph, of steroid sulphoconjugates and of other endometrial products of potential significance to embryonic growth and development before implantation. With regard to the origins of steroid sulphates in human uterine washings, the ratios of levels of steroid conjugate to total protein in flushings in the present study (median value about 1.2 pmol/mg for pregnenolone sulphate, about 63 pmol/mg for DHEA sulphate) approximate average values for plasma during the menstrual cycle (1.9 and 96 pmol/mg respectively; Milewich et al., 1978; Peretti & Mappus, 1983; the protein content of human plasma being about 67 g/l). These results support the view that steroid components of human uterine fluids derive from plasma, and are not specifically accumulated in the uterine lumen during the menstrual cycle.

The occurrence of prolactin in human uterine flushings is of interest in view of claims that this protein hormone is synthesized by the human endometrium (Maslar & Riddick, 1979) and is present in cervical mucus (Sheth, Vaidya & Raiker, 1976). Prolactin can promote the survival and transport of spermatozoa within the uterus (Sheth et al., 1976) and may be involved in implantation (Maslar & Riddick, 1979). The presence of steroid hormones can enhance these actions (Nicoll, 1974). However, the median proportion of total protein represented by prolactin in uterine washes in the present study ($~0.12 \times 10^{-6}$) is of a similar order to the calculated value for blood plasma of women ($0.214 \times 10^{-6}$; from Sinha, Selby, Lewis & Vanderlaan, 1973). This parallel suggests that prolactin is not actively accumulated in intrauterine fluids of the non-pregnant woman, and corroborates the steroid data in indicating that human uterine fluid is a filtrate of plasma.

Technical support from A. Weiss, J. Chappel and N. Kosmadopoulos is gratefully acknowledged.

References


Fowler, R.E., Johnson, M.H., Walters, D.E. & Eager,


Received 7 November 1985