Medroxyprogesterone acetate does not perturb the profile of steroid metabolites in urine during pregnancy

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ABSTRACT

The plasma concentrations of medroxyprogesterone acetate (MPA) in 14 women administered the progestagen for threatened abortion during the first 6 weeks of pregnancy were measured by specific radioimmunoassay. Treatment (52 nmol orally every 6 h) was continued to 18 weeks of gestation. The mean plasma concentration of MPA rose rapidly during day 1 of treatment to 14·1 ± 1·84 nmol/l. It reached 21·5 ± 2·3 nmol/l by 7 days and subsequently stabilized at around 26·8 ± 5·0 nmol/l by the end of week 2. Urinary steroid profiles were determined by gas–liquid chromatography and mass spectrometry for six of the MPA-treated women and compared with those of six untreated women of similar gestational age. No differences were detected between the two groups of women, suggesting that the administration of MPA during pregnancy did not alter qualitatively or quantitatively the metabolism and excretion into urine of progesterone and oestrogens.

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INTRODUCTION

The efficacy of progestagen support therapy in the form of either progesterone or synthetic progestagens in reducing the rate of spontaneous abortions remains controversial (Shearman & Garrett, 1963; Goldzieher, 1964; Klopper & MacNaughton, 1965; Jones, Aksel & Wentz, 1974; Soules, Wiebe, Aksel & Hammond, 1977; Fainstat & Bhat, 1983; Tognoni, Ferrario, Inzalaco & Crosignani, 1983). Progesterone or medroxyprogesterone acetate (MPA) has been administered to women diagnosed as having inadequate luteal function, manifested either as a short luteal phase and/or slow rate of endometrial maturation (Dizerega & Ross, 1981). In these circumstances, luteal progesterone secretion may be less than optimal for successful implantation of the embryo and maintenance of pregnancy before the onset of placental progesterone synthesis (Dizerega & Ross, 1981).

In previous studies, it has been shown that the rate of fetal abnormality is unaffected by the administration of MPA to sub-fertile women during pregnancy (Burstein & Wasserman, 1964; Yovich, Stanger, Willcox & Michael, 1983). However, the plasma steroid profiles in a group of women who received oral MPA until week 18 of gestation for threatened abortion differed in the second half of pregnancy from women who did not threaten to abort and who did not receive MPA (D. L. Willcox, J. L. Yovich, S. C. McColm & L. H. Schmitt, unpublished results). From 20 to 40 weeks, progesterone and 17α-hydroxyprogesterone were significantly higher, and oestradiol-17β was significantly lower, in the women who were given the progestagen. Although MPA did not affect the plasma concentrations of these steroids in the first 20 weeks of pregnancy compared with untreated women, it may have altered their metabolism. In this particular study, we have measured the concentration of MPA in the plasma of women treated from weeks 4 to 18 of gestation and have compared their urinary steroid profiles with those of untreated women at similar gestational ages.

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MATERIALS, SUBJECTS AND METHODS

Chemicals

All chemicals were analytical grade and solvents were redistilled before use. New England Nuclear (Boston, MA, U.S.A.) supplied [6α-1,2,9(n)-^{3}H]methyl-17α-hydroxyprogesterone acetate (60 Ci/mmol), which was routinely repurified at 8-week intervals by thin-layer chromatography on silica gel using a solvent system of 1:1 (v/v) cyclohexane and ethyl acetate. Medroxyprogesterone acetate (6-methyl-17α-hydroxyprogesterone acetate) was provided by Upjohn Pty Ltd (Kalamazoo, MI, U.S.A.). Reference steroids were purchased from Sigma (St Louis, MO, U.S.A.), British Drug Houses (Sydney, New South Wales, Australia), Searle (Chicago, IL, U.S.A.), Makor Chemicals (Jerusalem, Israel) or Steraloids (Wilton, NH, U.S.A.). Methoxylamine hydrochloride and pyridine were purchased from Pierce (Rockford, IL, U.S.A.), Sylon BTZ (a 3:2:3 (by vol.) mixture of bis (trimethylsilyl) acetamide, trimethylchlorosilane and trimethylsilyl imidazole) from Supelco (Bellefonte, PA, U.S.A.), hexamethyldisilazane and alkanes from Applied Science Laboratories (Waltham, MA, U.S.A.), suc d'helix pomatia from Industrie Biologique Français (Clichy, France), Sep-Pak C18 cartridges from Waters Associates (Milford, MA, U.S.A.) and Lipidex-5000 from Packard (Downers Grove, IL, U.S.A.).

Subjects and sampling

Twenty pregnant women, ranging in age from 27 to 38 years and of parity ranging from 0 to 2, were recruited into this study. Their pregnancies followed treatment for infertility, and in each case hormonal monitoring through the ovarian cycle was carried out so that the day of the luteinizing hormone (LH) surge was known. Some patients conceived after in-vitro fertilization and the day of ovulation was considered to be the day of oocyte recovery or the day after the LH surge for those not having oocytes recovered. This enabled consistent gestational dating by nominating the last menstrual period as 14 days before ovulation. Fourteen patients were included because they were being given MPA tablets (Provera; Upjohn Pty Ltd, Sydney, Australia) as part of an ongoing trial to assess the role of the progestagen in preventing abortion in women conceiving from infertility treatments. The 14 women had presented with signs of threatened abortion between weeks 4 and 6, and were prescibed MPA (20 mg orally) four times daily until week 16 of gestation. From weeks 16 to 18 the dose was progressively reduced to zero. Blood samples (10 ml, heparinized) were taken from the antecubital vein of these women before the commencement of progestagen therapy and thereafter at intervals of 2, 4, 6 and 8 h, 1, 2 and 3 days and weekly for 5 weeks. During the first week, the women v hospitalized and MPA was administered by nursing staff. Samples of 24-h urine collections v obtained from six of the women receiving MPA from six additional women matched for age, infert treatment and gestational stage, but who had threatened to miscarry and were not receiving M (Table 1). Urine and plasma samples were store aliquots at −120 °C until analysis. The women e delivered a healthy infant at between weeks 37 an of gestation.

Plasma analysis

Antiserum raised in goats against an MP/ oxime–bovine serum albumin complex was supp by Dr K. T. Kirton (Upjohn, Kalamazoo, MI, U.S and has been shown to be specific for MPA (Corn Kirton & Duncan, 1971). Progesterone did not cr react significantly (<0·001%) in the MPA ra immunoassay. Medroxyprogesterone was extra from plasma and urine with efficiencies of 80 and respectively, using diethyl ether. Isolation of steroids by chromatography indicated that pu cation of the organic extracts of plasma or urine be assay was unnecessary. Regression analysis of values obtained by direct extraction on those obta after thin-layer chromatography on silica gel correlation coefficients of 0·97 and 0·95 respecti Solvent blanks were equivalent to <15 fmol/tube the limit of sensitivity of the assay was 80 fmol/ corresponding to a detection limit of 0·6 nmol/l. plasma and urine samples were each analysed in assay to avoid interassay variation. The intera coefficients of variation were 5·8% for plasma sam and 7·2% for urine samples.

Urine analysis

Stock solutions were prepared as follows. The inte standard, cholesteryl butyrate (100·0 mg) was solved in ethanol pyridine (10·0 ml) and kept frigerated. A solution of cyclohexane, pyridine hexamethyldisilazane in the ratio of 98:1:1 (C was prepared. Tetracosane (5 mg) and dotricacon (5 mg) were dissolved in CPH (100 ml).

Aliquots (5 ml) of urine diluted with 5 ml 0· acetate buffer, pH 4·5, were hydrolysed overnight gentle shaking at 37 °C using 50 µl suc d'helix pom solution (Jarrige, 1962). The hydrolysate was bro to pH 1·0 with 10 M-HCl and allowed to stand fo min. Sep-Pak C18 cartridges were washed methanol (5 ml) and water (5 ml). The urine hydr sates (10 ml) were filtered using gentle vaccum, cartridges washed with 5 ml water, and the steroi the hydrolysate eluted with 5 ml methanol. Cholest butyrate solution (50 µl) was added as an inte

TABLE 1. Clinical details of pregnant women whose urine was analysed by gas chromatography–mass spectrometry

Case no.	Age (years)	Infertility treatment	Week MPA started	Urine collected: Volume (ml)	Urine collected: Week of gestation
1	28	Ovulation induction	5	2100	8
2	30	Ovulation induction	—	1160	10
3	28	IVF	4	1980	9
4	27	IVF	—	2015	12
5	33	AID	5	3395	10
6	31	AID	—	1038	12
7	33	Ovulation induction	6	1575	11
8	34	Ovulation induction	—	1160	13
9	29	AIH	6	1555	15
10	28	AIH	—	980	16
11	27	IVF	5	2650	17
12	31	IVF	—	1860	17

MPA, medroxyprogesterone acetate; IVF, in-vitro fertilization; AI, artificial insemination; D, donor; H, husband.

standard and the solutions were evaporated to dryness in silylation tubes under a stream of nitrogen (Shackleton & Whitney, 1980). The residues were dissolved in 100 µl methoxylamine hydrochloride solution and heated to 100 °C for 30 min in Teflon-lined screw-capped tubes. Most of the pyridine present was evaporated and 100 µl Sylon BTZ solution was added, after which the mixture was maintained at 100 °C for another 2 h (Thenot & Horning, 1972). The methoxime-trimethylsilyl ether steroid derivatives were purified on Lipidex-5000 (Axelson & Sjövall, 1974). Lipidex-5000 was suspended in methanol, filtered and washed with CPH solution. Columns were prepared in Pasteur pipettes using 1 ml slurry and a plug of cotton wool. The CPH solution (100 µl) was added to the derivatized steroid mixtures and the solutions were applied to Lipidex columns. The columns were eluted with 2 ml CPH. The eluants were evaporated to dryness under nitrogen and redissolved in 1 ml of the alkane standards solution.

Gas chromatography (GC) was carried out using a Hewlett-Packard 5840 instrument fitted with a SGE OCI-3 on-column injector (Scientific Glass Engineering, Melbourne, Australia) and a flame ionization detector. Aliquots (1·0 µl) of steroid solutions were injected at 40 °C into a wide-bore cross-linked fused silica WCOT column (SGE BP-1; Scientific Glass Engineering; 0·3 mm internal diameter × 25 m) coated with methyl silicone fluid. The column was kept at 40 °C for 1·0 min, heated to 200 °C at 20 °C/min and then to 300 °C at 2 °C/min and maintained at 30(for 11 min using He carrier gas at a linear flow velo of 34 cm/s.

The chromatograph was programmed to trans peak retention times and areas to a laboratory c puter based on an LSI-11/23 microprocessor (Dig Equipment Corp., Maynard, MA, U.S.A.) wh retention indices and peak concentrations were culated using the programme SSERCH (Wilkins Hähnel & Hähnel, 1980). Combined GC and m spectrometry (GC–MS) was carried out using a Var 2740 gas chromatogram fitted with glass capill columns as above directly coupled to a Varian M 311 mass spectrometer (Varian MAT, 1975, 1977). ion source was an electron-impact type operatec 70 eV and 200 °C. Spectra were acquired repetitiv with a Varian Spectrosystem 100 MS. Steroids w identified by comparison of their mass spectra w those of reference steroids and with published d (Varian MAT, 1975, 1977).

Statistical analysis

The plasma concentrations of MPA were tes against time by one-way analysis of variance (Snede & Cochran, 1967). Differences between gas chroma graphic peak areas in the urinary steroid analysis control women and women receiving progesta treatment were assessed by Student's *t*-test (Snede & Cochran, 1967).

RESULTS

The women administered MPA ceased vaginal bleeding generally within 36 h. The plasma concentration of MPA rose rapidly during the first day of treatment. After 1 week it was 21·5 ± 2·3 (S.E.M.) nmol/l and by the end of week 2 it had reached 26·8 ± 5·0 nmol/l (Fig. 1). There were no significant differences in the plasma concentrations of MPA between 1 and 5 weeks ($F(10, 92) = 8{\cdot}23$) after the commencement of progestagen support therapy in individual women.

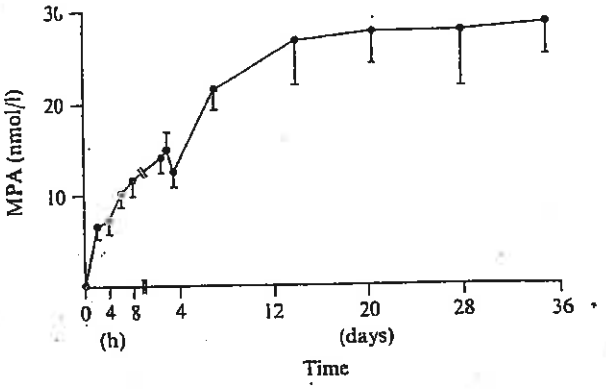

FIGURE 1. Profile of accumulation of medroxyprogesterone acetate (MPA) in the plasma of 14 pregnant women. Treatment was started between 4 and 6 weeks of gestation. Values are mean ± S.E.M.

Metabolic profiles of the urinary steroids in women with normal pregnancies were compared with those of pregnant women given MPA (Table 2). The steroids in urine were identified by comparison of their mass spectra and retention indices with data accumulated from reference steroids and with published data (Varian MAT, 1977). Typical urinary steroid profiles of a woman undergoing an uncomplicated pregnancy (Fig. 2*a*) and a woman treated with MPA (Fig. 2*b*) showed a complex pattern of steroids with pregnanediol (peak 20) as a prominent component. Androsterone (peak 1), etiocholanolone (peak 2), allopregnanediol (peak 19), 5α-pregnane-3α,17α,20β-triol (peak 21), pregnanetriol (peak 34) and 5β-pregnane-3α,11β,17α,21-tetrol-20-one (peak 39) were the other major steroid metabolites in the urine of both groups of women. There were some minor apparent differences between the profiles of women taking MPA compared to women who did not receive exogenous progestagen, but these differences were not statistically significant.

Medroxyprogesterone acetate and its principal urinary metabolite, 6α-methyl-4-pregnene-6β-21-diol-17-acetoxy-3,20-dione (Helmreich & Huseby, 1965), could not be detected in urine by GC or GC–MS. The

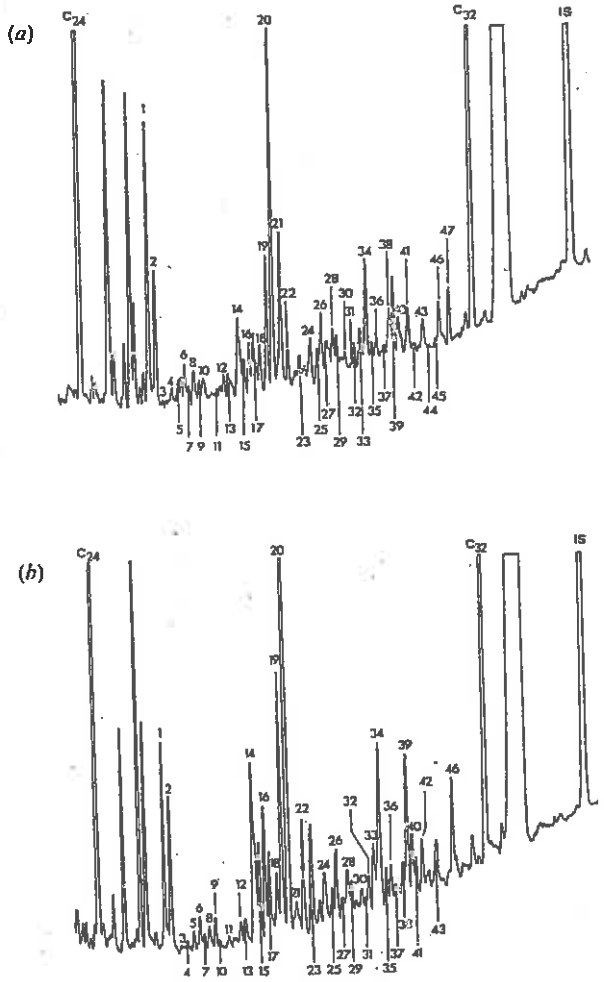

FIGURE 2. Capillary gas chromatographic profiles of methoxime-trimethylsilyl derivatized steroids isolated from 24-urine samples of pregnant women at 10 weeks of gestatior Profiles from (*a*) a woman receiving no progestagen suppc and (*b*) a woman treated orally with 80 mg medroxyprogesterone acetate daily from week 4 of pregnancy. IS, C_{24} and C_{32} denote cholesteryl butyrate, tetracosane and dotriacontane respectively, added as internal standards.

metabolite was not available as a reference standa but the mass fragmentation of the molecular ion cou be predicted confidently. Expected signals due to ic at mass : charge ratios of 605 (M-15) and 589 (M-: were not detected in the urine from women taki MPA. Medroxyprogesterone acetate was recover with 64% efficiency when 260 μmol were added to 5 urine from control women. However, the detecti limit of MPA by GC was approximately 250 nm whereas the concentration of MPA in the urine women who threatened to abort varied between and 39·2 nmol/l (Table 3). The MPA antiserum v

TABLE 2. Urinary gas chromatography–mass spectrometry (GC–MS) profile of 12 pregnant women whose gestational range was 8–17 weeks, six of whom were treated with medroxyprogesterone acetate (MPA)

Peak	Steroid	Amount excreted (μmol/24 h) Normal	MPA
1	Androsterone	7·5 (3·4–14·9)	9·8 (6·6–11·6)
2	Etiocholanolone	6·4 (3·7–14·9)	6·6 (4·2–8·6)
6	16α-Hydroxyetiocholanolone	17·4 (4·3–39·5)	16·7 (8·1–36·9)
7	Epiandrosterone[a]		
9	11-Oxoetiocholanolone	2·6 (0·3–6·0)	2·7 (1·3–5·0)
10	Androstenediol[a]		
14	11β-Hydroxyandrosterone	7·9 (3·9–13·1)	7·1 (2·8–14·5)
15	11β-Hydroxyetiocholanolone	2·5 (1·3–4·2)	3·2 (2·1–4·6)
16	Pregnanolone	8·1 (1·3–21·7)	5·5 (0·8–17·2)
17	5β-Pregnane-3β, 17α-diol-20-one	4·0 (1·6–6·7)	3·0 (0·3–6·0)
18	16α-Hydroxydehydroepiandrosterone		
19	Allopregnanediol	8·7 (2·2–8·0)	9·4 (4·1–18·7)
20	Pregnanediol	106·2 (32·2–220·3)	73·6 (30·4–105·9)
21	5α-Pregnane-3α,17α,20β-triol		
22	Pregnanetriol	5·0 (2·8–5·0)	6·8 (1·8–24·3)
23	16-Oxoandrostenediol		
24	Androstenetriol	5·1 (1·8–9·7)	3·3 (0·8–7·7)
25	5β-Pregnane-3α,20β-diol-11-one[a]	5·7 (0·3–24·4)	3·0 (2·4–4·6)
26	5α-Pregnane-3α,21-diol-20-one	4·6 (1·3–10·4)	4·7 (1·9–9·2)
27	Oestriol	11·6 (0·2–19·4)	5·4 (0·8–14·7)
28	5β-Pregnane-3β,16α,20β-triol[b]	2·6 (0·6–4·7)	2·4 (1·7–3·2)
32	16α-Hydroxypregnenolone	1·0 (0·9–1·2)	1·0 (0·4–1·7)
33	5α-Pregnane-3β,16α-diol-20-one	10·2 (3·7–20·4)	15·5 (11·6–18·1)
34	Pregnenetriol	4·8 (3·4–9·1)	7·0 (2·8–12·9)
35	5β-Pregnane-3α,21-diol-11,20-dione		
36	5α-Pregnane-3α,11β,21-triol-20-one		
37	5α-Pregnane-3β,16α,20α-triol		
39	5β-Pregnane-3α,11β,17α,21-tetrol-20-one	3·7 (1·8–7·0)	3·0 (1·5–6·6)
40	5α-Pregnane-3α,11β,17α,21-tetrol-20-one	1·1 (0·8–1·3)	1·0 (0·2–2·5)
41	Cortolone	5·4 (0·9–21·5)	1·8 (0·6–3·4)
46	5α-Pregnane-3β,20β,21-triol	6·4 (2·8–12·4)	4·3 (1·6–6·8)

Steroid peaks were characterized by comparing their retention indices during GC, and their fragmentation spectra during MS, with those of reference steroids. They were also compared to the spectra obtained from late-pregnancy urine (>30 weeks) in previous studies in this laboratory (Hähnel *et al.* 1982). When a peak comprised two components, only the major component is given. In two cases, identification was by retention index only (a), or by mass spectrometry only (b), due to the unavailability of suitable reference steroids. Blank entries indicate peaks either not detected or detected in only one or two samples; concentrations are given as the mean and range of five to six observations. An additional 16 peaks are of unknown identity.

TABLE 3. Daily excretion of medroxyprogesterone acetate (MPA) into the urine of women treated with MPA (Provera) for threatened abortion

Woman	Gestation (weeks)	Concentration (nmol/l)	Excretion (nmol/24 h)
A	17	24·4	64·7
B	9	19·8	31·2
C	11	25·7	40·0
D	9	39·2	82·3
E	10	31·0	61·4
F	11	9·1	30·9

highly specific (Cornette *et al.* 1971) so that metabolites of MPA, which may have been present in greater concentration than MPA, would not have been measured in the radioimmunoassay. Thus the cc centration of MPA and its metabolites in the urine the treated women was insufficient for detection GC.

DISCUSSION

The measurement of steroids in urine by GC–MS h been applied successfully in recent years to the qua tative and quantitative assessment of sterc metabolism (Trocha & D'Amato, 1978). T technique has been used in the diagnosis of patie with abnormal steroid biosynthesis (Curtius, Völlm Zagalak & Zachmann, 1975) and during pregnancy reveal steroid sulphatase deficiency as part of t

prenatal diagnosis of X-linked ichthyosis (Hähnel, Hähnel, Wysocki *et al.* 1982). In this study, the plasma concentration of MPA was maintained at 26·8 ± 5·0 nmol/l after 2 weeks in 14 women taking 207 nmol MPA daily between weeks 4 and 18 of pregnancy. However, 23 identified urinary steroids measured in six of these women did not differ significantly, either qualitatively or quantitatively, from those of six untreated women of similar gestational age (Fig. 2 and Table 2). Thus, it is likely that the administration of MPA did not disturb the metabolism of progesterone (peaks 17 and 20 in Table 1), oestrogens (especially oestriol, peak 27 in Table 1) and other steroids within either the maternal or feto-placental compartments between weeks 4 and 18 of gestation.

Radioimmunoassay of MPA in the urine of six of the MPA-treated women showed that 15–40% (mean of 25%, Table 3) of the total MPA ingested each day was excreted directly into urine. There are 11 known metabolites of MPA (Fukushima, 1979), of which four at least are excreted into urine in the form of glucuronides (Castegnaro & Sala, 1971). However, the metabolites have not been reported to date with the GC–MS technique and were not detected within the urinary steroid profiles of the women treated in this study with MPA. Our methodology was adequate for the hydrolysis and detection of conjugated steroids, including glucuronides, but the amounts of MPA and MPA metabolites were only about 10% of that required for analysis of unconcentrated urine.

It is known that a number of progestagens, including MPA, given during the luteal phase of the menstrual cycle will significantly depress plasma concentrations of progesterone (Johansson, 1971). This effect has been investigated *in vitro* using human corpora lutea from the menstrual cycle. Shinada, Yokota & Igarashi (1978) showed that MPA and other synthetic progestagens, as well as progesterone itself, inhibited competitively the conversion of pregnenolone to progesterone by the 3β-hydroxysteroid dehydrogenase/isomerase systems. Medroxyprogesterone acetate treatment introduced during the luteal phase and continued into the first trimester of pregnancies achieved by in-vitro fertilization caused reduced serum concentrations of progesterone and oestradiol-17β during the latter half of the luteal phase, rising to normal values during pregnancy (Yovich, Stanger, Yovich & Tuvik, 1984). The recovery to normal plasma concentrations of progesterone and oestradiol-17β presumably occurred in response to circulating human chorionic gonadotrophin (hCG), since hCG is known to reverse the suppressive effect of exogenous progestagens on progesterone synthesis (Johansson, 1971). Thus, progestagen support in the form of MPA to women at week 4 or more of pregnancy, when hCG synthesis is substantial, does not appear to ovarian steroidogenesis.

Our findings do not exclude the possibility embryopathic action of MPA, although it ha observed clinically that the rate of fetal abnorm unaffected by the administration of MPA in earl nancy (Burstein & Wasserman, 1964; Yovich 1983). However, we have shown that the meta of ovarian, placental and adrenal steroids is una by treatment with MPA up to week 18 of ges This provides evidence of the safety of proge support by MPA in early pregnancy, even thou efficacy of progestagen support to reduce fetal w remains unproven.

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REFERENCES

Axelson, M. & Sjövall, J. (1974). Separation and computeris chromatography–mass spectrometry of unconjugated neu steroids in plasma. *Journal of Steroid Biochemistry* 5, 733–

Burstein, R. & Wasserman, H. C. (1964). The effect of Prove the foetus. *Obstetrics and Gynecology* 23, 931–934.

Castegnaro, E. & Sala, G. (1971). Pharmacokinetics and met lism of medroxyprogesterone acetate, influence of the rout administration and its physical stage. *Steroidologia* 2, 13–2

Cornette, J. C., Kirton, K. T. & Duncan, G. W. (1971). Mea ment of medroxyprogesterone acetate (Provera) by radioir munoassay. *Journal of Clinical Endocrinology and Metabo.* 33, 459–466.

Curtius, H. Ch., Völlmin, J., Zagalak, M. J. & Zachmann, M (1975). Gas chromatography of steroids and its clinical ap tions, including loading tests with deuterated compounds. *Journal of Steroid Biochemistry* 6, 677–684.

Dizerega, G. S. & Ross, G. T. (1981). Luteal phase dysfunctic *Clinics in Obstetrics and Gynaecology* 8, 733–751.

Fainstat, T. & Bhat, N. (1983). Recurrent abortion and prog one therapy. In *Progesterone and progestins*, pp. 259–276. I C. W. Bardin, E. Milgrom & P. Mauvais-Jarvis. New York Raven Press.

Fukushima, D. (1979). Isolation and partial synthesis of a ne metabolite of medroxyprogesterone acetate. *Steroids* 34, 57

Goldzieher, J. W. (1964). Double-blind trial of a progestin in habitual abortion. *Journal of the American Medical Associc* 188, 651–654.

Hähnel, R., Hähnel, E., Wysocki, S. J., Wilkinson, S. P. & H A. (1982). Prenatal diagnosis of X-linked ichthyosis. *Clinic Chimica Acta* 120, 143–152.

Helmreich, M. L. & Huseby, R. A. (1965). Factors influencin absorption of medroxyprogesterone acetate. *Steroids* Supp 79–85.

Jarrige, P. (1962). Purification et propriétés des sulphatases d digestif d'helix pomatia. *Thèse de Docteur ès sciences nature* Paris.

Johansson, E. D. B. (1971). Depression of the progesterone le in women treated with synthetic gestagens after ovulation. *Endocrinologica* 68, 779–792.

Jones, G., Aksel, S. & Wentz, A. C. (1974): Serum progesterone values in luteal phase defects. *Obstetrics and Gynecology* **44**, 26–34.

Klopper, A. & MacNaughton, M. (1965). Hormones in recurrent abortion. *Journal of Obstetrics and Gynaecology of the British Commonwealth* **72**, 1022–1028.

Shackleton, C. H. L. & Whitney, J. O. (1980). Use of SEP-PAK cartridges for urinary steroid extraction: evaluation of the method for use prior to gas chromatographic analysis. *Clinical Chimica Acta* **107**, 231–243.

Shearman, R. P. & Garrett, W. J. (1963). Double-blind study of the effect of 17-hydroxyprogesterone caproate on abortion rate. *British Medical Journal* **1**, 292–295.

Shinada, T., Yokota, Y. & Igarashi, M. (1978). Inhibitory effect of various gestagens upon the pregnenolone 3β-ol-dehydrogenase 5-4-isomerase system in human corpora lutea of menstrual cycles. *Fertility and Sterility* **29**, 84–87.

Snedecor, G. W. & Cochran, W. G. (1967). *Statistical methods*, edn 6. Ames, Iowa: Iowa State University Press.

Soules, M. R., Wiebe, R. H., Aksel, S. & Hammond, C. B. (1977). The diagnosis and therapy of luteal phase deficiency. *Fertility and Sterility* **28**, 1033–1037.

Thenot, J. P. & Horning, E. C. (1972). Methoxime-trimethylsilyl derivatives of human urinary steroids for GC and GC–MS studies. *Analytical Letters* **5**, 21–33.

Tognoni, G., Ferrario, L., Inzalaco, M. & Crosignani, P. (
Controlled clinical trials and medical practice: the case f
progestogens in threatened abortion. In *Progesterone an
tins*, pp. 127–131. Eds C. W. Bardin, E. Milgrom & P. M
Jarvis. New York: Raven Press.

Trocha, P. & D'Amato, N. A. (1978). Method for screenin
steroids by gas chromatography. *Clinical Chemistry* **24**,

Varian MAT, Bremen, F. R. G. (1975). Libraries of mass s
on magnetic tape: library 'A' from EPA and NIH, Beth
MD, U.S.A.

Varian MAT, Bremen, F. R. G. (1977). The Mass Spectro
Data Centre, Atomic Weapons Research Establishment
maston, Reading, Berks.

Wilkinson, S. P., Hähnel, R. & Hähnel, E. (1980). A comp
library search program for steroid profiles by capillary g
matography. *Australian and New Zealand Journal of Ob
and Gynaecology* **20**, 193.

Yovich, J. L., Stanger, J. D., Willcox, D. L. & Michael, C.
(1983). Medroxyprogesterone in in-vitro fertilization. *L
711.

Yovich, J. L., Stanger, J. D., Yovich, J. M. & Tuvik, A. I. (
Assessment and hormonal treatment of the luteal phase
vitro fertilization cycles. *Australian and New Zealand Jo
Obstetrics and Gynaecology* **24**, 125–130.