Human Reproduction vol.9 no.1 pp.41–48, 1994

Regulation of corpus luteum function

M.R.Johnson[1], A.A.Abbas[2], R.Irvine, A.F.Riddle[3], J.Q.Norman-Taylor[4], J.G.Grudzinskas[4], W.P.Collins[3] and K.H.Nicolaides[2]

The Department of Obstetrics and Gynaecology, The Chelsea and Westminster Hospital, Fulham Road, London SW10 9NH, [3]The Department of Obstetrics and Gynaecology and [2]The Harris Birthright Centre for Research into Fetal Medicine, King's College School of Medicine and Dentistry, Denmark Hill, London SE5 8RX and [4]Academic Unit of Obstetrics and Gynaecology, The Royal London Hospital, Whitechapel, London E1 1BB, UK

[1]To whom correspondence should be addressed

The effects have been studied of different ovulation induction regimens [either clomiphene citrate or buserelin in combination with human menopausal gonadotrophin (HMG)] on the circulating concentrations of progesterone, oestradiol, relaxin and human chorionic gonadotrophin (HCG) during the first trimester of pregnancy. Ovulation induction with clomiphene resulted in elevated concentrations of gonadotrophins in both phases of the cycle, while during ovulation induction with buserelin, gonadotrophin concentrations were elevated in the follicular phase only. The concentrations of all corpus luteum products were greater in clomiphene pregnancies compared with spontaneous pregnancies, but only oestradiol and relaxin concentrations were greater in clomiphene pregnancies compared with buserelin pregnancies. The concentrations of HCG were significantly reduced in clomiphene pregnancies compared to natural pregnancies. Relaxin concentrations were significantly higher from 7 weeks gestation in buserelin compared with spontaneous pregnancies, while progesterone, oestradiol and HCG concentrations were not consistently different. Consistent associations were found between relaxin and HCG concentrations in spontaneous pregnancies and between the concentrations of relaxin and both progesterone and oestradiol in pregnancies achieved after ovulation induction. These data suggest that (i) given the similarity in the circulating concentrations of HCG, the relatively lower circulating gonadotrophin concentrations during the luteal phase of the cycle of conception result in reduced circulating concentrations of oestradiol and relaxin; while in the case of relaxin this effect is partially reversible, there is no evidence that this is so for oestradiol; (ii) synthesis of progesterone in the corpus luteum is less affected by lower concentrations of gonadotrophins during the luteal phase; (iii) ovulation induction with clomiphene results in pregnancies with lower concentrations of HCG, suggesting that trophoblast function may be impaired; and (iv) corpus luteum function is linked with placental steroidogenesis.

Key words: buserelin/clomiphene citrate/corpus luteum/ovulation induction/pregnancy

Introduction

The corpus luteum is considered to be redundant after 6–7 weeks gestation (Csapo and Pulkkinen, 1978). However, prior to this time, corpus luteum dysfunction is thought to be one of the many causes of early pregnancy loss through reduced concentrations of progesterone. Studying corpus luteum function during early pregnancy with the measurement of steroid concentrations alone is complicated by the ever increasing placental contribution to the circulating pool. Even 17-hydroxyprogesterone, once held to be the product of corpus luteum alone (Yoshimi *et al.*, 1969; Tulchinsky and Hobel, 1973), has now been shown to be synthesized and released by the developing placenta in considerable amounts (Nowroozi *et al.*, 1991). As implied above, placental steroid synthesis must be capable of supporting a pregnancy from between 6 and 7 weeks (Csapo and Pulkkinen, 1978), but the exact time of the luteo-placental shift has been difficult to define, and probably varies between pregnancies and steroids studied. Certainly, the placenta seems to become the dominant source of oestradiol earlier than it does for progesterone in in-vitro fertilization (IVF) pregnancies (Johnson *et al.*, 1993a). Moreover, the timing of the luteo-placental shift is likely to be of critical importance only when the life of the corpus luteum is reduced or placental development impaired.

In addition to oestradiol and progesterone, the corpus luteum produces the peptide relaxin. This hormone is thought to play a role in the modulation of uterine contractility and cervical compliance at term (Johnson *et al.*, 1992). Circulating relaxin during pregnancy is derived solely from the corpus luteum (Johnson *et al.*, 1991a). Thus relaxin concentration may provide a more accurate marker of corpus luteum function. However, during the menstrual cycle, relaxin concentration is strongly correlated with oestradiol but not progesterone concentration (Johnson *et al.*, 1993b). Furthermore, in naturally conceived pregnancies, progesterone synthesis by the corpus luteum has been shown to peak at ~8 weeks gestation and to decline sharply thereafter (Yoshimi *et al.*, 1969; Tulchinsky and Hobel, 1973), while the relaxin peak tends to occur later at between 12 and 14 weeks (Johnson *et al.*, 1993c). Thus, it seems that differences may exist in the regulation of the synthesis of these compounds.

Ovulation induction for IVF may be achieved using exogenous gonadotrophins in combination with either clomiphene citrate or a gonadotrophin-releasing hormone agonist, the former providing

higher concentrations of gonadotrophins in both phases of the menstrual cycle and the latter only in the follicular phase. Thus, by comparing the concentrations of progesterone, oestradiol and relaxin during the first trimester of pregnancies achieved naturally or following ovulation induction using one of these regimens, the objectives were: (i) to investigate the factors which are important in the regulation of corpus luteum function in early pregnancy; (ii) to assess the association between concentrations of progesterone, oestradiol and relaxin to establish the time of the luteo-placental shift; and (iii) to establish whether relaxin may be a useful indicator of steroid synthesis by the corpus luteum. In addition, in order to assess the early luteotrophic stimulus of each pregnancy and to have an estimate of placental function in the different types of pregnancy, human chorionic gonadotrophin (HCG) was measured in the same samples.

Materials and methods

Patients

Three groups of women were recruited: (i) subjects from the Fertility Clinic of the Royal London Hospital with naturally conceived singleton pregnancies (n = 18, age range 26–37 years, median 31 years); (ii) singleton (n = 52) and twin pregnancies (n = 22) achieved following ovulation induction with clomiphene citrate (Clomid; Merrel Dow Pharmaceuticals Ltd, Uxbridge, Middlesex, UK) 100 mg orally, days 2–6 of the menstrual cycle and human menopausal gonadotrophin (HMG; Pergonal, Serono Laboratories UK Ltd, Welwyn Garden City, Herts, UK) and IVF/embryo transfer (age range 22–39 years, median 32 years) (Sharma *et al.*, 1988); (iii) singleton (n = 17) and twin pregnancies (n = 14) achieved following pituitary desensitization with buserelin acetate (Suprafac; Hoescht UK Ltd, Hounslow, Middlesex, UK), ovulation induction with HMG and IVF/embryo transfer (age range 22–41 years, median 34 years) (Waterstone and Parsons, 1992). Both ovulation induction groups were recruited following a positive pregnancy test from the Assisted Conception Unit, King's College Hospital. Blood samples were obtained at weekly intervals, serum from the clomiphene pregnancies and plasma from the natural and buserelin pregnancies. The number of oocytes collected from each patient was assessed at the time of oocyte retrieval.

Assays

Progesterone and oestradiol were extracted from the samples with diethyl ether and measured by radioimmunoassay using tritiated antigens and monoclonal antibodies to P-11 α-succinyl-bovine serum albumin (BSA) and oestradiol-6-carboxymethyl oxime-BSA respectively. The samples were diluted to check for parallelism against the dose–response curve and analysed in batches with appropriate quality control. HCG was measured by a non-competitive fluoroimmunoassay (Pharmacia Wallac, Milton Keynes, UK). The precision (intra- and inter-assay coefficients of variation) for these assays, over the period of study, were <10%.

The concentration of relaxin was measured in unextracted plasma or serum by a non-competitive enzyme labelled immunoassay (Ferrailo *et al.*, 1991). The standards, from 1 to 1250 pg/ml, were prepared in pooled normal male plasma or serum. The working range of the assay (defined as the lowest and highest value with a coefficient of variation of <10%) was 35–1100 pg/ml. For plasma samples the inter- and intra-assay variations at a concentration of 500 pg/ml were 12.1 and 5.2% respectively; all serum samples were assayed in one batch, the intra-assay variation was 5.1% at 400 pg/ml. The cross-reactivity with insulin, nerve growth factor and porcine relaxin was <0.1%.

Statistical methods

The data were not normally distributed and comparisons were made between concentrations of substances analysed in different groups at the same time points, using a Mann–Whitney U test. Associations between concentrations of the substances analysed were assessed by a simple regression analysis.

Results

Natural versus clomiphene citrate pregnancies

Progesterone, oestradiol and relaxin concentrations were significantly higher in clomiphene than in naturally conceived pregnancies: for progesterone this occurred in weeks 5–12 (P = 0.05–0.0001); for oestradiol in weeks 5–10 (P = 0.003–0.0004); and for relaxin in weeks 4–14 (P = 0.009–0.0001) (Figure 1a–c). HCG concentrations were lower in the clomiphene pregnancies (P < 0.05) in weeks 9, 10, 11, 13 and 14 (Figure 1d).

Natural versus buserelin acetate pregnancies

Progesterone concentrations were higher in buserelin than in naturally conceived pregnancies throughout the first trimester, but these differences were only significant in weeks 7, 9 and 11 (P = 0.04–0.02) (Figure 2a). Oestradiol concentrations seemed higher in buserelin than in naturally conceived pregnancies but were only significantly so in week 5 (P = 0.03) (Figure 2b). Relaxin concentrations were similar in both groups until week 7, from which time they were significantly higher in buserelin pregnancies (weeks, 7, 9, 10, 11, 13 and 14; P = 0.02–0.0002) (Figure 2c). There were no differences in HCG concentration in buserelin and naturally conceived pregnancies (Figure 2d).

Clomiphene citrate versus buserelin acetate pregnancies

Progesterone concentrations were similar in clomiphene and buserelin pregnancies (Figure 3a), but oestradiol concentrations were consistently higher in the clomiphene pregnancies (weeks 5, 6, 8, 10 and 12; P = 0.01–0.0009) (Figure 3b). Relaxin concentrations were higher in clomiphene pregnancies (weeks 4, 5, 6, 8, 10 and 11; P = 0.04–0.001) (Figure 3c). HCG concentrations appeared higher in the buserelin pregnancies, but at no time point was the difference statistically significant (Figure 3d).

Singletons versus twins following ovulation induction

Progesterone concentrations were higher in twin than in singleton pregnancies in weeks 5, 6, 8 and 10–13 (P = 0.03–0.0008) (Figure 4a). Similarly for oestradiol, concentrations were the higher in twin pregnancies from weeks 5 to 14 (P = 0.03–0.0001) (Figure 4b). There was no difference in the relaxin

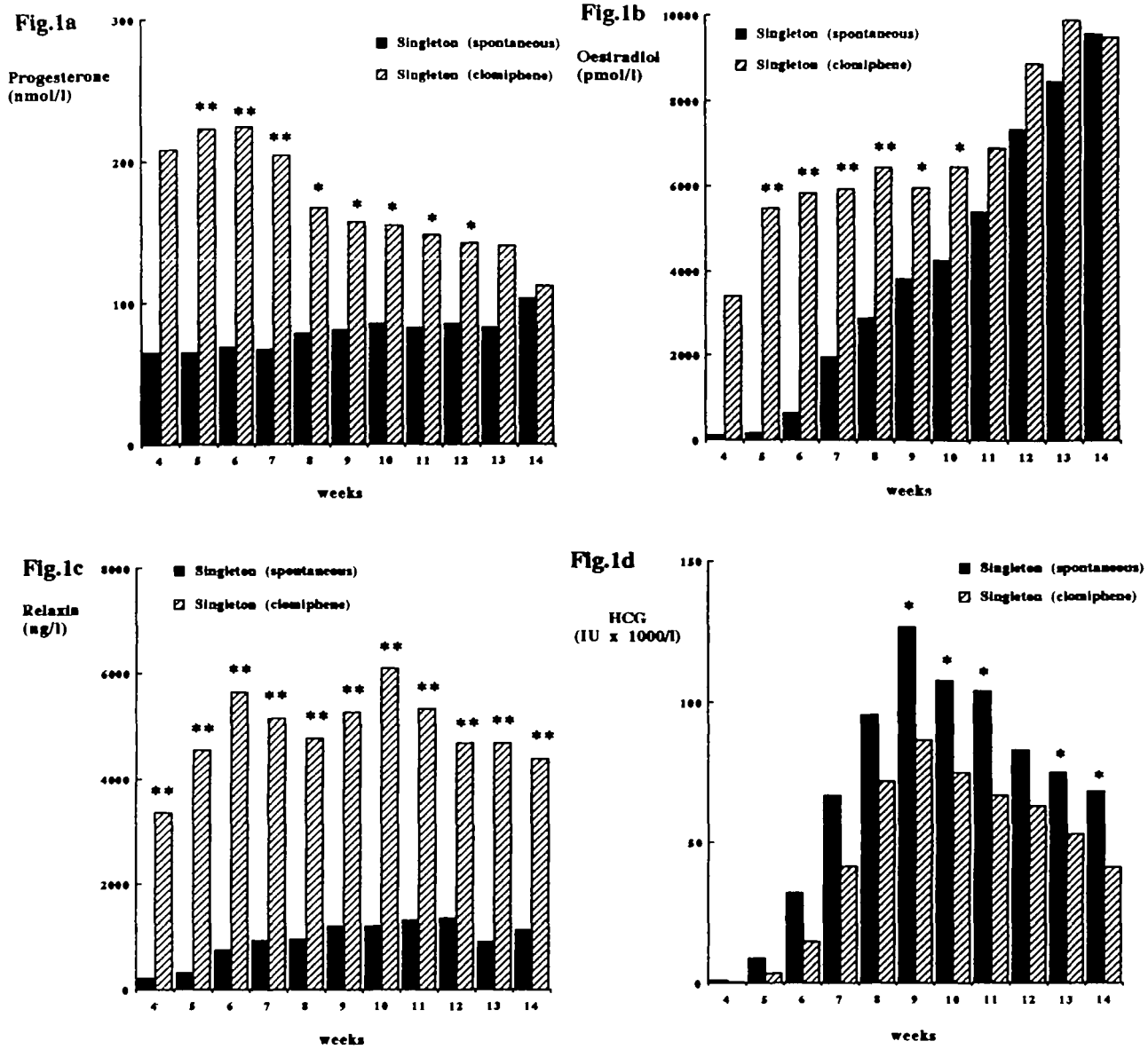

Fig. 1. The geometric mean of (**a**) progesterone, (**b**) oestradiol, (c) relaxin and (**d**) human chorionic gonadotrophin (HCG) in patients who became pregnant either spontaneously or following ovulation induction with human menopausal gonadotrophin and clomiphene citrate. * Denotes a difference of $P < 0.05$ and ** a difference of $P < 0.01$ in the circulating concentrations of the substances analysed.

concentrations (Figure 4c). HCG concentrations were higher in twin pregnancies from weeks 4 to 14 ($P = 0.03-0.0001$) (Figure 4d).

Correlations

Natural pregnancies

There were no consistent associations between the concentrations of HCG and either progesterone or oestradiol, although the concentrations of HCG and relaxin were associated in weeks 5–11 ($r = 0.47-0.72$); and relaxin concentrations were associated with those of progesterone in weeks 5, 7, 8, 9 and 12 ($r = 0.5-0.875$), and with those of oestradiol in weeks 5, 7, 10 and 11 ($r = 0.44$, 0.56, 0.47 and 0.38 respectively) (Figure 5a).

Clomiphene citrate pregnancies

There were no consistent associations between the concentrations of HCG and those of progesterone, oestradiol or relaxin over the same time points until both oestradiol and progesterone had become predominantly placental in origin (for oestradiol weeks 7–13, $r = 0.3-0.51$; for progesterone weeks 11–13, $r = 0.3-0.86$), these data were reported previously (Johnson *et al.*, 1993a) and are not shown. Relaxin concentration was correlated with that of progesterone in weeks 4 and 6–11 ($r = 0.45-0.82$) and with oestradiol in weeks 4, 6 and 8 ($r = 0.4-0.87$) (Figure 5b).

Buserelin pregnancies

There were no consistent associations between the concentrations of HCG and those of progesterone, oestradiol or relaxin at the same time points until both oestradiol and progesterone had become predominantly placental in origin (for progesterone week 12, $r = 0.65$; for oestradiol weeks 6, 8, 10–12, $r = 0.43-0.69$; data not shown). Relaxin concentration was correlated with that of progesterone in weeks 4–12 ($r = 0.5-0.78$) and with oestradiol in weeks 4, 5 and 7 ($r = 0.4-0.75$) (Figure 5c).

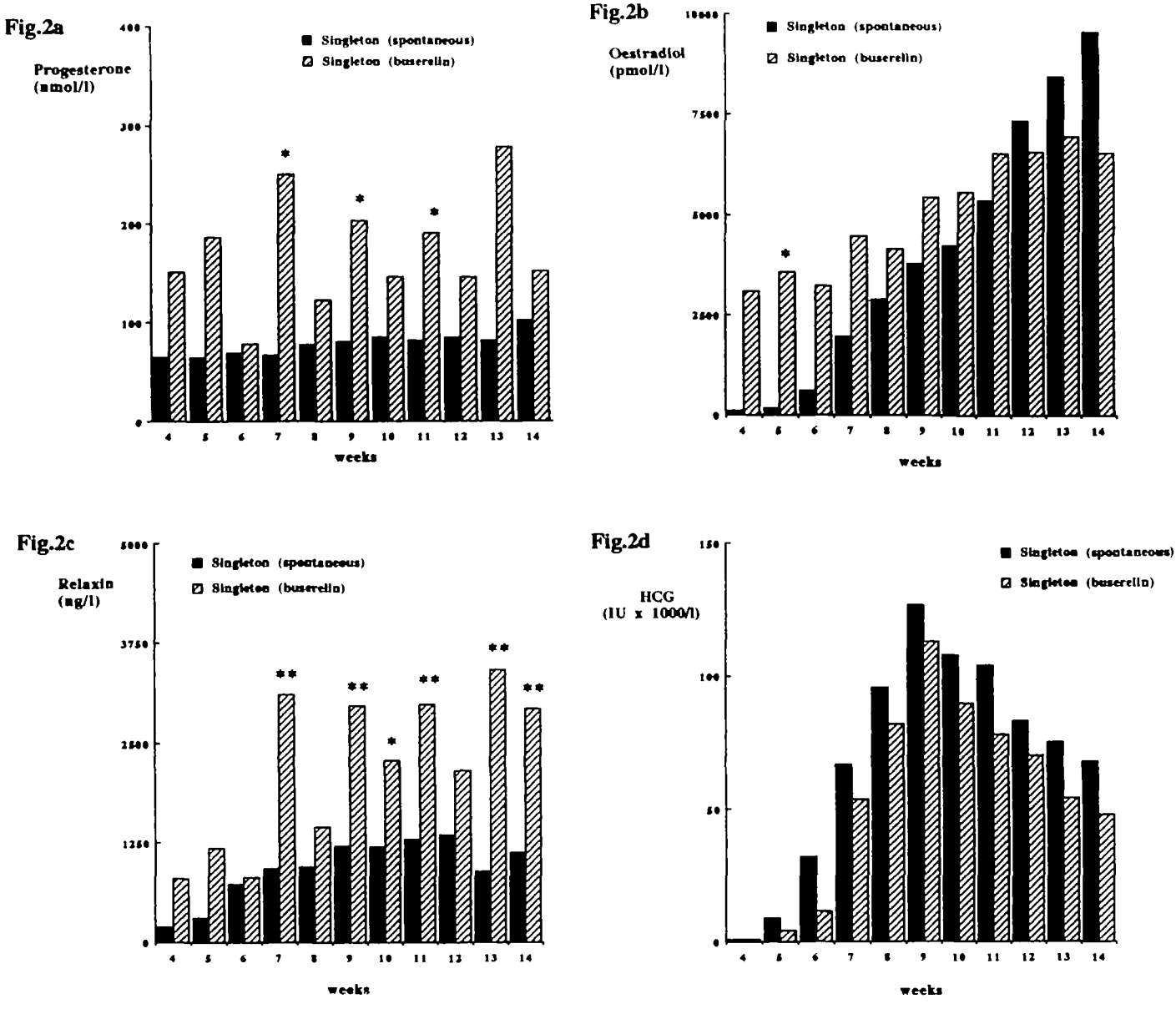

Fig. 2. The geometric mean of (a) progesterone, (b) oestradiol, (c) relaxin and (d) human chorionic gonadotrophin (HCG) in patients who became pregnant either spontaneously or following ovulation induction with human menopausal gonadotrophin and buserelin acetate. * Denotes a difference of $P < 0.05$ and ** a difference of $P < 0.01$ in the circulating concentrations of the substances analysed.

Combination of all groups

There were no consistent associations between the concentration of HCG and those of progesterone, oestradiol or relaxin at the same time points, although relaxin concentration was correlated with progesterone concentration in weeks 4 and 6–12 ($r = 0.48–0.69$) and with oestradiol in weeks 4–8 ($r = 0.42–0.78$) and in weeks 11 and 12 ($r = 0.55$ and 0.66, respectively) (Figure 5d).

Oocyte numbers

In the singleton clomiphene pregnancies, the range of oocyte numbers collected was 3–9 with a median of 4, while for the singleton buserelin pregnancies, the range was 3–29 with a median of 14 ($P = 0.0001$).

Discussion

Ovulation induction using buserelin and HMG resulted in greater numbers of oocytes than the combination of clomiphene and HMG. Thus, it might be expected that the circulating concentrations of the products of the corpus luteum would be higher in buserelin pregnancies than in clomiphene pregnancies. However, while the values for progesterone were similar, those for oestradiol and relaxin were significantly lower in the buserelin pregnancies. As HCG concentrations were similar in clomiphene and buserelin pregnancies, the likely explanation for these differences lies in the gonadotrophin concentrations in the luteal phase of the two ovulation induction regimens. Gonadotrophins in the luteal phase of buserelin pregnancies are reduced (Smitz *et al.*, 1990) and may result either in the involution of some of the multiple corpora

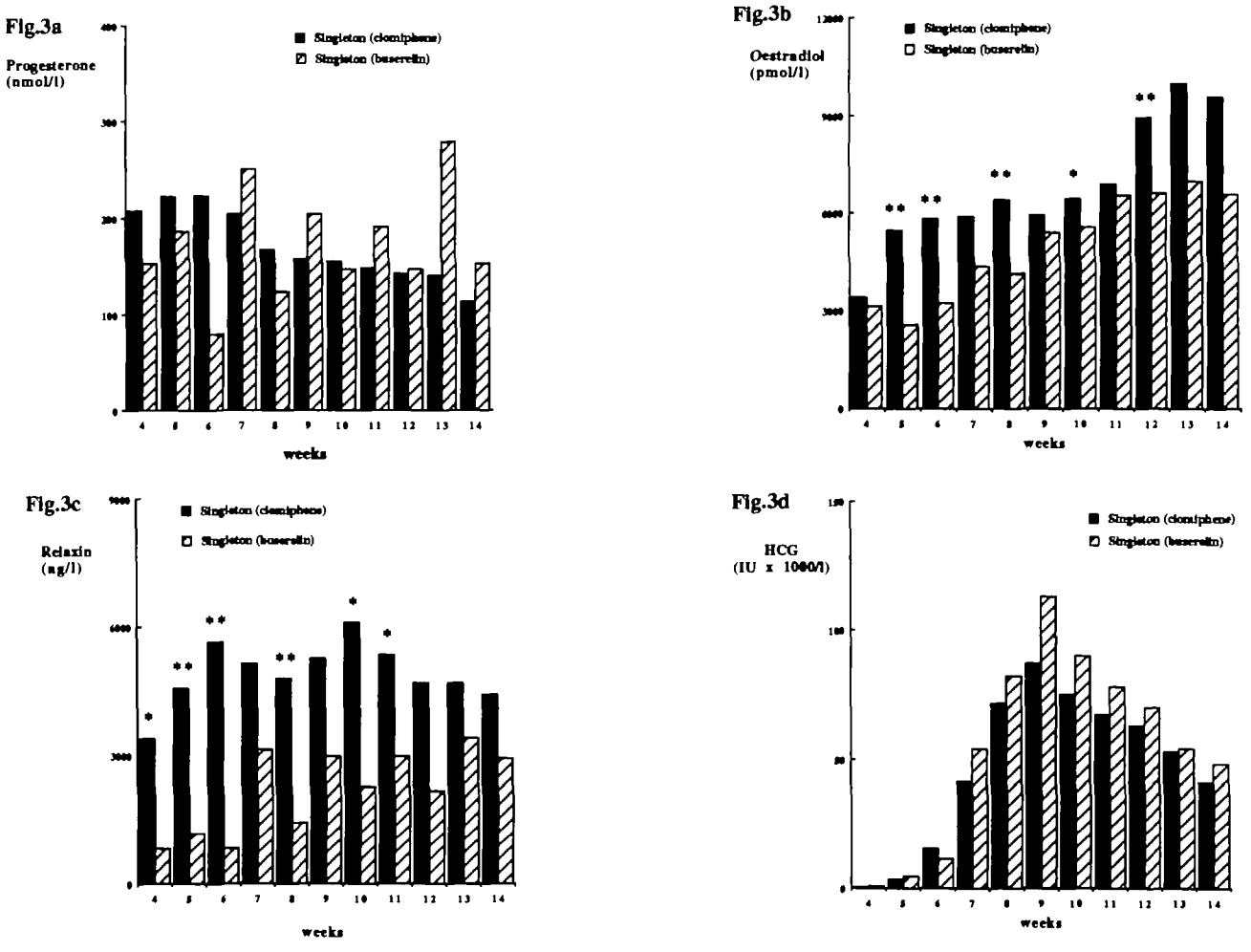

Fig. 3. The geometric mean of (**a**) progesterone, (**b**) oestradiol, (**c**) relaxin and (**d**) human chorionic gonadotrophin (HCG) in patients who became pregnant following ovulation induction with human menopausal gonadotrophin and either clomiphene citrate or buserelin acetate. * Denotes a difference of $P < 0.05$ and ** a difference of $P < 0.01$ in the circulating concentrations of the substances analysed.

lutea or in a reduction of the synthetic and/or secretory capacity of each corpus luteum. In contrast, the concentrations of luteinizing hormone (LH) are greater in the luteal phase of clomiphene cycles than in the natural menstrual cycle (Archer *et al.*, 1989). The primate corpus luteum is able to withstand periods of gonadotrophin withdrawal, shown experimentally in monkeys (Hutchinson and Zeleznik, 1985). Indeed, in terms of progesterone, corpus luteum function was similar in both clomiphene and buserelin pregnancies, while concentrations of oestradiol and relaxin were reduced in buserelin pregnancies. While the higher concentrations of oestradiol in clomiphene pregnancies may be a result of the recruitment of follicles during the luteal phase, those of relaxin are derived only from luteinized granulosa cells. Thus it seems unlikely that this is the explanation for the concentration differences of oestradiol and relaxin in clomiphene and buserelin pregnancies. Therefore, one may conclude that the relative importance of luteal phase gonadotrophin maintenance of corpus luteum function is least for progesterone and of greater but equal importance for relaxin and oestradiol.

The effect of the luteotrophic stimulus of pregnancy is difficult to assess for progesterone and oestradiol, as the placental contribution to the concentrations of each is impossible to define. Assuming that placental steroid production increases progressively with gestation, then the decline in the circulating concentrations of progesterone suggests that the ovary remains the predominant source of progesterone. In contrast, the increase in the circulating concentrations of oestradiol may be derived either from the ovary or, more probably (in view of the early associations between circulating HCG and oestradiol, and the decreasing progesterone concentrations which suggest a reduction in ovarian steroidogenesis) from the placenta. Nevertheless, the higher concentrations of oestradiol in clomiphene compared with buserelin pregnancies, when placental function (as assessed by HCG production) is similar in the two groups, suggest that the ovaries continue to produce a significant amount of oestradiol. However, the effect of HCG on corpus luteum production of oestradiol is uncertain (Wilks and Noble, 1983). Thus, ovarian oestradiol production may be determined in the cycle of conception or be regulated by another pregnancy-related factor, as has been suggested previously (Johnson *et al.*, 1993d). That ovarian steroidogenesis is regulated by a conceptus-derived/regulated factor is shown by the higher concentrations of progesterone and

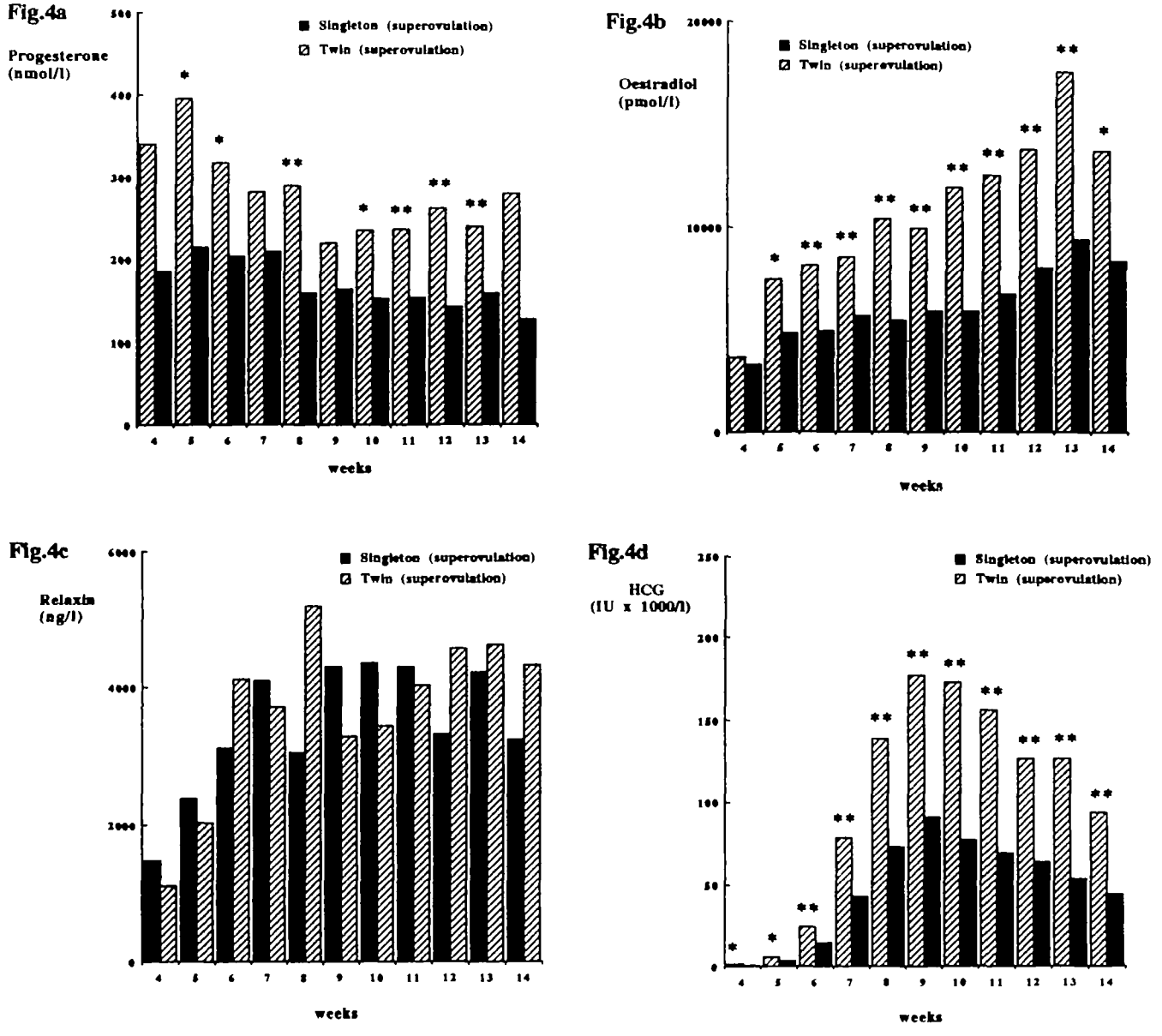

Fig. 4. The geometric mean of (a) progesterone, (b) oestradiol, (c) relaxin and (d) human chorionic gonadotrophin (HCG) in patients who became pregnant with a singleton or twin pregnancy following ovulation induction with human menopausal gonadotrophin and either clomiphene citrate or buserelin acetate. * Denotes a difference of $P < 0.05$ and ** a difference of $P < 0.01$ in the circulating concentrations of the substances analysed.

oestradiol from as early as 5 weeks in twin pregnancies. In contrast, there is no discernible difference in the circulating concentrations of relaxin in twin and singleton pregnancies, suggesting that gonadotrophin concentrations during the cycle of conception are of greater importance in the determination of the circulating concentrations of relaxin during the first trimester, than those of HCG. Initially, however, circulating relaxin concentrations in buserelin pregnancies are similar to those of naturally conceived pregnancies and significantly lower than in clomiphene pregnancies; with time they rise to be greater than those of naturally conceived pregnancies and similar to those of clomiphene pregnancies. The relative increase in circulating relaxin probably reflects rescue of suppressed corpus luteum function induced by the relatively low concentration of LH in the luteal phase of buserelin ovulation induction cycles. Thus, ovarian relaxin synthesis does respond to HCG, but the response is delayed. Indeed, in natural conceptions a consistent association was identified between HCG and relaxin, which supports earlier studies that reported an association between the mean concentrations of these compounds (Johnson *et al.*, 1993c), and an increase in relaxin in response to the administration of exogenous HCG (Quagliarello *et al.*, 1980; Johnson *et al.*, 1991b).

The lower HCG in the clomiphene group of pregnancies suggests that trophoblast function is impaired in these pregnancies. Indeed, the greater incidence of low birth weight babies conceived following ovulation induction and IVF/embryo transfer suggests that placental function may not be normal in these pregnancies (MCR working party, 1990). Whether this is due to the poor reproductive function of infertile women (Williams *et al.*, 1991),

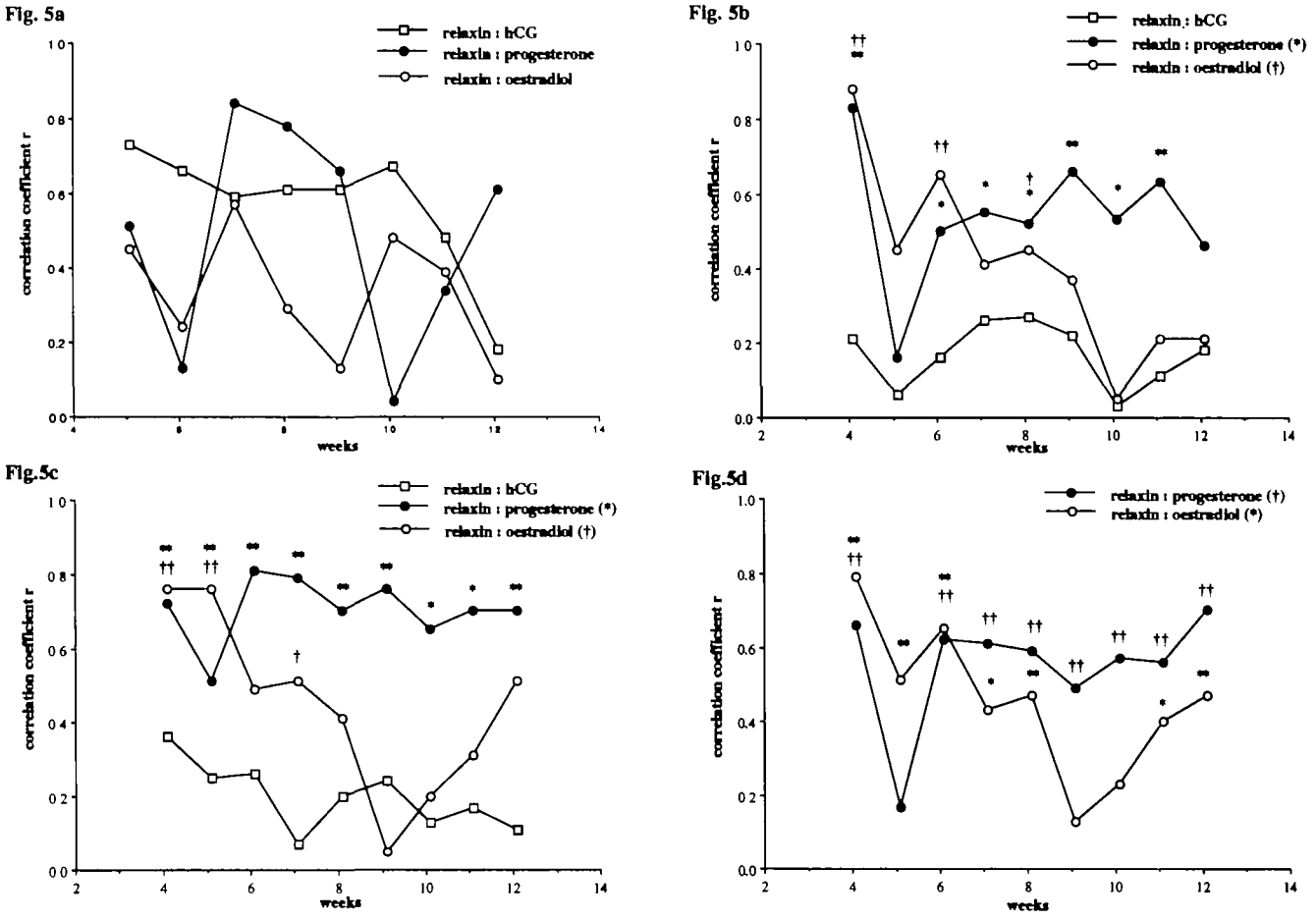

Fig. 5. The correlation coefficient (r) between relaxin and human chorionic gonadotrophin (HCG), oestradiol and progesterone for pregnancies conceived spontaneously (**a**), or following ovulation induction with human menopausal gonadotrophin and either clomiphene citrate (**b**), or buserelin acetate (**c**); and the correlation coefficient r between relaxin and oestradiol and progesterone for all pregnancies combined (**d**). */† Denotes a difference of $P < 0.05$ and **/†† a difference of $P < 0.01$.

or to an effect of the mode of ovulation induction is not clear, but the intermediate concentrations of HCG in the buserelin group of pregnancies suggests that it is more likely to be due to the mode of ovulation induction. Such an effect on trophoblast function may be mediated through an impaired endometrial function, demonstrated by reduced PP14 (Johnson *et al.*, 1993e) and PP12 (M.R.Johnson, unpublished observations) in pregnancies achieved following ovulation induction, or due to impaired conceptus development *in vitro*, although any such effect on conceptus development appears to be reversed early in pregnancy (Khazen *et al.*, 1986). The lower concentrations of HCG in pregnancies achieved after ovulation induction may be of importance in situations where HCG is used to assess the relative risk of a chromosomal abnormality in the fetus.

The correlations between relaxin and both progesterone and oestradiol concentration in the different groups seem to suggest that the luteo-placental shift of oestradiol synthesis occurs in natural pregnancies at 7 weeks and for pregnancies achieved after ovulation induction at 9 weeks. For progesterone synthesis in natural pregnancies, the timing of the luteo-placental shift seems to be 10 weeks, but in the pregnancies achieved following ovulation induction the association persists throughout the first trimester. This may mean that the luteo-placental shift for the synthesis of progesterone occurs after the end of the first trimester, or that placental synthesis of progesterone is linked to that of the corpus luteum, which, in turn, is linked to corpus luteum synthesis of relaxin. Such a linkage between placental and corpus luteum synthesis of progesterone has been proposed previously (Johnson *et al.*, 1993a). Further support for such a link is seen in the correlation between oestradiol and relaxin concentrations in weeks 11 and 12 in the combined data.

These data suggest that corpus luteum function is determined primarily during the cycle of conception and that although HCG enhances corpus luteum production of progesterone, it has a limited effect on relaxin synthesis, and an indeterminant effect on oestradiol synthesis. In addition, they suggest that corpus luteum and placental function are linked and that trophoblast function is impaired in clomiphene pregnancies.

References

Archer,D.F., Hofmann,G., Brzyski,R., Ross,B., Scott,R.T., Philiput,C.B., Oehninger,S. and Ware,C. (1989) Effects of clomiphene citrate on episodic luteinising hormone secretion throughout the menstrual cycle. *Am. J. Obstet. Gynecol.*, **161**, 581–592.

Csapo,A.I. and Pulkkinen,M. (1978) Indispensibility of the human corpus

luteum in the maintenance of early pregnancy luteectomy evidence. *Obstet. Gynecol. Surv.*, **33**, 69–81.

Ferrailo,B.L., Winslow,J., Laramee,G., Celnicker,A. and Johnston,P. (1991) The pharmacokinetics and metabolism of human relaxins in rhesus monkeys. *Pharm. Res.*, **8**, 1032–1038.

Hutchinson,J.S. and Zeleznik,A.J. (1986) The corpus luteum of the primate menstrual cycle is capable of recovering from a transient withdrawal of pituitary gonadotropin support. *Endocrinology*, **117**, 1043–1049.

Johnson,M.R., Abdalla,H., Allman,A.C.J., Wren,M.E., Kirkland,A. and Lightman,S.L. (1991a) Relaxin levels in ovum donation pregnancies. *Fertil. Steril.*, **56**, 59–61.

Johnson,M.R., Okokon,E., Collins,W.P., Sharma,V. and Lightman, S.L. (1991b) The effect of human chorionic gonadotropin and pregnancy on the circulating level of relaxin. *J. Clin. Endocrinol. Metab.*, **72**, 1042–1047.

Johnson,M.R., Allman,A.C.J., Steer,P.J. and Lightman,S.L. (1992) Circulating levels of relaxin may influence the time of onset and progression of labour. *J. Endocrinol.*, **135**, 31.

Johnson,M.R., Riddle,A.F., Grudzinskas,J.G., Sharma,V., Campbell, S., Collins,W.P., Lightman,S.L., Mason,B. and Nicolaides,K.H. (1993a) Endocrinology of IVF pregnancies during the first trimester. *Hum. Reprod.*, **8**, 316–322.

Johnson,M.R., Carter,G., Grint,C. and Lightman,S.L. (1993b) Relaxin in the menstrual cycle. *Acta Endocrinol.*, **129**, 121–125.

Johnson,M.R., Allman,A.C.J., Norman-Taylor,J.Q., Pickering,S.J., Grudzinskas,J.G., Steer,P.J. and Lightman,S.L. (1993c) Relaxin and hCG levels during pregnancy: and their relationship to outcome. *Acta Obstet. Gynecol.*, in press.

Johnson,M.R., Bolton,V.N., Riddle,A.F., Sharma,V., Nicolaides,K.H., Grudzinskas,J.G. and Collins,W.P. (1993d) Interactions between the embryo and corpus luteum. *Hum. Reprod.*, **8**, 1496–1501.

Johnson,M.R., Abbas,A., Norman-Taylor,J.Q., Riddle,A.F., Grudzinskas,J.G., Chard,T. and Nicolaides,K.H. (1993e) Circulating placental protein 14 (PP14) in the first trimester of spontaneous and IVF pregnancies. *Hum. Reprod.*, **8**, 323–326.

Khazen,N.E., Puissant,F., Camus,M., Lejeune,B. and Leroy,F. (1986) A comparison between multiple and single pregnancies obtained by in-vitro fertilization. *Hum. Reprod.*, **1**, 251–254.

MRC working party on children conceived by *in vitro* fertilisation (1990) Births in Great Britain from assisted conception, 1978–1987. *Br. Med. J.*, **300**, 1229–1233.

Nowroozi,K., Check,J.H., O'Shaughnessy,A. and Shapse,D. (1991) Measurement of 17-hydroxyprogesterone levels during the first trimester of donor oocyte pregnancies. *Hum. Reprod. Abstracts of the 7th Annual Meeting of ESHRE*, p. 27.

Quagliarello,J., Goldsmith,L., Steinetz,B., Lustig,D.S. and Weiss,G. (1980) Induction of relaxin secretion in non-pregnant women by human chorionic gonadotropin. *J. Clin. Endocrinol. Metab.*, **51**, 74–77.

Sharma,V., Riddle,A., Mason,B., Pampiglione,J. and Campbell,S. (1988) An analysis of factors influencing the establishment of a chemical pregnancy in an ultrasound based ambulatory in vitro fertilization program. *Fertil. Steril.*, **49**, 458–468.

Smitz,J., Devroey,P. and Van Steirteghem,A.C. (1990) Endocrinology in luteal phase and implantation. *Br. Med. Bull.*, **46**, 709–719.

Tulchinsky,D. and Hobel,C.J. (1973) Plasma human chorionic gonadotropin, estrone, estradiol, estriol, progesterone and 17a-hydroxyprogesterone in human pregnancy. *Am. J. Obstet. Gynecol.*, **117**, 884–893.

Waterstone,J.J. and Parsons,J.H. (1992) A prospective study to investigate the value of flushing follicles during transvaginal ultrasound-directed follicle aspiration. *Fertil. Steril.*, **57**, 221–223.

Wilks,J.W. and Noble,A.S. (1983) Steroidogenic responsiveness of the monkey corpus luteum to exogenous chorionic gonadotrophin. *Endocrinology*, **112**, 1256–1266.

Williams,M.A., Goldman,M.B., Mittendorf,R. and Monson,R.R. (1991) Subfertility and the risk of low birth weight. *Fertil. Steril.*, **56**, 668–671.

Yoshimi,T., Strott,C.A., Marshall,J.R. and Lipsett,M.B. (1969) Corpus luteum function in early pregnancy. *J. Clin. Endocrinol. Metab.*, **29**, 225–230.

Received on June 28, 1993; accepted on August 31, 1993