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Is luteal function maintained by factors other than chorionic gonadotrophin in early pregnancy?

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Women with ectopic pregnancy (n = 14) and early embryonic arrest ('blighted ovum') (n = 9) were studied 16 days after conception, at a time when they were asymptomatic and serum concentrations of β-human chorionic gonadotrophin (HCG) were in the normal range and increasing at an apparently normal rate. Serum progesterone and oestradiol concentrations were compared with those from normal women matched for gestational age and serum β-HCG concentration whose singleton intra-uterine pregnancies proceeded normally beyond 20 weeks. Mean serum progesterone concentrations were significantly lower in the women with ectopic pregnancies than in matched controls (P < 0.002); however, there was no difference in the serum progesterone concentrations between women with blighted ova and matched controls. Statistically significant differences were not seen in serum oestradiol concentrations between either group and matched controls. Similarly there was no difference in serum progesterone or oestradiol concentrations in 20 women who conceived ectopic pregnancies and 20 women conceiving blighted ovum pregnancies and their matched intra-uterine controls when conception followed ovarian stimulation. The low serum progesterone concentrations seen in ectopic pregnancy suggest that there is a specific and selective deficiency in progesterone synthesis, which implies that factors other than HCG may influence luteal function.

Key words: corpus luteum/early pregnancy failure/ectopic pregnancy

Introduction

Implantation of the human embryo is the trigger for a dramatic increase in the synthesis of human chorionic gonadotrophin (HCG), the action of which ensures that the cells of the corpus luteum continue to produce progesterone, which maintains the integrity of the endometrium until placental steroid synthesis supervenes. There have been several reports describing depressed serum concentrations of progesterone in ectopic pregnancy and other abnormalities such as embryonic arrest (blighted ovum) (Grudzinskas *et al.*, 1986; Hubinont *et al.*, 1987; Norman *et al.*, 1988; Hartshorne, 1989; Wang *et al.*, 1990), but it is not entirely clear why the circulating concentrations of progesterone and other steroids are low at the time of clinical presentation. One proposed explanation is the decrease in luteotrophic drive consequent to the depressed rate of HCG synthesis by the failing trophoblast. In this context previous investigators (Hubinont *et al.*, 1987; Norman *et al.*, 1988; Lindblom *et al.*, 1989) have examined the association between serum HCG concentrations and luteal function at the time of clinical presentation when the pregnancy was obviously failing or had failed. Norman *et al.* (1988) concluded that depressed luteal function could not be explained either by reduced circulating concentrations of HCG or diminished HCG bioactivity. They speculated that depressed luteal function was either a manifestation of a primary defect of ovulation, or due to the absence or abnormality of other regulatory factors.

In order to test these hypotheses we have systematically examined circulating β-HCG, progesterone and oestradiol in women 16 days after spontaneous or assisted ovulation and conception, who were subsequently found to have an ectopic gestation (spontaneous ovulation, n = 14; ovarian stimulation, n = 20) or early embryonic arrest ('blighted ovum') (spontaneous ovulation, n = 9; ovarian stimulation, n = 20). These women were matched for gestational age and clinical features to a control group whose pregnancies proceeded beyond 20 weeks.

Materials and methods

Women attending PIVET Medical Centre for early pregnancy surveillance were recruited for this study after informed consent. Venous blood was taken from the antecubital fossa at regular intervals during the luteal phase. The diagnosis of pregnancy was made 16 days after conception. Serum was separated by centrifugation within 1 h of collection and assayed the same day for oestradiol, progesterone and β-HCG by radioimmunoassay or immunofluorimetric assay described in detail elsewhere (Yovich and Grudzinskas, 1990). Subsequently the pregnancies were monitored by weekly measurements of the serum concentrations of oestradiol, progesterone and β-HCG. An ultrasound scan was performed between 7 and 8 weeks of gestation to confirm viability, unless endocrine evidence or clinical signs demanded earlier examination.

Spontaneous ovulation and conception

Women with ectopic pregnancies (n = 14) and blighted ova (n = 9) were identified for analysis. These women were selected

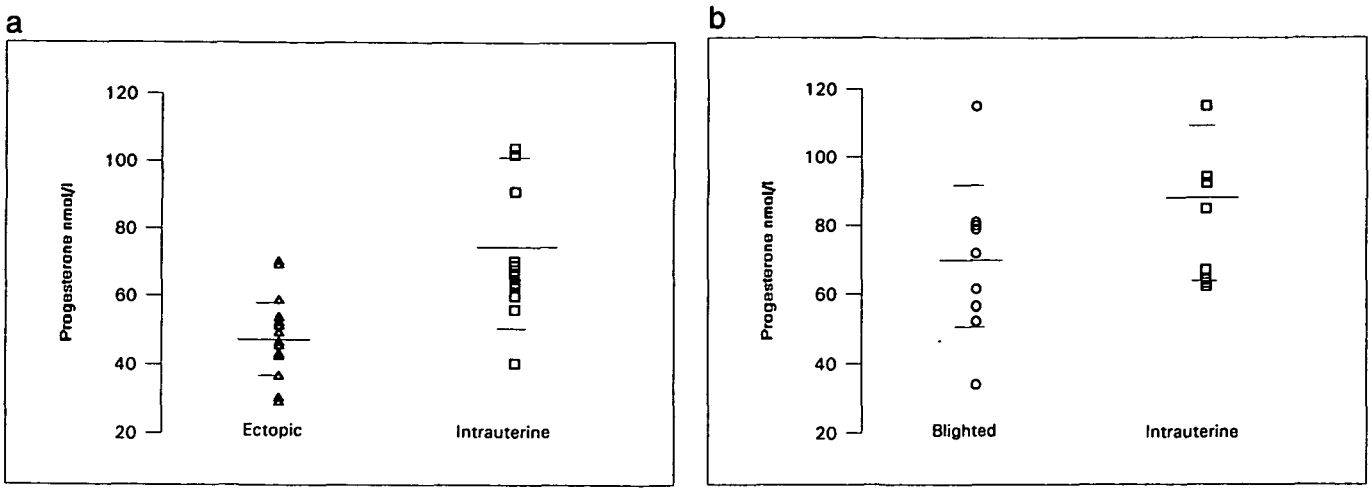

Fig. 1(a). Serum progesterone concentrations after spontaneous ovulation in 14 asymptomatic women with ectopic pregnancies at 4–5 weeks gestation (△) compared with 14 women with normal intra-uterine pregnancies matched for human chorionic gonadotrophin concentration and gestational age (□). **(b)** Serum progesterone concentrations after spontaneous ovulation in nine women with blighted ova at 4 weeks gestation (○) compared with nine women with normal intra-uterine pregnancies i.e. matched normal controls at the same gestation (□).

from a larger group of patients (ectopic pregnancies, $n = 33$; blighted ova, $n = 58$) with these complications because their menstrual dating was reliable and they were asymptomatic at the time of the study (14–21 days post-conception). The diagnoses became clinically apparent 2–4 weeks later. Women with abnormal pregnancies were each matched with women who had normal singleton intra-uterine pregnancies selected as paired controls, and matched for gestational age and initial serum β-HCG concentration within 10% of the women with abnormal pregnancies. In addition, these women were selected only if serum β-HCG levels doubled in the week subsequent to the time of study. The diagnosis of ectopic pregnancy or early embryonic arrest was made at routine ultrasound examination performed at 7 weeks of gestation or earlier if the clinical condition dictated. The surgical diagnoses were confirmed by histological examination.

Ovarian stimulation and assisted conception

Similar study and control groups were identified from women who conceived after in-vitro fertilization (IVF) and embryo transfer. Serum progesterone and oestradiol concentrations were studied in 20 women with an ectopic pregnancy and 20 women with blighted ova, selected according to the same criteria described above. The paired controls were selected from women subsequently demonstrated to have intact intra-uterine pregnancies and matched for serum β-HCG levels, gestational age, stimulation regimen and luteal support.

The ovulation induction regimen, which included gonadotrophin-releasing hormone (GnRH) agonists, human menopausal gonadotrophin (HMG) and HCG as the luteinizing hormone (LH) trigger, is described in detail elsewhere (Yovich and Grudzinskas, 1990). Luteal phase support was administered routinely to these women. The regimen comprised twice weekly i.m. injections of 5000 IU HCG and weekly injections of Proluton (hydroxy-progesterone hexanoate, Schering Health Care, West Sussex, UK) (Yovich *et al.*, 1984; Yovich and Grudzinskas, 1990).

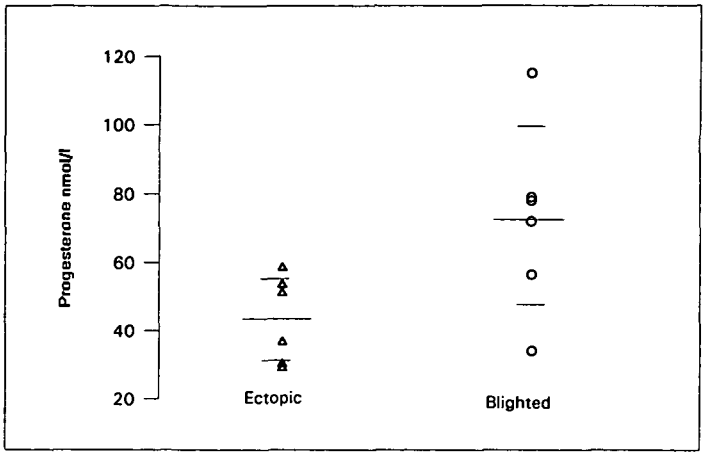

Fig. 2. Serum progesterone concentrations after spontaneous ovulation in six women with ectopic pregnancies at 4 weeks gestation (△) compared with six women with blighted ova (○) matched for serum human chorionic gonadotrophin concentration and gestational age.

The differences observed between the study and control groups were tested for statistical significance by using Student's *t*-test.

Results

Spontaneous ovulation and conception

Figure 1a and b demonstrates serum progesterone concentrations in women who had conceived either an ectopic pregnancy or blighted ovum after spontaneous ovulation. At week 4–5 of gestation the serum progesterone concentration was significantly lower in women conceiving an ectopic pregnancy than in matched controls ($P = 0.001$; $t = 3.55$). Plasma levels of progesterone concentration were not significantly different from the control value for women with blighted ovum pregnancies ($P = 0.187$; $t = 1.38$). Moreover, in the six pairs of women with ectopic

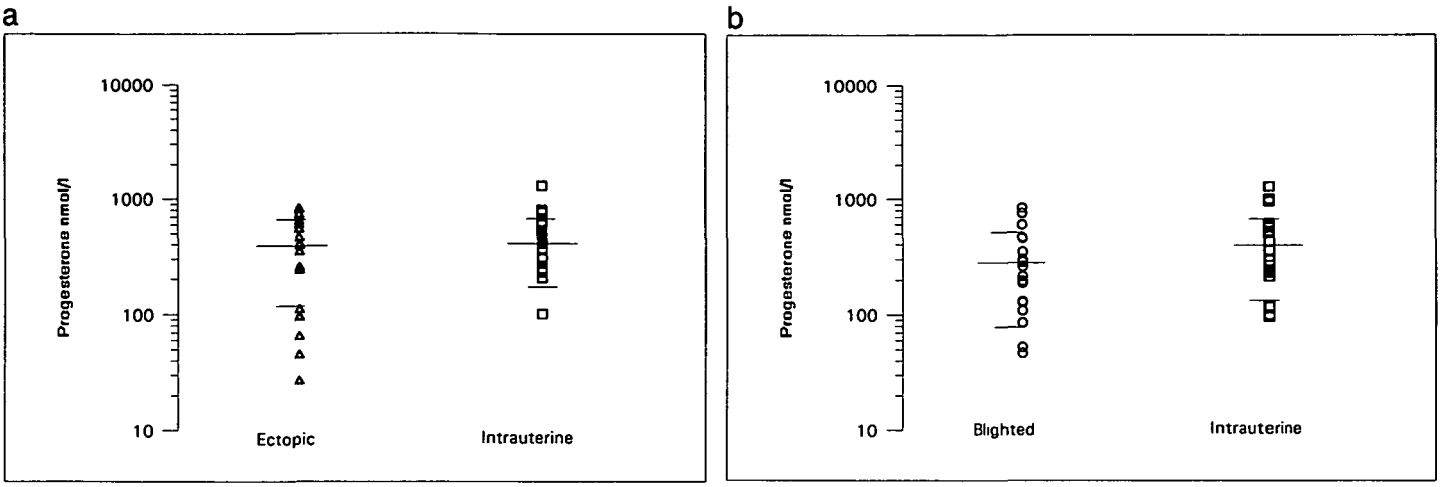

Fig. 3(a). Serum progesterone concentrations after ovulation induction in 20 asymptomatic women with ectopic pregnancies at 4–5 weeks gestation (△) compared with 20 matched women with normal intra-uterine pregnancies at the same gestation after similar stimulation (□). **(b)** Serum progesterone concentrations after ovarian stimulation in 20 women with blighted ova at 4 weeks gestation (○) compared with 20 matched normal controls at the same gestation (□).

pregnancy and blighted ovum in whom it was possible to match for serum β-HCG levels at 4 weeks of gestation (Figure 2), the mean serum progesterone concentration was significantly lower in ectopic pregnancy than in blighted ova ($P = 0.036$; $t = 2.418$).

By contrast the mean level of serum oestradiol was not significantly different from matched paired controls for either ectopic pregnancies or blighted ova (data not shown).

Ovarian stimulation and assisted conception

Figure 3a and b shows the serum progesterone concentrations for women conceiving ectopic pregnancies and blighted ova after ovarian stimulation. There was no significant difference between the plasma levels of progesterone seen in abnormal pregnancies and matched controls after ovarian stimulation.

Similarly no significant differences were observed between the serum oestradiol concentrations in women conceiving ectopic or anembryonic pregnancies and their matched controls after ovarian stimulation (data not shown).

Discussion

Our data suggest that the depressed serum progesterone concentrations seen in ectopic pregnancies and perhaps blighted ova described here and in earlier studies cannot be attributed to failing trophoblastic function alone for at least two reasons. Firstly, women were studied while asymptomatic and some weeks before the diagnosis became clinically evident. Rupture of the Fallopian tube occurred at the time of diagnosis in only one of 14 women with ectopic pregnancy. Secondly, we selected only those women in whom there was some evidence of 'normal' trophoblastic function as measured by rising rather than static or falling levels of β-HCG. The design of this study is in contrast to earlier reports in which the hormone measurements were obtained at the time of symptoms of complications such as imminent tubal rupture and without consideration for the dynamic changes in HCG levels.

The synthesis of progesterone by the cells of the corpus luteum has been thought to be primarily dependent upon the concentration of circulating HCG (Kadar, 1983; Grudzinskas *et al.*, 1986). Our results provide further evidence that control of the corpus luteum of pregnancy may be influenced by additional factors. Our findings supplement the observations of Norman *et al.* (1988) which demonstrated depressed serum concentrations of ovarian steroids at a mean gestational age of 40 days in women presenting with ectopic pregnancy when compared with normally pregnant women matched for plasma levels of HCG. They concluded that their findings could not be explained by depressed serum HCG concentrations or altered HCG bio-activity. They postulated a primary defect of the corpus luteum, the absence of another stimulator of ovarian steroid biosynthesis, or more subtle variations in HCG glycosylation which they could not examine (Norman *et al.*, 1988). Another explanation is that in extra-uterine pregnancies, some particular isoforms of HCG are produced which are less steroidogenic (Cole and Kardana, 1992). That this could be the case is shown by the fact that in IVF patients with luteal support in the form of exogenous HCG, levels of plasma progesterone in patients with extra-uterine pregnancies are not different when compared to the matched controls. By contrast, in a similar study Kratzer and Taylor (1990) confirmed these findings with respect to HCG bio-activity. They found a considerable overlap in serum progesterone concentrations in women with ectopic and normal intra-uterine pregnancies and concluded that the rate of change of HCG was the most important influence upon corpus luteum function, independent of the activity of other serum factors. It is also noteworthy that women were recruited for study in the symptomatic phase and no mention was made of gestational age. Liu *et al.* (1991) found that both the serum oestradiol and progesterone concentrations were lower in women with ectopic pregnancies than in controls with intra-uterine pregnancies. They suggested that embryonic HCG may act via the endometrium through intermediate substances which then stimulate the corpus luteum. Our findings support the possibility of a biochemical or metabolic signal consequent to the direct contact between trophoblast and endometrium.

A significant difference in serum plasma oestradiol levels between ectopic pregnancies and their matched intra-uterine pregnancies was not seen, suggesting that oestradiol synthesis by the luteal cells is not altered in ectopic pregnancy or blighted ovum during the asymptomatic phase.

A statistically significant difference between the levels of plasma progesterone following ovulation induction was not seen, although five of 20 women with ectopic pregnancies had lower serum progesterone concentrations than any of the control group. The luteal response is complicated as the ovaries may contain a large number of follicles after ovarian stimulation by exogenous gonadotrophins. They may not be capable of responding to any further luteotrophic influence if one did exist. Plasma levels of ovarian steroids seen after ovarian stimulation are considerably elevated and do not fall to levels comparable with spontaneous conceptions until 8–10 weeks following conception. The high concentrations may mask any subtle influence on progesterone synthesis that may be lacking in women with ectopic pregnancy in the absence of ovarian stimulation, or alternatively no difference may have been observed because HCG had been administered for luteal phase support.

It is interesting to note that serum progesterone levels appear to be slightly lower in ectopic pregnancies than in blighted ova. It may be that the abnormal trophoblasts of blighted ova are still capable of eliciting a luteotrophic response when contact with the endometrium is maintained. Furthermore, blighted ova are a heterogeneous group, and the time of embryonic arrest may vary. It is possible that this is the reason why there is a less significant difference between the serum progesterone concentrations of normal pregnancy controls and this group, than that seen in the ectopic pregnancy group.

The data presented here provide new evidence of the complexity of the endocrine response during early pregnancy. Specific studies are required to provide further clarification of the messengers involved, with particular reference to the secretory products of the endometrium, embryo, trophoblast and their interaction. These studies may well develop from continuing research in centres offering assisted conception, where there is unique access to early pregnancy material, especially in natural cycle programmes.

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