Pituitary-Ovarian Function in Mothers Who Have Had Two Sets of Dizygotic Twins

NICHOLAS G. MARTIN, PH.D., MARILYN E. OLSEN, HELEN THEILE, B.SC., JOSE L. EL BEAINI, M.B., DAVID HANDELSMAN, M.B., PH.D., AND AJAY S. BHATNAGAR, PH.D.

Reprinted from June 1984 Fertility and Sterility

PUBLISHED MONTHLY BY THE AMERICAN FERTILITY SOCIETY, BIRMINGHAM, ALABAMA
Copyright © 1983 The American Fertility Society
Pituitary-ovarian function in mothers who have had two sets of dizygotic twins

Nicholas G. Martin, Ph.D.*†
Marilyn E. Olsen*
Helen Theile, B.Sc.‡
Jose L. El Beaini, M.B.§
David Handelsman, M.B., Ph.D.‖
Ajay S. Bhatnagar, Ph.D.¶

Australian National University, Canberra, Prince of Wales Hospital, Sydney, University of New South Wales, Sydney, University of Sydney, Sydney, New South Wales, Australia, and Medical College of Virginia, Richmond, Virginia

Serum gonadotropin and estradiol levels were measured in 14 women with regular menses during the early and midfollicular phases. Early follicular follicle-stimulating hormone and, to a lesser extent, luteinizing hormone levels were significantly higher in a group of eight women who had had at least one set of dizygotic twins (six of whom had had two sets) than in a control group of six women with no dizygotic twins. Estradiol levels also tended to be higher in mothers of twins, particularly in the midfollicular phase. The two groups of women did not differ in age, height, weight, or parity. Fertil Steril 41:878, 1984

Little is known about the etiology of dizygotic (DZ) twinning in humans, although there is extensive evidence that it tends to be familial.1 Animal evidence and the increased rate of multiple pregnancies following ovulation induction in anovulatory women2 suggest that the immediate physiologic cause of the tendency to multiple births is alteration in the mother’s hormone levels. It has been hypothesized that mothers of DZ twins have higher gonadotropin levels than mothers of singletons,3 but the only such evidence is for nonsignificant elevations of follicle-stimulating hormone (FSH) at midcycle.4 However, because follicles are recruited for growth early in the menstrual cycle,5 it is unlikely that a higher midcycle FSH peak is directly relevant to the cause of multiple ovulation. We hypothesize that the tendency to DZ twinning is associated with higher than normal FSH levels in the early follicular phase. In order to test this hypothesis, we have chosen a group of women who have had two sets of DZ twins under natural circumstances and compared their gonadotropin levels in the early follicular phase with those in control mothers of singletons. We also measured estradiol (E2) to see whether any elevation of FSH results from reduced feedback inhibition by this ovarian hormone.

878 Martin et al. Dizygotic twins Fertility and Sterility
MATERIALS AND METHODS

Mothers with two sets of twins were ascertained who (1) were 18 to 40 years of age (i.e., not postmenopausal), (2) were not using oral contraceptives or other hormone preparations, (3) were not pregnant or lactating, and (4) had not had a hysterectomy. Seven mothers who satisfied these criteria were tested. A further mother of a single set of DZ twins was included in the sample because she had two sets of DZ twin siblings and was judged to be from a high-risk family. If twin pairs were not of the opposite sex, then mothers answered questions on their physical characteristics, including eye color, hair color, and height, or produced photographs to satisfy the investigators that their twins were in fact DZ. One mother of an opposite-sex pair produced photographs of her second pair of female twins, which strongly suggested that they were monozygotic (MZ); but she has nevertheless been included. Practical considerations, including the young age of many of the twins, prevented blood typing of the same-sex pairs to confirm zygosity diagnoses. Six women who had had no DZ twins but who satisfied all the other screening criteria were tested as a control group. One of these women had a single pair of MZ twins but no other twins in her family.

Subjects informed us immediately at the onset of menses, and each woman had blood samples collected on 5 days of this cycle. Blood collection took place on 3 days of the early follicular phase (cycle days 1 to 3 or 2 to 4), or 1 day in the midfollicular phase (day 8 or 9), and on 1 day in the midluteal phase (days 19 to 23). On each of the first 4 sampling days, 4 × 10-ml intravenous blood samples were drawn at 20-minute intervals (i.e., at 0, 20, 40, and 60 minutes). On the fifth sampling day (cycle days 19 to 23), only a single blood sample was drawn, which was assayed for progesterone (P) to check that the cycle had been ovulatory. All sampling took place in the morning, and serum samples were frozen at −20°C until being assayed for FSH, luteinizing hormone (LH), E₂, prolactin (PRL), and P.

Hormone levels were estimated in duplicate for each sample by radioimmunoassay using kits from Diagnostic Products Corporation, Los Angeles, CA, for LH and FSH and from Radio-isotope Service, Wurenlingen, Basel, Switzerland, for E₂. Peptide assay standards were MRC 68/40 (LH) and 69/104 (FSH). Coefficients of variation were < 6.4% within the batch and < 13% between batches for all hormones. Means of the four samples taken each day were computed in order to minimize variation due to the pulsatility of gonadotropin release.

RESULTS

The mothers of twins and control groups were well matched for age, height, weight, and number of pregnancies (Table 1). Midluteal P levels were all consistent with ovulatory cycles. PRL levels were not significantly different in the two groups of women, and the levels of all the subjects were within the normal range.

Because our prior hypothesis is that mothers of twins have greater early follicular FSH and LH concentrations than control subjects, we may perform one-tailed t-tests on the differences between their means. These are significant at the 10% level for both hormones on the first three sampling days and at the 5% level for LH on day 1 and for FSH on days 1 and 2 (Fig. 1A and B).

DISCUSSION

A recent study suggests that the early stages of folliculogenesis are primarily dependent on gonadotropin (especially FSH), but that in the later stages of recruitment of the dominant follicle, both gonadotropins and follicular estrogen biosynthesis are important. The hypothesis that mothers of DZ twins have higher serum gonadotropin levels in the early follicular phase of the menstrual cycle than mothers of singletons is strongly supported by our data. It is unlikely that these results are artifacts produced by differences in PRL levels, age, weight, or parity in the two groups. In selecting women for study who had had

Table 1. Comparability of Mothers of Twins and Control Subjects for Age, Height, Weight, and Number of Pregnancies

<table>
<thead>
<tr>
<th></th>
<th>Mothers of twins (n = 8)</th>
<th>Control subjects (n = 6)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>Mean 37.3, SD 2.7</td>
<td>Mean 36.3, SD 3.0</td>
<td>0.60</td>
<td>0.56</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Mean 169.6, SD 3.5</td>
<td>Mean 169.5, SD 6.8</td>
<td>0.04</td>
<td>0.97</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Mean 68.0, SD 12.9</td>
<td>Mean 68.5, SD 13.9</td>
<td>0.06</td>
<td>0.95</td>
</tr>
<tr>
<td>Pregnancies</td>
<td>Mean 3.38, SD 1.30</td>
<td>Mean 3.00, SD 1.79</td>
<td>0.46</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*SD, standard deviation.

Martin et al.  Dizygotic twins 879
two sets of DZ twins, we have given ourselves the optimum chance of detecting hormonal differences. We believe that this is the first report of gonadotropin levels measured in the very early part of the menstrual cycle of DZ twin mothers.

Our subsidiary hypothesis, if FSH levels were indeed higher in mothers of DZ twins, was that this would be due to lower E2 levels causing less feedback inhibition of pituitary gonadotropin production. However, E2 levels are actually higher in DZ mothers than in control subjects on all four sampling days (Fig. 1C). The difference increases with sampling day and is significant at the 10% level (two-tailed test) on day 8 or 9. This is consistent with very recent results, suggesting that early follicular gonadotropin levels may be predominantly under negative feedback inhibition by inhibin, a peptide of ovarian origin, and not by E2. Since a recruited follicle produces all the E2 circulating in the midfollicular phase, elevated levels in the DZ mothers at this time suggest that the occurrence of multiple follicle recruitment remains common in these women after the birth of DZ twins, although we have no direct evidence (such as ovarian ultrasound) for this yet.

Our sample size is small, and the information on the stage of the menstrual cycle in our subjects is not precise. We must therefore regard our results as tentative. Nevertheless, they are in accord with theoretic expectations and provide strong support for further research along these lines into the causes of DZ twinning.

Acknowledgments. We are grateful to our subjects for their willing cooperation and to our colleagues who collected the samples. We thank the Australian Multiple Birth Association for their help in finding volunteer subjects. The Australian Twin Registry, through which most of the mothers of twins were ascertained, is maintained by a grant from the National Health and Medical Research Council of Australia.

REFERENCES

5. McNatty KP: Ovarian follicular development from the onset of luteal regression in humans and sheep. In Follicular Maturation and Ovulation, Edited by Rolland, EV van Hall, SG Hillier, KP McNatty, J Schoemaker. Amsterdam, Excerpta Medica, 1982, p 1