A Comparison of Adult Female Twins from Opposite-Sex and Same-Sex Pairs on Variables Related to Reproduction

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In several litter-bearing species, prenatal exposure of a female fetus to hormones from adjacent male fetuses can lead to later effects on various anatomical and behavioral characteristics of the female, including a number related to reproduction. To see if such traits are also affected in humans, adult female twins from a large Australian sample who had male cotwins were compared to females with female cotwins on 90 questionnaire items related to reproductive functions. No substantial effects could be clearly demonstrated, although some weak effects remained a possibility. Some variables, such as age at first menstruation, age at first pregnancy, and height, were consistent in direction with results from the animal literature, although the effect sizes were small and not statistically significant.

KEY WORDS: Opposite-sex twins; prenatal effects; male hormones; reproductive processes.

INTRODUCTION

For several litter-bearing mammals, a female fetus exposed prenatally to male hormones because it is located between two male fetuses may show effects on a variety of anatomical and behavioral characteristics. These include several related to reproduction, such as a longer estrous cycle, less attractiveness to males, and a later age of sexual maturity (vom Saal, 1989). It has been suggested that females from opposite-sex fraternal twin pairs might provide evidence for similar effects among humans (Resnick *et al.*, 1993; Miller, 1994).

Some results consistent with such effects have been reported. Resnick *et al.* (1993) found that females from opposite-sex fraternal twin pairs averaged higher on the trait of sensation-seeking than

did females from same-sex pairs (this is a trait typically higher in males). Miller (1994) found females from opposite-sex twin pairs to have more masculine attitudes than females from same-sex pairs. However, as both authors noted, in these cases one cannot rule out postnatal effects of having a male rather than a female sibling, a plausible source of influence on characteristics such as these. McFadden (1993) reported that females from a small sample of opposite-sex twin pairs were lower (more like males) in their level of spontaneous otoacoustic emissions than were females from same-sex pairs. Postnatal effects of sex of sibling are less likely here, as typical sex differences in the prevalence of otoacoustic emissions can be observed in month-old infants (Burns *et al.*, 1992).

However, not all investigations have obtained positive results. Rose *et al.* (1994) found no difference on a scale of feminine interests between 16-year-old female twins from opposite-sex and same-sex pairs in a Finnish sample. These authors also failed to find a difference in the age at which

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the first child was born to women from the two kinds of pairs. Both femininity and age at first reproduction are traits for which one might have expected a difference, given the animal research.

The present study investigates the possible effects of prenatal androgen exposure, using measures of reproductively related characteristics in adult female twins from the Australian sample studied by Martin and his colleagues (e.g., Martin and Jardine, 1986; Treloar and Martin, 1990). The data come from an 8-year follow-up of the initial sample. In this follow-up, a mailed questionnaire on health, attitudes, and personality was filled out by approximately 3000 twin pairs. Part of the questionnaire, completed only by the females, included questions on such topics as menstruation, premenstrual symptoms, pregnancies, births, and menopause. In studies of rats and mice, females prenatally exposed to male hormones by their location next to male fetuses were found to differ from females located next to female fetuses in length of the estrous cycle, duration of estrus, timing of puberty and reproductive senescence, and sexual receptiveness and attractiveness to males (vom Saal, 1989). Therefore, a comparison between the responses of females from opposite-sex and same-sex pairs on this part of the questionnaire seemed appropriate.

METHOD

All females from opposite-sex pairs in the sample were compared with females from same-sex pairs. Where two females from a same-sex pair both had completed the questionnaire, only one—the first listed in the data file—was used, as a simple means of avoiding the statistical dependencies introduced by including both members of a twin pair. The number of subjects available varied from item to item: questions regarding births, for example, were responded to only by women who had had children, and the question on age at menopause only by those who had passed through it. For the items most widely applicable, typical sample sizes were of the order of 600–700 women from opposite-sex pairs and 1400–1500 women from same-sex pairs.

RESULTS AND DISCUSSION

Table I contains data for women in the opposite-sex and same-sex twin groups on a variety of variables related to reproduction, including menstruation, premenstrual symptoms, pregnancies, contraception, and menopause. Means and percentages are given, along with the number of individuals on which each is based. Thus, for example, 685 women from opposite-sex twin pairs reported heights that averaged 163.5 cm, and 1642 women from same-sex pairs reported heights that averaged 162.8 cm. In the case of dichotomous (yes–no) answers, the percentage answering "yes" is given. Thus 16% of the 676 women from opposite-sex pairs responding to the question, "Have you reached menopause?" said "yes," and 21% of 1616 women from same-sex pairs did.

In Table II, the items refer to the births of the twins' own children—up to three for each woman (the first three, if she had more). For example, the last three rows in the table indicate that for 418 first or only children of women from opposite-sex twin pairs, solid foods were introduced at an average age of 4.57 months, whereas for 347 second and 195 third children the average ages at which this happened were 4.64 and 4.50 months, respectively.

The overall impression from these tables is that for most items the average differences are small between women with male and women with female cotwins. The first item in Table I, age, is an exception. The women from the opposite-sex pairs (OS) average almost 3 years younger than those in the same-sex group (SS). This appears to reflect the fact that the male twins initially recruited to the study were younger than the females; opposite-sex pairs, of course, necessarily involve a male. Among the DZ pairs in the initial sample of which this is a follow-up, female–female pairs averaged 35.35 years of age, male–male pairs averaged 32.26 years, and opposite-sex pairs averaged 32.90 years (Martin and Jardine, 1986). Because of this age difference, age was statistically controlled in the analysis of other variables. To allow for possible nonlinear relationships with age, both linear and quadratic effects of age were adjusted for, by including both age and age squared as covariates.

The last column in Tables I and II provides the p value. In the case of the dichotomous variables, this is based on a logistic regression, with the "yes–no" response predicted from twin group with age and age squared also present. For the remaining items, it is based on a one-way analysis of variance with age and age squared as covariates. In interpreting these p values, it should be kept in mind

Table I. Comparison of Australian Twin Females on Variables Related to Menstruation and Reproduction

Variable	OS	N	SS	N	p
Age (years)	39.66	747	42.63	1748	.000
Height (cm)	163.5	685	162.8	1642	.058
Age at first menstrual period (years)	13.27	679	13.23	1615	.529
Have you reached menopause? (yes)	16%	676	21%	1616	.785
Age at menopause (years)	47.70	97	49.07	325	.162
Problems with menopause (1=none, to 4)	1.90	104	1.88	331	.896
Have you had a hysterectomy? (yes)	12%	680	15%	1627	.426
Age at hysterectomy (years)	40.68	81	40.47	248	.559
If past menstruating, hormone treatment?	25%	160	24%	513	.896
Premenstrual symptoms (ever had)					
Sensation of bloating or weight gain	83%	701	83%	1606	.552
Headache	59%	679	62%	1541	.288
Changes in appetite (up or down)	48%	662	43%	1488	.147
Changes in amount of sleep (up or down)	39%	658	33%	1466	.004
Craving for certain foods	35%	649	31%	1457	.142
Breast fullness, tenderness, or pain	79%	691	80%	1582	.753
Clumsiness	34%	648	31%	1462	.137
Continual irritability or anger	71%	681	69%	1545	.855
Changes in mood (e.g., suddenly tearful)	72%	684	69%	1555	.354
Difficulty in concentrating	40%	651	41%	1490	.929
Constipation	39%	645	39%	1471	.662
Anxiety, tension, or feeling "on edge"	72%	683	75%	1578	.312
Feeling flat, down in moood, hopeless	69%	672	68%	1539	.739
Tiredness, low energy	77%	688	76%	1575	.691
Backache	63%	675	62%	1542	.665
Less interest in usual activities, friends	47%	657	45%	1495	.398
Joint or muscle pain	42%	665	42%	1491	.959
If any premenstrual symptoms ever					
At what age became a problem?	20.42	466	21.54	1019	.224
Were they limiting? (1=no, to 3=very)	1.57	596	1.58	1371	.925
Interfered with daily activities? (1 to 3)	1.51	598	1.52	1389	.773
Do you feel you suffer from PMS? (yes)	52%	638	54%	1459	.360
Have you sought treatment? (yes)	16%	636	16%	1442	.620
If periods more or less regular					
Average days of bleeding	5.01	511	4.87	986	.105
Periods are light (=1) to heavy (=3)	2.03	543	2.01	1076	.332
No trouble (=1) to very painful (=3)	1.55	535	1.60	1068	.079
Not limiting (=1) to very limiting (=3)	1.38	529	1.41	1050	.344
Average days from start to start of next	27.31	507	27.26	977	.988
Days since start of last (if not pregnant)	15.43	432	16.01	922	.363
Using contraception at present? (yes)	44%	549	45%	1128	.454
If any pregnancies					
Number of full-term pregnancies (if some)	2.56	524	2.65	1327	.812
Number of miscarriages (if some)	1.63	146	1.49	383	.155
Number of terminations (if some)	1.28	87	1.29	158	.923
Total number of pregnancies (if some)	3.03	558	3.10	1386	.708
Age at first pregnancy (years)	24.44	545	24.31	1353	.374
Are you pregnant now? (yes)	4%	534	3%	1331	.766
Are you breastfeeding now? (yes)	8%	493	6%	1262	.489

Note. Raw means and percentages; *p* values are with age and age squared controlled. OS, females with male cotwins; SS, females with female cotwins.

that 90 significance tests are being made, and of this many, it is likely that several would achieve conventional levels of statistical significance purely by chance. One way of protecting against this is by means of a Bonferroni correction (see, e.g., Darlington, 1990). To do this, one divides the desired

Table II. Comparison of Australian Twin Females on Variables Related to Births and Childrearing

Variable	OS	N	SS	N	p
For 1st, 2nd, & 3rd births was there					
High blood pressure, toxemia					
1	24%	478	23%	1221	.557
2	14%	412	12%	1069	.329
3	14%	238	14%	604	.767
Premature baby (>2 weeks early)					
1	14%	476	10%	1217	.022
2	15%	412	9%	1069	.003
3	11%	238	8%	603	.442
Epidural anesthetic (block)					
1	22%	477	17%	1222	.143
2	11%	411	10%	1069	.944
3	7%	237	9%	604	.330
Induced labor					
1	28%	478	30%	1219	.435
2	23%	412	22%	1070	.884
3	20%	238	25%	605	.102
Stitches (episiotomy or tear)					
1	72%	478	69%	1224	.334
2	57%	412	52%	1072	.196
3	50%	238	40%	603	.023
Stillborn child					
1	2%	478	3%	1217	.193
2	1%	412	1%	1068	.708
3	0%	238	1%	603	.429
Forceps delivery					
1	34%	479	32%	1222	.451
2	14%	412	11%	1069	.086
3	6%	238	7%	604	.626
Cesarean section					
1	10%	477	9%	1218	.894
2	7%	412	8%	1068	.174
3	3%	238	7%	603	.017
Length of labor (h)					
1	14.13	418	14.35	1051	.867
2	7.86	333	8.49	838	.351
3	6.40	192	7.85	460	.062
Birth weight (g)					
1	3310	439	3337	1107	.371
2	3336	366	3390	925	.138
3	3415	202	3402	500	.841
Difficult labor (1=not really, to 3)					
1	2.07	449	2.08	1153	.770
2	1.68	371	1.75	943	.103
3	1.64	216	1.66	532	.749
Postnatal depression (yes)					
1	24%	501	24%	1261	.648
2	20%	430	19%	1099	.704
3	12%	251	17%	619	.041
Breastfeeding (months)					
1	6.24	480	5.48	1172	.008
2	6.10	391	5.34	955	.030
3	5.84	225	5.64	517	.998
Age formula introduced (months)					
1	3.88	349	3.77	928	.706
2	3.56	290	3.40	794	.547
3	3.62	169	3.37	435	.569
Age solids introduced (months)					
1	4.57	418	4.63	1084	.563
2	4.64	347	4.45	914	.159
3	4.50	195	4.39	506	.513

Note. Raw means and percentages; *p* values are with age and age squared controlled. OS, females with male cotwins; SS, females with female cotwins.

overall p value by the number of tests to be made. Thus if one wants to ensure a 95% chance of avoiding false claims in the analysis as a whole, one would use a .00055 p level for individual tests (90 × .00055 = .05).

In the present case, one might well want to consider results from more than one perspective, e.g., by using a fairly strict standard, against which one could regard a given finding as definitely established, and a more tolerant one, against which a result would be considered worth checking on in future research, or speculating about, but not claimed as a firm finding from the present study. For simplicity, we use a .0005 and a .05 p value for these two purposes.

Based on the stricter criterion, there are no differences between opposite-sex and same-sex twins in Tables I and II that we can consider firmly established in these data (other than the age difference).

What about the more tolerant criterion? Here we consider comparisons nominally significant at the .05 level. There are eight such items altogether—in excess of the four or five that would be expected by chance from among 90 comparisons if these reflected solely random sampling fluctuation, but not dramatically so. These include only one item from Table I, which contains most of the items relevant to the findings from the animal studies. The one item from this table relates to premenstrual symptoms: the females with twin sibs of the opposite sex (OS) more often report sleep changes as a premenstrual symptom. This is not a variable for which animal data are available. Moreover, the women with OS twin sibs do not in general report more severe or disabling premenstrual symptoms, nor is it obvious why this particular one should be affected.

In Table II there are seven items that qualify at the individual .05 level. Somewhat more OS females had their first or second child born early, more required stitches in the third birth, fewer had cesarean sections for the third birth or postnatal depression after it, and the first and second child were breastfed longer. One might want to take more seriously items that show some degree of consistency across births. These would include prematurity and length of breastfeeding. One might make a case that prenatal exposure to male hormones could affect the development of physiological or anatomical features later involved in the timing of birth, but it is not obvious why such exposure should result in breastfeeding longer when one comes to

be a mother oneself. Obstetric and infant-care practices are subject to social and cultural influences, and in the absence of some specific plausible mechanism, one must be cautious about explanations that invoke the mother's own prenatal environment. For instance, having a sister one's own age could arguably have an effect on one's knowledge and attitudes about childrearing. Women from same-sex twin pairs have such a sister, women from opposite-sex pairs do not.

In short, these data do not lead to a strong conclusion that exposure of female twins to a male cotwin's hormones *in utero* has any substantial effect across the range of variables here surveyed. This does not, of course, mean that such effects never occur in humans, but at least they seem not to be large and compelling on traits related to reproduction, a place where one would expect to find them, given the results from research with lower animals.

Such negative conclusions must be qualified by the fact that the variables in this study are measured by self-report questionnaires—in some cases, such as age at first menstruation, after a lapse of several decades. [See Treloar and Martin (1990) for a discussion of this particular variable in these data.] However, it must be kept in mind that with large samples such as these, a high level of precision in individual measurement is not required in order to detect average effects, if they are substantial enough to be of practical importance.

Although the overall screening of reproductively related variables just described fails to yield strong evidence of the prenatal effect of a male cotwin's hormones, it is perhaps worth looking at just those variables most directly analogous to those for which results have been reported in the animal literature, in order to see if any consistent directional tendencies might be present. These variables include age at first menstruation and at first pregnancy, for which females with male cotwins would be predicted to be older, and length of menstrual cycle, predicted to be longer for these females. It is perhaps reasonable to add to these height, as analogous to sexually dimorphic bodily measures for which effects have been reported in rodents.[4] By hypothesis, females with male cotwins should be taller.

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[4] These include anogenital distance and weight. In one study in mice, body length differences were in the appropriate direction, although they were not statistically significant (Kinsley et al., 1986).

Table III. Age-Adjusted Means for Four Selected Variables for Females from Opposite- and Same-Sex Pairs, with Standard Deviation and Effect Size

Variable	OS	SS	SD	ES
Age at first menstruation (years)	13.27	13.23	1.47	.03
Age at first pregnancy (years)	24.48	24.30	4.16	.04
Length of menstrual cycle (days)	27.28	27.28	3.46	.00
Height (cm)	163.39	162.81	6.66	.09

Note. OS, females with male cotwins; SS, females with female cotwins; SD, standard deviation (within groups); ES, effect size, (OS−SS)/SD.

Table III presents the results for these selected variables. Shown in the table are means statistically adjusted for respondent's age (rather than the raw means given in Tables I and II, although the differences are in the same direction in either case). For three of the four variables in the table, the difference is in the direction expected if there had been an influence of male hormones upon the fetus; the fourth, length of menstrual cycle, shows no difference. The four variables are fairly independent of one another—only one of the six pairwise correlations has an absolute value greater than .05 (a correlation of +.13 between height and age at first menstruation). Thus the consistency of the mean differences is not simply an artifact of highly correlated traits. Also shown in Table III are effect sizes—the differences in means expressed as a fraction of the standard deviation of the trait. They are all less than .10, i.e., quite small. These results suggest the possibility of weak effects of the cotwin's hormones in opposite-sex pairs, effects that would require very large samples of twin pairs to be consistently demonstrable and would be of little or no practical (as opposed to theoretical) concern. Moreover, one of these variables, age at first pregnancy, would be expected to resemble age at first birth, which was studied by Rose *et al.* (1994). (The 4% greater frequency of premature births in the OS group would decrease the difference a little for age at birth, but not very much.) In the Rose *et al.* data, for 1144 first births to female members of opposite-sex twin pairs, the mean age of the mother was 25.97 years; for 1689 first births to members of same-sex female twin pairs, it was 26.05 years. The difference, such as it is, is opposite in direction to that predicted by the prenatal androgen hypothesis, suggesting even more caution in interpreting the present results.

Finally, the effects of uterine position on reproductively related variables among litter-bearing mammals are far from monolithic (vom Saal, 1989). The effects may differ across species: pigs show effects on duration of estrus but not on length of estrous cycles or age at first reproduction; gerbils show effects on litter size and sex ratio that mice and rats do not. The effects may be sensitive to environmental conditions: females housed with several other females may show effects of uterine position on age at sexual maturity and length of estrous cycle that are opposite in direction to those found in individually housed animals. Moreover, maternal stress can eliminate the effects (vom Saal *et al.*, 1990). Clearly, extrapolations to human twins need to be undertaken with considerable care. Nonetheless, we expect interest in this question to continue.

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