RESEARCH ARTICLE

# Reproductive Life Events of Twins with Opposite Sex

Jahanfar S.

**Authors' affiliations:**

PhD, Assistant Professor, School of Health Sciences Building 2239, Central Michigan University, Mount Pleasant, MI 48859, jahan2s@cmich.edu

**Corresponding author:** Shayesteh Jahanfar, PhD, Reproductive Epidemiologist, PhD (OB&GYN , Australia), PhD (Epi &Health Care, Canada)

Assistant Professor | School of Health Sciences | MPH Program
Health Professions 2239 | Central Michigan University | Mount Pleasant, MI 48859
P: 989-774-3909 | E: jahan2s@cmich.edu | F: 989-774-2908 | www.cmich.edu

**Abstract**

**Introduction**: It is hypothesized that androgens are transferred between fetuses across membranes, and have shown that female fetuses in female-male twin pairs have higher concentrations of serum testosterone than female fetuses in female-female pairs. The question is whether or not this hormonal transfer has any impact on adult reproductive life events.

**Objective**: The study objective was to test the hypothesis that females from female-female twins and females from female-male twins differ with respect to reproductive life events.

**Design and method**: We conducted a cross sectional study using volunteer female twins aged 15 and above from two settings of Malaysian and Iranian twin registries.

**Result**: Female-female twins had a higher frequency of congenital abnormality and hirsutism than male-female twins. Other reproductive events were not found to be statistically significantly different between the two groups.

**Conclusion**: Our study was distinctive in comparing specific reproductive events among females from female-male twins compared with female-female twins. Our results indicates that hormonal transition from male to female in a female-male gestation cannot be a valid explanation for reproductive ill health during adulthood.

## Introduction

Human studies of prenatal hormone effects were initially motivated by experimental studies in animals. Animal studies[1,2] have hypothesized that androgens are transferred between fetuses across membranes, and have shown that female fetuses in female-male twin pairs have higher concentrations of serum testosterone than female fetuses of female-female pairs. For instance, female rodent fetuses located between male fetuses have higher testosterone concentrations than do their sisters located between female fetuses. These hormonal imbalances can cause behavioral as well as morphological differences in adult rodents.[3] Human studies show similar phenomena occur in twins with opposite genders. The hypothesis behind these studies is called "intrauterine position effect" and suggests there is diffusion of steroid hormones from one fetus to another through direct transfusion or through the fetal membrane, placenta or amniotic fluid that could cause similarities in terms of hormonal balance.

The main body of findings in the studies shows that there is a degree of masculinization of women who had a male co-twin, and include differences in the auditory system, disinhibition or adventure seeking behavior.[4,5] Morphological differences including alterations in craniofacial growth and the size and type of molar teeth have been shown as examples of these changes.[6,7] Ryan (2002) suggests intrauterine position effect may impact a number of fields of scientific research such as endocrine disruption, toxicology, population biology, animal production and health.[8]

Phoenix et al. (1959) were the first to show that prenatal exposure to the steroid testosterone could alter brain structure and function and result in behavioral differences.[8] Prenatal testosterone exposure as a result of having an opposite gender twin in utero is suggested to cause eating disorders.[8] To examine whether in-utero testosterone exposure has masculinizing effects on disordered eating, the authors set up a comparison between male-female (n=59) and female-female twins (n=304). Results suggested that the disordered eating behavior in females from female-female twins is unlikely to be due to socialization effects alone. Biological factors, such as the masculinization of the central nervous system by prenatal testosterone exposure, may also contribute to sex differences in eating disorder prevalence.

Reproductive success has been also investigated in twins with different gender.[8] Results showed that daughters born with a male co-twin have reduced lifetime reproductive success compared to those born with a female co-twin. This reduction arises because such daughters have decreased probabilities of marrying as well as reduced fecundity. Mothers who produce opposite-sex twins consequently have fewer grandchildren (and hence lower fitness) than mothers who produce same-sex twins. These results are unlikely to be a consequence of females born with male co-twins receiving less nutrition because such females do not have reduced survival, and increases in food availability fail to improve their reproductive success. After-birth social factors (females growing up with similarly aged brothers) could not explain these results either because females born with a male co-twin have reduced success even when their co-twin died shortly after birth and they were raised as singletons after birth. These findings suggest that hormonal interactions between opposite-sex fetuses which are known to influence female morphology and behavior can also have negative effects on daughter fecundity and, hence, maternal fitness, and bear significant implications for adaptive sex allocation in mammals. This study concludes that female fetuses that are exposed to testosterone from adjacent male fetuses in utero can have masculinized anatomy and behavior.

The effect of in-utero hormonal influences on later behavioral development is also shown in a study of sensation seeking in 422 British twin pairs, where analysis demonstrated the predicted increase in sensation seeking in female members of opposite-sex pairs.[4] In contrast, male opposite-sex twins were not significantly different from male same-sex twins.

It is worth noting that previous studies did not take the role of age, sex and various aspects of masculinity-femininity in to consideration. Loehlin et al. (2000) rejected the previous findings as their investigation of a large cohort of 2,647 pairs of Australian twins did not support the female-male in utero effect hypothesis.[9] They suggested that no simple masculinization hypothesis—prenatal or postnatal—will adequately account for all the evidence. Since the literature lacks data on reproductive life events, the main objective of this study was to test the hypothesis that female same-sex and female opposite-sex twins differ with respect to reproductive events.

## Method

This was a cross-sectional and comparative study. Volunteer twins from two study settings (Iran and Malaysia) were included in the study. Details of these settings, recruitment of subjects, and data collection may be found elsewhere.[10] Female twins from 15-45 years of age (reproductive active years) were included in the study. There were 17 females who belonged to female-male twin pairs and 176 females from female-female twin pairs. This study was funded by University of Kuala Lumpur (UniKL), Royal College of Medicine, Perak in Ipoh and Avicenna Research Centre, Tehran.

Data collected were as follows: age, sex of twin, weight and height, birth weight, reproductive events, menstrual history [including duration (day), interval (day) and amount of menstruation (amount was clarified by the number of socked pads used per day)], and irregular menstrual bleeding. Regular menstrual cycles were defined as having regular periods every 21 to 35 days. Clinical symptoms for polycystic ovary syndrome (PCOS) were considered positive if subjects were suffering from hyper-androgenemia (hirsutism, acne), and chronic unovulation (amenorrhea, oligomenorrhea or irregular menstruation). Gynecological problems were considered inclusive of signs and symptoms (bleeding, pelvic pain, vaginal infection) that required referral to a gynecologist.

Reproductive behavior was assessed by asking the following questions: age of first pregnancy, sex of children, number of pregnancies and deliveries and abortions, and number of children. All participating twins in this study were living together during childhood. This was verified by asking them "were you living together during childhood?"

Descriptive analysis was done using SPSS version 20. A descriptive analysis was done using t-tests, Mann-Whitney U tests for quantitative variables and chi-square, and Fisher's Exact tests for qualitative data comparisons. A p-value less than 0.05 was considered significant.

## Result

A comparison was made between Female-Male (FM) twin sets and Female-Female (FF) twins (Table 1).

 

**Table 1.** A comparison between the mean ± SD of quantitative variables related to reproductive health (n=196). Mann-Whitney U test used when necessary *.

| Variable | FM (n=17) | FF (n=176) | P value |
|---|---|---|---|
| Age (Y) | 13.35±2.03 | 13.16±1.56 | 0.635 |
| Birth Weight (gr) | 2275±607 | 2375±421 | 0.650 |
| Age of menarche (Y) | 13.35±2.03 | 13.16±1.56 | 0.635 |
| Menstrual duration (days) | 5.88±1.69 | 5.81±1.24 | 0.814 |
| Menstrual interval (days) | 30.82±11.63 | 29.04±5.53 | 0.382 |
| Weight (kg) | 73.31±11.38 | 56.61±10.78 | 0.001 |
| Height (cm) | 162.29±8.88 | 157.53±7.24 | 0.004 |
| Age of marriage (Y) | 20.88±4.60 | 20.48±4.88 | 0.677 |
| Age of first pregnancy | 20.00±5.13 | 21.35±4.51 | 0.277 |
| Number of pregnancies | 4.38±2.22 | 2.67±1.91 | 0.001 |
| Number of deliveries | 3.69±1.99 | 2.32±1.58 | 0.002 |
| Number of abortions | 0.25±0.577 | 0.25±0.537 | 0.986 |
| Number of living children | 3.69±1.99 | 2.33±1.57 | 0.003 |
| Number of boys | 1.73±0.884 | 1.22±1.00 | 0.061 |
| Number of girls | 2.20±1.52 | 1.20±1.24 | 0.005 |

There was no significant difference between average age of FM twins (n=17) (13.35±2.03) and FF twins (n=176) (13.16±1.56) (p=0.635). There was no significant difference in the duration and interval of menstruation between the two groups of FM and FF twins, as shown in Table 1. Age of marriage and first pregnancy was also similar ($p > 0.05$). Females within the opposite-sex group were heavier (p=0.001) and taller (p=0.004) than females in the same-sex group. This was despite the fact that average birth weight was similar between the two groups.

The numbers of pregnancies, deliveries, living children, and gender of their children were found to be significantly higher among the FM group than the FF group. Adjustment with age was done for all the above comparisons (using linear regression analysis, where age was not found to be a significant element in determination of other variables), and results were found to be similar.

The number of subjects with congenital abnormality and hirsutism was significantly higher among FF twins. None of the subjects developed any type of cancer. FM subjects were heavier (73.31±11.38 v. 56.61±10.78; p=0.003) and taller than FF twins (162.29±8.88 vs. 157.53±7.24; p=0.001). BMI was found to be higher among FM twins than FF twins (28.19±3.67 vs. 22.66±4.67; P=0.001) (Table 2).

**Table 2:** A comparison between FM and FF group related to reproductive ill health using Chi-square test.

| Variable | FM (n=17) Number (%) | FF (n=176) Number (%) | P value |
|---|---|---|---|
| Congenital abnormality | 1(0.4) | 31(11.8) | 0.001* |
| PMS | 10(5.8) | 66(38.6) | 0.209 |
| Irregular menstruation | 2(1.1) | 23(12.2) | 0.781 |
| Abnormal menstrual amount | 4(2.2) | 23(12.8) | 0.306 |
| Acne | 3(1.4) | 30(14.4) | 0.250 |
| Hirsutism | 14(6.7) | 30(14.4) | 0.001 |
| Baldness | 3(1.4) | 15(7.2) | 0.566* |
| Infertility | 2(1.3) | 11(6.9) | 0.516* |
| PCOS | 1(0.5) | 17(8.9) | 0.504* |
| Gynecological problems | 6(3.1) | 44(22.9) | 0.459 |

*Fishers Exact test

Female-female twins had a higher rate of acne compared with that of FM twins but this difference was not statistically significant. Subjects with clinical symptoms of polycystic ovary syndrome (PCOS) had a higher BMI than PCOS negatives (24.56±3.36 vs. 22.67±4.85) but this difference was not significant and is not shown in the tables (p=0.132). Number of pregnancies (p=0.001) and deliveries (p=0.002) were higher among females from FM twin pairs compared to that of females from FF twin pairs. Females from FM twin pairs had more girls (p=0.005) than the comparative group.

## Discussion

Our study is unique in that we investigated variables related to reproductive events. Frequency of acne and hirsutism was found to be much higher among females from FF twin pairs than females from FM twin pairs, but the difference was not found to be significant.

 

Sex differences in congenital birth defects are often confounded by environmental risk factors. Opposite-sex twins provide a unique model for detecting sex differences in birth defects while maximally controlling environmental risk factors in a natural setting. A rather large retrospective study in the Florida Birth Defect Registry (n=4,768) has found that sex differences exist between opposite sex twins[11]. Our results, with limited sample size, found a higher prevalence of congenital abnormalities among females from FF twin pairs compared with females from FM twin pairs. This could be due to the impact of zygosity rather than gender.

Hirsutism was also found to be higher among females of FF twin pairs compared to the other group. We could not find any publication that investigates the association between hirsutism during adulthood with the sex at birth. Hirsutism in women is one of the early manifestations of virilization that is associated closely with elevated testosterone production. Testosterone production rates in normal women average 0.2 mg/day, secreted by the ovaries (25%), adrenals (25%), and peripheral metabolism of pre-hormones, notably androstenedione (50%). Increased testosterone from adrenal and/or ovarian sources induces 5 alpha-reductase activity within the susceptible hair follicle. This results in the local production of di-hydro-testosterone, which is responsible for the growth and stimulation of the hair follicle, and leads to hirsutism. An increase in exogenous androgens, primarily from diseases of the adrenals or ovaries, will lead to hirsutism. Our results refute the hypothesis that male hormones passed from male to female in utero changes the testosterone production in the future life of a female co-twin.

The frequency of reproductive events such as PMS, irregular menstruation, abnormal menstruation amount, baldness, infertility, clinical symptoms of PCOS (a combination of hyper-androgenism and irregular menstruation), and gynecological problems was much higher among FF twins than FM twins, but the difference was not found to be significant.

Animal work has suggested that opposite-sex fetuses can affect each other in utero. Differential hormone exposure during development may modulate brain structure and function, and thereby contribute to subsequent physiological variation within and between sexes. The study of opposite-sex twins provides a means of investigating prenatal hormonal influences on reproductive life events in humans. The best established and most theoretically explicable effects are of male fetuses on female fetuses (and male twins on female co-twins). A study of Australian twins investigated whether females in opposite-sex pairs were more masculine or less feminine than other females on sexually dimorphic traits.[12] The variables examined included sexual orientation, childhood sex-typical behavior, gender identity, personality traits, interest in casual sex, attitude toward homosexuality, juvenile delinquency, and handedness. Overall results indicated that opposite-sex twins were more sex-atypical in some respects than their same-sex dizygotic counterparts. There was a general tendency for males in opposite-sex twin pairs to be more feminized on measures of childhood sex-typical behavior, gender identity, and neuroticism. Males were significantly more masculine on the measure of juvenile criminal behavior. Females from opposite-sex twin pairs were more masculine on the measure of childhood sex-typical behavior.

The current study hypothesized that opposite sex twins could have an effect on reproductive events (such as premenstrual syndrome) and reproductive diseases (such as PCOS and gynecological problems). The pathogenesis could be the effect of sex hormones such as free testosterone and estrogen transferred from either twin to the other. These hormones are fat-soluble steroids and can be transported in the blood stream and through diffusion. Twin fetuses can receive a tremendous amount of sex hormones through the placenta and amniotic membranes from each other.[13,14] Both animal and human studies have shown significant consequences of individual morphology, physiology and behavioral changes within the recipient.[14,15] For instance, female rodents positioned between two males had higher serum testosterone levels than the control group (positioned between two female rodents).[14] According to the literature, human twins can also be influenced hormonally by the presence of an opposite sex co-twin.[16] Increased female growth in utero has been reported for those who have a male co-twin[17]; reported characteristics included masculinization of sexually dimorphic anatomical traits known to be sensitive to free serum testosterone level such as craniofacial growth, auditory system, canine size, visual acuity, and male-like behavior.[6,15]

Our results suggested that reproductive behavior characteristics such as number of pregnancies and deliveries were higher among females from FM twin pairs compared to that of females from FF twin pairs.

One study was found that looked at life-time reproductive success in humans. Lummaa et al. compared opposite sex twins with same sex co-twins.[16] This retrospective study of parishes during 1734-1888 from church registries of 377 twin births in Finland suggested that daughters born with a male co-twin have reduced lifetime reproductive success compared to those born with a female co-twin. Mothers who produced opposite-sex twins consequently had fewer grandchildren. The authors concluded that hormonal interactions between opposite-sex fetuses known to influence female morphology and behavior can have negative effects on daughter fecundity. Our findings, however, do not support Lummaa's study. It seems that hormones disturbing menstrual regularity or hyperandrogenism in females who shared an intrauterine environment with their brothers are under the influence of other factors such as environmental factors during early or late adulthood. Only a longitudinal study of twins' reproductive life study can confirm this hypothesis.

It has been shown that intrauterine over-exposure to androgens in the non-human primate leads to female offspring with PCOS-like traits.[18,19] Acquisition of testosterone from the male co-twin was suggested as a possible cause. If females from FM twin pairs have reduced fecundity through androgen excess produced by the male co-twin, and such overexposure causes PCOS-like symptoms, then a higher prevalence of PCOS among the females of FM twin pairs is expected. In our sample, contradictory to this theory, the frequency of PCOS was found to be higher in females from FF twin pairs compared with females from FM twin pairs. This high frequency among FF twin pairs was despite the fact that FM twins were heavier and had a higher BMI than FF twins ($p=0.001$). It can be concluded not only that intra-uterine hormonal influences are not responsible for PCOS phenotype, but also anthropometric measures are possibly not the determinant factors for PCOS clinical symptoms.

We found a higher frequency of pregnancies and deliveries among females of FM twin pairs compared with females of FF twin pairs. This result is contradictory to that found in the literature. Medland et al.[20] showed that in populations from Australia, The Netherlands and the United

 

States, there were no reproduction differences among female twins from same-sex (n=1,979) and opposite-sex (n=913) dizygotic (DZ) pairs. In all three samples, there were no differences in the number of children, age of first pregnancies, or psychological femininity between women from same-sex or opposite-sex twin pairs.[20]

**Summary**

Females from FF twin pairs had a higher possibility of congenital abnormality and hirsutism than females from FM twins. Our study was unique in comparing specific reproductive events in terms of twin gender. Hormonal interactions claimed to influence females were not seen in reproductive events and behavior studied in our opposite-sex twins. Further studies can compare opposite-gender twins who shared a placenta during intra-uterine life with those who had separate placentas.

**Conflict of interest:** None

**References**


1. Di Renzo, G. C. G. . *et al.* Does Fetal Sex Affect Pregnancy Outcome ? *Gend. Med.* **4,** 19–30 (2007).
2. Sheiner, E. *et al.* Gender does matter in perinatal medicine. *Fetal Diagn. Ther.* **19,** 366–9
3. KINSLEY, C. Intrauterine contiguity influences regulatory activity in adult female and male mice. *Horm. Behav.* **20,** 7–12 (1986).
4. Resnick, S. M., Gottesman, I. I. & McGue, M. Sensation seeking in opposite-sex twins: an effect of prenatal hormones? *Behav. Genet.* **23,** 323–329 (1993).
5. McFadden, D. A masculinizing effect on the auditory systems of human females having male co-twins. *Proc. Natl. Acad. Sci. U. S. A.* **90,** 11900–11904 (1993).
6. Dempsey, P. J., Townsend, G. C., Martin, N. G. & Neale, M. C. Genetic covariance structure of incisor crown size in twins. *J. Dent. Res.* **74,** 1389–1398 (1995).
7. Boklage, C. E. Interactions between opposite-sex dizygotic fetuses and the assumptions of Weinberg difference method epidemiology. *Am. J. Hum. Genet.* **37,** 591–605 (1985).
8. Ryan, B. C. & Vandenbergh, J. G. Intrauterine position effects. *Neurosci. Biobehav. Rev.* **26,** 665–678 (2002).
9. Loehlin, J. C. & Martin, N. G. Dimensions of psychological masculinity-femininity in adult twins from opposite-sex and same-sex pairs. *Behav. Genet.* **30,** 19–28 (2000).
10. Jahanfar, S., Lye, M.-S. & Krishnarajah, I. S. The heritability of premenstrual syndrome. *Twin Res. Hum. Genet.* **14,** 433–6 (2011).
11. Cui, W. *et al.* Sex differences in birth defects: a study of opposite-sex twins. *Birth Defects Res. A. Clin. Mol. Teratol.* **73,** 876–880 (2005).
12. Barbo, D. M. Reproductive health over the life phases: an overview. *Clin. Obstet. Gynecol.* **45,** 1181–1188 (2002).
13. Even, M. D., Dhar, M. G. & vom Saal, F. S. Transport of steroids between fetuses via amniotic fluid in relation to the intrauterine position


 

phenomenon in rats. *J. Reprod. Fertil.* **96,** 709–716 (1992).

14. Ryan, B. C. & Vandenbergh, J. G. Intrauterine position effects. *Neurosci. Biobehav. Rev.* **26,** 665–78 (2002).

15. Cohen-Bendahan, C. C. C., van de Beek, C. & Berenbaum, S. A. Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings. *Neurosci. Biobehav. Rev.* **29,** 353–384 (2005).

16. Lummaa, V., Pettay, J. E. & Russell, A. F. Male twins reduce fitness of female co-twins in humans. *Proc. Natl. Acad. Sci. U. S. A.* **104,** 10915–10920 (2007).

17. Blumrosen, E., Goldman, R. D. & Blickstein, I. Growth discordance and the effect of a male twin on birth weight of its female co-twin: a population-based study. *J. Perinat. Med.* **30,** 510–3 (2002).

18. Manuscript, A., Blood, P., Changes, P. & Retinopathy, D. NIH Public Access. **23,** 83–88 (2010).

19. Abbott, D. H., Padmanabhan, V. & Dumesic, D. A. Contributions of androgen and estrogen to fetal programming of ovarian dysfunction. *Reprod. Biol. Endocrinol.* **4,** 17 (2006).

20. Dash, P. K. *et al.* NIH Public Access. *Quest* **31,** 3148–3157 (2011).

 