

HHS Public Access

Author manuscript *Horm Behav*. Author manuscript; available in PMC 2015 May 27.

Published in final edited form as:

Horm Behav. 2015 March ; 69: 123-131. doi:10.1016/j.yhbeh.2015.01.007.

Academic performance of opposite-sex and same-sex twins in adolescence: A Danish national cohort study

Linda Ahrenfeldt^a, Inge Petersen^a, Wendy Johnson^b, and Kaare Christensen^{a,c}

Linda Ahrenfeldt: lahrenfeldt@health.sdu.dk; Inge Petersen: ipetersen@health.sdu.dk; Wendy Johnson: wendy.johnson@ed.ac.uk; Kaare Christensen: kchristensen@health.sdu.dk

^aThe Danish Twin Registry, Epidemiology, Biostatistics and Biodemography, The University of Southern Denmark, 5000 Odense, Denmark

^bDepartment of Psychology, The University of Edinburgh, Edinburgh, Scotland, UK

^cDepartment of Clinical Biochemistry and Pharmacology and Department of Clinical Genetics, Odense University Hospital, 5000 Odense, Denmark

Abstract

Testosterone is an important hormone in the sexual differentiation of the brain, contributing to differences in cognitive abilities between males and females. For instance, studies in clinical populations such as females with congenital adrenal hyperplasia (CAH) who are exposed to high levels of androgens in utero support arguments for prenatal testosterone effects on characteristics such as visuospatial cognition and behaviour. The comparison of opposite-sex (OS) and same-sex (SS) twin pairs can be used to help establish the role of prenatal testosterone. However, although some twin studies confirm a masculinizing effect of a male co-twin regarding for instance perception and cognition it remains unclear whether intra-uterine hormone transfer exists in humans. Our aim was to test the potential influences of testosterone on academic performance in OS twins. We compared ninth-grade test scores and teacher ratings of OS (n = 1812) and SS (n = 1812) 4054) twins as well as of twins and singletons (n = 13,900) in mathematics, physics/chemistry, Danish, and English. We found that males had significantly higher test scores in mathematics than females (.06–.15 SD), whereas females performed better in Danish (.33–.49 SD), English (.20 SD), and neatness (.45-.64 SD). However, we did not find that OS females performed better in mathematics than SS and singleton females, nor did they perform worse either in Danish or English. Scores for OS and SS males were similar in all topics. In conclusion, this study did not provide evidence for a masculinization of female twins with male co-twins with regard to academic performance in adolescence.

Keywords

Twins; Sex-difference; Testosterone; Behavior; Academic performance; Mathematics; School engagement

Correspondence to: Linda Ahrenfeldt, lahrenfeldt@health.sdu.dk. **Conflicts of interest** No conflicts of interest.

^{© 2015} Elsevier Inc. All rights reserved.

Introduction

In human foetuses, large sex differences in testosterone levels exist from early in gestation, and this hormone difference exerts permanent effects on brain development and behaviour (Hines, 2010). The literature regarding sex differences in cognitive abilities is reasonably consistent. Differences in quantitative abilities have received most attention because of the large sex differences in choice of professional careers in natural science and mathematics favouring males (Halpern et al., 2007). Males tend to outperform females on most measures of visuospatial abilities (though distributions overlap considerably, as is the case for all sex differences in cognitive abilities), which may contribute to the sex differences in test scores in mathematics and natural science (Halpern et al., 2007). However, the magnitude of this sex difference appears to increase with age (Bharadwaj et al., 2012; Hyde, 2005). It seems that the male advantage tends to emerge as the mathematical concepts being taught require more reasoning, more spatial abilities, and more complex problem-solving (Haworth et al., 2010; Hyde et al., 1990), though recent changes in the patterns suggest that cultural expectations also play a role (Lindberg et al., 2010). Conversely, sex-differences favouring females in verbal abilities such as reading, writing, and language usage are well documented in the literature (Halpern et al., 2007; Hedges and Nowell, 1995), and the superiority of females in verbal abilities continues into adulthood (Strand et al., 2006). While school achievement measures are not direct measures of abilities, they are generally strongly correlated with them (Bartels et al., 2002; Naglieri and Bornstein, 2003).

Human studies of prenatal hormone effects were initially motivated by experimental studies in animals. The study by Phoenix et al. (Phoenix et al., 1959) was the first to show that prenatal exposure to steroid testosterone could alter brain structure and function and result in behavioural differences (Phoenix, 2009). The study found that female guinea pigs that were exposed to testosterone prenatally showed masculinized behaviour in adulthood. Since then, several studies of non-human mammals have demonstrated effects of testosterone on neurobehavioural sexual differentiation (Constantinescu and Hines, 2012). Evidence that testosterone also influences human neurobehavioural development is to a great extent derived from studies of individuals who develop in atypical hormone environments (Constantinescu and Hines, 2012), and the best-studied clinical condition is congenital adrenal hyperplasia (CAH) (Cohen-Bendahan et al., 2005). Females with CAH, who produce high levels of adrenal androgens from early in gestation due to an autosomal recessive disorder, show increased male-typical behaviour and decreased female-typical behaviour despite postnatal hormone treatment (Hines, 2011). The most consistent findings have emerged from studies of childhood play. These studies found that females with CAH show increased male-typical and decreased female-typical toy, activity and playmate preferences (Cohen-Bendahan et al., 2005; Hines, 2011). Additionally, androgens also appear to affect cognition in females with CAH. A meta-analysis of nine samples (Puts et al., 2008) found that CAH females show higher spatial performance than do control females.

OS twins have been suggested to provide another opportunity to test the effects of prenatal testosterone exposure (Miller, 1994; Resnick et al., 1993), and higher testosterone in OS females is inferred on the basis of animal studies in e.g. rats and mice which have demonstrated that exposure to sex hormones is influenced by the intrauterine foetal position

(Ryan and Vandenbergh, 2002). Male foetuses have greater concentrations of testosterone than females, and females produce higher amounts of estradiol than males (vom Saal, 1989), but any foetus (male or female) located between two male foetuses has a higher concentration of testosterone than a female foetus located between two female foetuses (Ryan and Vandenbergh, 2002; vom Saal, 1989). This phenomenon results in females appearing masculinized in several anatomical, physiological and behavioural traits such as aggressive behaviour and reproductive organs (Ryan and Vandenbergh, 2002). Likewise, female foetuses that develop between other female foetuses show more feminized traits as adults, for example earlier vaginal opening (Ryan and Vandenbergh, 2002). Thus, intrauterine position and the possibility of steroid transfer of especially testosterone from one foetus to another during foetal life have effects in animals (Ryan and Vandenbergh, 2002; vom Saal, 1989).

The twin testosterone transfer (TTT) hypothesis reflects the possibility that human sex hormones is transferred between twins, most likely by diffusing across foetal membranes (Even and vom Saal, 1992). However, there is no direct evidence that females with a male co-twin have been exposed to sex-atypical hormone levels, and the literature on masculinization in OS female twins is inconsistent (Tapp et al., 2011). Some twin studies confirm the masculinizing effect of a male co-twin on females, for instance regarding physiological traits such as tooth size (Dempsey et al., 1999; Ribeiro et al., 2013), otoacoustic emissions (sounds produced by the inner ear) (McFadden, 1993), second-tofourth-finger-length ratio (van Anders et al., 2006), maternal fitness (Lummaa et al., 2007), and leukocytes telomere length (Benetos et al., 2014), but other large studies have reported negative findings (Gaist et al., 2000; Medland et al., 2008a; Medland et al., 2008b). Moreover, several studies have failed to find behavioural differences between OS and SS females including toy preferences (Henderson and Berenbaum, 1997; Rodgers et al., 1998), eating disorders (Baker et al., 2009; Raevuori et al., 2008), social behaviour and friendship in preschool children (Laffey-Ardley and Thorpe, 2006), and autistic symptomatology (Ho et al., 2005). However, two studies have found greater sensation-seeking, including experience-seeking, in OS females compared with SS females (Resnick et al., 1993; Slutske et al., 2011). These latter findings suggest effects of hormone exposures on later behavioural development, although psychosocial explanations cannot be excluded (Resnick et al., 1993).

More evidence exists for the effect of being an OS twin on cognitive and perceptual abilities than for other sex-typed characteristics (Tapp et al., 2011). In agreement with earlier CAH studies (Berenbaum et al., 2012; Puts et al., 2008), recent co-twin studies (Heil et al., 2011; Tapp et al., 2011; Vuoksimaa et al., 2010) provide evidence for effects of prenatal testosterone on cognitive abilities. According to two twin studies that included 200 and 471 study participants, respectively (Heil et al., 2011; Vuoksimaa et al., 2010), OS females were found to have higher mental rotation (MRT) ability (the ability to imagine objects from a perspective other than the one depicted) than SS and singleton females. One of the studies (Heil et al., 2011) also demonstrated that OS females have higher mental rotation performance than non-twin females raised with a slightly elder brother (born within 18 months) which helps exclude possible socialisation effects of growing up with a twin brother.

Most studies that have investigated co-twin effects in males, have failed to identify differences between OS and SS male twins in the direction predicted by the TTT hypothesis (Cohen-Bendahan et al., 2005; Tapp et al., 2011). However, animal studies (Ryan and Vandenbergh, 2002; vom Saal, 1989) and limited evidence from human studies suggest that excess testosterone in males might further masculinize traits such as disordered eating (Culbert et al., 2008) and brain volume (Peper et al., 2009). Moreover, one study (Ho et al., 2005) reported that sub-threshold autistic symptomatology rated by parents was higher in SS than in OS male twins aged 7 to 15, which may support the TTT hypothesis.

Outcome used in the present study is ninth grade test scores and teacher assessment of academic performance (outcomes which are of great importance for both the twins and their parents). Academic achievement is different from abilities and potentially more likely to be influenced by environmental/social factors (Bacete and Remirez, 2001). However, a high correlation (.70 to .74 in average) between standardised achievement tests and IQ had been shown (Naglieri and Bornstein, 2003), and the correlation between IQ and national achievement tests, such as the test used here, have similar correlations among 12-year-old school children (Bartels et al., 2002). Studies comparing the academic performance of OS and SS twins are scarce. Only one recent study of 13,368 twins and 837,752 singletons born during 1973–1981 in Sweden has investigated differences between OS and SS twins in grade point average (Hjern et al., 2012). The study found that SS twins of both sexes had slightly higher average scores in ninth grade than OS twins, and that they more often than OS twins had attained a university degree by the age of 27–35. However, the differences in grade point average were very small and non-significant, and the authors did not include topic specific analyses and did not discuss possible reasons for their results.

The overall aim of this study was to test the potential influences of testosterone on academic performance in OS twins. The reason for studying OS vs SS twins was to test behavioural effects of prenatal hormone, and therefore the effects of co-twin sex should be tested on measures that show sex differences such as academic performance in adolescence. Additionally, the evidence on spatial abilities in females with CAH supports arguments for prenatal testosterone effects on mathematical abilities (Halpern et al., 2007). This study relies on a large sample size with the existence of control variables such as parental education. Furthermore academic performance was analysed as topics, resulting in more detailed investigation of possible sex differences and whether these differences are transmitted to differences of OS and SS twins. Our primary objective was to compare academic performance in mathematics, physics/chemistry, Danish and English for female and male members of OS and SS twin pairs to test for an interaction between sex and OS/SS status. Secondly, we examined whether the OS/SS twin groups differed in performance from the general population. This was achieved by comparing the OS twins and the SS twins born in Denmark during 1986-1990 for each sex, with a 5% random sample of singletons born in Denmark during the same period and surviving until January 1, 2003.

Material and methods

Material

We included information from four registers: the Danish Demographic Database, including information on parental identities, deaths, migrations, and adoptions (Petersen, 2000); the Integrated Database for Labour Market Research containing information on the highest obtained parental education (Petersson et al., 2011); the register of Compulsory School Completion Assessments and Test Scores compiled by the Danish Ministry of Education (Education, 2000) and the Danish Twin Registry, which comprises more than 85,000 Danish twin pairs born since 1870 (Skytthe et al., 2011), with all twins born after 1973 identified through the Medical Birth Registry (Skytthe et al., 2002). Zygosity determination of samesex twin pairs was based on four standard questions about similarity of appearance, a method with less than 5% misclassification (Christiansen et al., 2003), though responses to these questions were missing for 27% of the SS twin pairs, who were classified as twins of unknown zygosity (ssUZ). Linkage of registers was enabled due to the Danish Civil Registration System where each citizen is assigned a personal identification number at birth (Pedersen, 2011).

School achievements were available for the years 2002–2006 for the ninth-grade students aged 15–16 at test, corresponding to the 1986–1990 birth cohorts. Most Danish students in ninth grade completed standardised, nationwide tests of academic achievement in several subject areas, scored on a scale of 0–13. Average performance was rated as 8, higher scores indicating better performance (Christensen et al., 2006). Teacher ratings in ninth grade supplemented the test scores, also scored on the 0–13 scale. These ratings were subjective evaluations of the students' overall academic performance during the academic year whereas test scores reflected actual student performance rated by the teachers and/or external examiners at specific times on specific tests (Petersen et al., 2009). The test scores and teacher ratings covered major domains of academic achievement such as mathematics and Danish, which were the main topics included in the present study. Oral and written exercises, neatness, and Danish spelling were graded. In addition, physics/chemistry and English oral exercise grades were recorded.

We distinguished between non-attainment and missing scores. Non-attainment refers to the condition of not having attained any scores at all. Reasons for non-attainment were multiple and included drop-out or attendance at a specialised school due to disabilities. Schools for children with learning disabilities were not required to report test scores and teacher ratings used for statistics to the Ministry of Education. In addition, some private schools such as those following the Waldorf pedagogy as a principle did not test at all (Christensen et al., 2006; Petersen et al., 2009). Test scores were considered missing when an individual had attained some scores but not all. As the ninth grade exams were not mandatory between 1993 and 2006 in Denmark (Education, 1993; Lærerforening, 2014), a small proportion of students in public schools opted not to take some of the tests. Finally, there could be missing teacher ratings due to failures in reporting to the Ministry of Education. For individuals with missing scores, the available data were retained in analyses.

Participants

We used data from all twins whose co-twins had survived to the age of at least 17 to make sure that virtually all of the twins in the present study had grown up together in Denmark as members of intact twin pairs. Individuals who had lived abroad for more than two years during the age range 6–14 as well as those who had emigrated but returned to Denmark after age 14 were excluded from the analyses. Eighty-four twin individuals were excluded from the outset because the co-twin was stillborn. In addition, 396 individuals were excluded due to death before age 17, including 196 twins (45 twin pairs and 106 twin individuals); 106 twins were excluded due to death of the co-twin, and 508 individuals were excluded as they had emigrated during the study period. One twin was excluded because the twin pair had been separated by emigration. Thus, the study base consisted of 2941 female and 2926 male twins as well as 6771 female and 7129 male singletons. All analyses of OS and SS twins as well as of twins and singletons were conducted separately for each sex.

Statistical methods and confounders

In this study, both monozygotic (MZ) and dizygotic (DZ) twins were included in the SS twin group. This was done because of the rather large proportion (approximately 27%) of the sample being twins of unknown zygosity (ssUZ), which made it impossible to allocate all the SS twins to a zygosity group. Supporting the inappropriateness of dropping the ssUZ twins, a study consisting of 2,413 Danish twin pairs from birth cohorts 1986–1990 found that ssUZ twins averaged lower school achievement scores than did twins of known zygosity (Petersen et al., 2009). Because OS twins are always identifiable, these lower-achieving twins would not be dropped from the OS group, as exclusion of the ssUZ twins from the SS group would have biased the results towards better performance for the SS twins. Considering MZ and DZ twins together should not bias group comparisons assuming that the MZ and ssDZ twins did not differ on the outcome variables. To check the robustness of the results we repeated all analyses excluding twins with known MZ status (results not shown).

Analyses of differences in categorical baseline characteristics (mortality, emigration, death of a co-twin and parental education) for OS and SS twins and for twins and singletons were tested by chi-square tests. Parental educational attainments were coded as categorical variables for the highest obtained education by December 31, 2002. By that date, the median ages were 42.5 years for mothers and 45.1 years for fathers. The educational variables were coded from 0 to 5, corresponding to the following categories: Basic school 8th-10th grades, vocational school, secondary education, short higher education, medium higher education or Bachelor's degree, and higher academic or professional degree. Differences in continuous background variables (birth weight and parental age) were investigated using t-tests. Effect size differences for covariates (maternal and paternal age and education) for twins and singletons with at least one test score were performed applying ordinary linear least-square regression (OLS) to both the raw data and the data adjusted for the covariates maternal and paternal age and education. Parental age and education were independently associated with test scores (Supplementary Tables 1 and 2). Controlling them in our models did slightly change the effect estimates. Thus, we present all results both with and without adjustments for parental age and education.

We considered birth weight an intermediate factor in the association between OS/SS twins and academic performance. There is some indication that the birth weight of twins might be influenced by the sex of their co-twin (Glinianaia et al., 1998; Luke et al., 2005), and we found significantly higher birth weight for OS compared with SS twins for both sexes (Table 1). Moreover, previous studies have demonstrated a positive relationship between birth weight and intelligence in later life (Matte et al., 2001; Richards et al., 2001; Sorensen et al., 1997). The same trend has been observed in studies of school achievements among Danish twins and singletons from the birth cohorts 1986–1990 (Christensen et al., 2006; Petersen et al., 2009). In the present study we were interested in the overall effects on academic performance of having a co-twin of the opposite sex compared with having a co-twin of the same sex, and therefore adjustment for variables on the causal pathway were unwarranted (Hernandez-Diaz et al., 2006). However, all analyses were repeated including birth weight (results not shown), and the results were similar.

Logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) for test score non-attainment. We did this separately for males and females: OS and SS twins, and OS/SS twins and singletons, respectively. Analysis of differences in test scores and teacher rating between females and males, OS and SS twins, and OS/SS twins and singletons was done using OSL, with separate models for mathematics (oral, written, and neatness), oral physics/chemistry, Danish (oral, written, spelling, and neatness) and oral English. We used the standardised mean difference, d, to evaluate differences in means. This was calculated as the difference between the mean scores controlling for covariates divided by the within-sex standard deviation in the total study population in the specific topic (i.e. d is the measure of mean difference in units of the SD). For gender, a positive value of d implies that males scored higher, and a negative value implies that females scored higher on average. For OS vs SS twins a positive value of d means that OS scored higher on average, and for twins vs singletons, a positive value of d means that twins scored higher on average.

Sex differences were investigated for the full sample consisting of both twins and singletons. In addition, sex differences were investigated in the sample of all twins and repeated in the sample of only SS twins. To account for the intra-pair correlations when estimating standard errors, the cluster option in Stata (release 13) was used.

Results

Table 1 presents the numbers of live births among twins and the 5% random sample of singletons born in Denmark 1986–1990, along with birth weight, parental age and education information. Mortality in the entire period (before age 17) was not significantly different between OS and SS female twins: difference (in percentage) –1.08% (95% CI: –2.31 to . 14%), but mortality was lower for OS than SS males: –1.59% (95% CI: –2.76 to –.42%). Mortality before age 17 was significantly higher for twins than for singletons: difference 2.30% (95% CI: 1.66 to 2.94%) for females and 1.82% (95% CI: 1.17 to 2.48%) for males. There was a tendency towards higher proportions of OS twins compared with SS twins who emigrated: difference .87% (95% CI: –.05 to 1.79%), but the proportions of twins and singletons who emigrated were similar: .14% (95% CI: –.33 to .62%).

than mothers of OS twins:

Page 8

Mothers of SS twins were significantly younger at childbirth than mothers of OS twins: mean difference (in years) -.88y (95% CI: -1.13 to -.62y). A similar pattern was observed in fathers: -1.09y (95% CI: -1.41 to -.77y). Mothers of singletons were significantly younger than mothers of twins: mean difference -1.15y (95% CI: -1.29 to -1.00y), as were fathers of twins: -.99y (95% CI: -1.17 to -.81y). Mothers of SS twins had slightly shorter education than mothers of OS twins (p < .001), and the same pattern was found for fathers (p = .03). Mothers of singletons had slightly shorter education than mothers of twins (p = .02). The same pattern was found for fathers (p = .08).

Non-attainment

Table 2 presents OR and 95% CI for test score non-attainment, before and after adjustment for parental age and education. Males had significantly higher risk of test score non-attainment than females, adjusted OR 1.70 (95% CI: 1.54 to 1.88). Risks of non-attainment were similar in OS and SS females, adjusted OR 1.05 (95% CI: .76 to 1.47) and in OS and SS male twins, adjusted OR 1.10 (95% CI: .83 to 1.46). Likewise, no significant differences in risks of non-attainment were found between OS/SS twins and singletons.

Similar patterns of differences in test scores and teacher ratings for those with missing scores and those with all scores available were found for OS and SS twins as well as for singletons, suggesting that the relative frequencies of missingness did not differ with twin type or singleton birth status (not shown).

Academic performance

Table 1 also presents summary statistics for the academic performance scores. Overall, there were high degrees of similarity between OS and SS twins and between twins and singletons for both sexes. Table 3 and Supplementary Table 3 provide further information regarding the effect sizes of differences, before and after statistical adjustment for potentially confounding variables.

Males vs females

Overall, male twins and singletons showed significantly higher ninth-grade test scores in mathematics than females (Table 3); however, the effect sizes were small (adjusted difference .6–.15 SD). Conversely, females had slightly higher teacher ratings in mathematics than males. Test performances in physics/chemistry were similar for males and females. Unlike mathematics, males had significantly lower scores in Danish than females. All mean differences in Danish oral, written, spelling, and neatness for test scores and teacher ratings were significant (all p-values < .001) with moderate effect sizes (.33–.49 SD). A similar pattern was found in English, with males having significantly lower scores than females, but the effect size was small (.20–.27 SD). In addition, males received lower marks for neatness than females in both mathematics (.64–.72 SD) and Danish (.45–.67 SD) test scores as well as teacher ratings. The patterns of sex differences were the same in singletons and in the full sample consisting of twins and singletons. In twins, the overall patterns of sex differences were also identical with those of the full sample even when excluding the OS twins (not shown).

OS vs SS twins, and twins vs singletons

Female OS twins attained significantly lower scores in mathematics than SS twins (adjusted difference .12–.14 SD) (Table 3). There were similar differences in the teacher ratings. SS female twins had similar mathematics scores as singleton females, whereas OS females had significantly lower test scores than singleton females after adjustments in both oral and written mathematics (.13–.14). After excluding female twins with known MZ status we still found that OS females had slightly lower performance than SS females in mathematics; however, the estimates were not significant due to slightly reduced effect size and smaller sample size (not shown).

Performance was similar in OS and SS female twins in all aspects of Danish and English before and after adjustments; however, there was a slight tendency, though not significant, towards OS females having lower performance than SS and singleton females in all adjusted analyses, which would be consistent with a slightly lower OS performance overall. OS females had significantly lower adjusted test scores in Danish oral, written and spelling (. 08–.09 SD) compared with singleton females, as well as in the adjusted written Danish and spelling test and teacher-rating scores (.08–.13 SD). SS females had similar performance as singleton females except for a slightly lower performance in written Danish (.07 SD).

OS females had slightly lower teacher ratings than SS females in neatness in mathematics: adjusted difference .12 SD. SS females had the same mean scores as singletons, but OS females scored lower than singletons (.12 SD).

OS and SS male twins showed no significant differences in any area of performance, either before or after adjustments (Supplementary Table 3). Performance was similar for twins and singletons after adjustments in all aspects of mathematics, physics/chemistry, and Danish. Only in English, twin males attained significantly lower test scores than singleton males, but the effect sizes were small (.08–.09 SD).

Parents' age and education

The raw associations between the potential confounding variables and the test scores showed significantly higher performance with higher maternal and paternal age and education in both sexes (Supplementary Tables 1 and 2). When all the potential confounders were included in the regression model, the associations among confounders and test scores were attenuated. However, although these variables are overlapping and adjustment for all of them simultaneously will reduce their effects individually, it does not mean that they are no longer important. The tables show, e.g., that most of the effect of maternal age persisted after adjustment, and the effect of parental education on test scores was still highly significant after adjustment for all the other potential confounders.

Discussion

In this nationwide study of Danish adolescent twins and singletons, we found, as expected, that sex differences in academic achievement were dependent on topics. Males had higher test scores in mathematics than females (.06–.15 SD), whereas females performed significantly better in Danish (.33–.49 SD), English (.20 SD), and neatness (.44–.64 SD).

We hypothesized that OS female twins may be masculinized in academic performance due to testosterone exposure from the male co-twin in utero. However, we did not find that OS females performed better in mathematics than SS females. In addition, in Danish and English, where females on average performed better than males, no significant difference was found between OS and SS females. Only with regard to teacher ratings of neatness in mathematics did OS females resemble males when compared with SS females, but the difference was small (.12 SD). Scores for OS and SS males were similar in all topics.

The effect sizes for sex differences in mathematics in the present study were very small (. 06–.15) in magnitude, which was in accordance with previous literature showing that sex differences in average mathematical tests tend to be small (Else-Quest et al., 2010; Lindberg et al., 2010; Stoet and Geary, 2013). A small sex difference makes it harder to detect potential differences between OS and SS twins (Cohen-Bendahan et al., 2005). However, the size of the difference in mathematics scores between OS and SS females was comparable with that of the sex difference, but in the opposite direction. Sex differences in reading favouring females (app .2–.6 SD) exist across the globe (Hedges and Nowell, 1995; Reilly, 2012; Stoet and Geary, 2013). We did not investigate reading directly, but the standard mean difference between males and females in oral Danish was .35 SD. For writing we found a difference of .49 SD corresponding to the literature showing that sex differences in writing tasks are even larger than those of reading (Hedges and Nowell, 1995).

Although this study did not provide evidence of masculinization of female OS twins in academic performance in adolescence, our findings did not necessarily contradict earlier positive findings of prenatal masculinization in other traits, such as those replicating the male advantage in mental rotation ability in OS females (Heil et al., 2011; Vuoksimaa et al., 2010). The reason is that the tasks on which the students' scores and ratings were based did not rely upon the same ability as the mental rotation scores in any direct way. The finding of no differences between OS and SS males was in accordance with other studies investigating perceptual and cognitive traits (McFadden, 1993; McFadden et al., 1996; Vuoksimaa et al., 2010).

The values of twins for testing the effects of prenatal testosterone can be challenged. Transfer of testosterone is assumed, based on animal studies of intrauterine position, but these studies show that masculinization is most likely for female foetuses that gestate between two male foetuses, with smaller effects for those that gestate next to just one male foetus (Ryan and Vandenbergh, 2002), raising scepticism about sufficient hormone transfer in human pregnancies to affect physiology or behaviour. The literature is far from consistent and recent studies fail to see masculinization for a variety of personality and fertility traits such as anthropometric measures and fertility (Korsoff et al., 2014), birth weight (Sorensen et al., 2013; Tul et al., 2012), eating disorders (Lydecker et al., 2012), and age at menarche (Sorensen et al., 2013). However, the recent review on OS vs SS studies (Tapp et al., 2011) found the most consistent evidence for hormonal transfer in studies investigating cognitive traits in line with recent evidence from clinical studies linking early androgen exposure to spatial abilities (Puts et al., 2008). Nevertheless, there are evidence that females with CAH show larger and more consistent effects on activity interests than on spatial ability (Hines, 2010), which may show psychological mechanisms because prenatal testosterone might

affect predispositions to engage in activities, e.g. childhood plays that influence spatial ability (Berenbaum et al., 2012). Thus, recent evidence indicates nuanced interpretations of how prenatal androgens influence brain development (Miller and Halpern, 2014).

An alternative explanation to prenatal hormone effects is postnatal socialization effects. Apart from possible differences in prenatal hormone exposure, OS and SS twins may also be different due to different psychosocial rearing environments (Cohen-Bendahan et al., 2005). OS twins have more frequent interaction with, and have the opportunity for social imitative learning from a sibling of the opposite sex (Pulkkinen et al., 2003), and evidence from nontwin families suggest sex-typed effects from an older sibling (McHale et al., 2001). However, two twin studies of activity interests (toy play), one of which used sib comparisons as a control group for the social environment (Henderson and Berenbaum, 1997), failed to detect differences between children with OS vs SS co-twins regarding the amount of time spent playing with feminine, masculine, and neutral stereotyped toys (Henderson and Berenbaum, 1997; Rodgers et al., 1998). Additionally, twin studies with siblings as a control group for the psychosocial environment also failed to find evidence for socialization effects (Heil et al., 2011; Slutske et al., 2011). In general, there is sparse evidence regarding twin social interactions; however, indications of differences in socialisation in OS compared with SS twins are present (Pulkkinen et al., 2003).

Many factors may influence test scores. A previous Danish study comparing academic performance between twins and singletons born 1986 –1988 confirmed a number of potential confounding factors, including parental age and education (Christensen et al., 2006). Evidence suggests that parental age may influence cognitive ability of offspring (Edwards and Roff, 2010; Malaspina et al., 2005; Saha et al., 2009) and that parental education, a surrogate of socioeconomic status, is associated with offspring academic performance as well (Sirin, 2005). In this study, we showed that higher parental age and education were associated with higher academic achievement in both sexes (Supplementary Tables 1 and 2), and that parents of OS twins were older on average and had slightly higher education than the parents of SS twins (Table 1). However, the crude and adjusted estimates were approximately similar, indicating that the small differences in parental age and education did not change the results to any substantial degree.

In this study comparing OS and SS twins it was also important to investigate differences between singletons and OS and/or SS twins, because these comparisons can disclose whether the OS/SS twin groups differed in performance from the general population. Our results were consistent with those of a recent Swedish study showing similarity between twins and singletons in ninth-grade school achievement (Hjern et al., 2012). Moreover, they were in accordance with our previous Danish study that showed similar mean difference in academic performance in adolescence in twins and singletons (Christensen et al., 2006). However, because the present study investigated more birth cohorts and investigated academic performance in specific topics and subtopics as well as differences between subgroups of twins compared with singletons, there were small differences between the results of the present study and the study by Christensen et al.

Among the strengths of the study were the large nationwide register-base with minimal selection criteria and inclusion of information on important potential confounders for the majority of twins. Another advantage was the classification of academic performance into topics and subtopics, resulting in a more detailed investigation of the masculinizing effects among OS females and SS males.

The large group of twins with unknown zygosity (approximately 27%) was a limitation of this study because the unknown zygosity of these SS twins made it impossible to exclude the MZ twins and thus make the most valid test of the TTT hypothesis, which is a comparison of only ssDZ with OS twins (Cohen-Bendahan et al., 2005). However, excluding twins with known MZ status does only change the point estimates marginally. According to a meta-analysis, differences in intelligence between twins and singletons were not influenced by zygosity status (Voracek and Haubner, 2008), and this also applied to academic achievement in a recent study of approximately 10,000 Dutch twins (de Zeeuw et al., 2012).

A further limitation was that control for fertility treatments such as in vitro fertilisation (IVF) was not possible. A higher proportion of IVF children would be expected in the OS than in our SS group that consisted of both MZ and DZ twins, because many multiple births in IVF take place as a result of implantation of multiple fertilized eggs. These generate DZ twins, and all OS twins are DZ. Parents who undergo IVF treatment tend to have higher socioeconomic position than average (Hjern et al., 2012). This is consistent with the higher average age and educational level of parents of OS twins than parents of SS twins (Table 1), which may favour OS twins regarding academic performance. On the other hand, if IVF treatment influences cognitive outcomes negatively, we would expect lower performance in OS than SS twins. However, natural multiple ovulation in older age is another reason for the increase in twinning rate since the mid-1980s as females have waited longer to start their families (Blondel and Kaminski, 2002), and it is most likely that only a small proportion of the twins in our sample had been born through in vitro fertilisation because the dramatic rise in its use took place in the 1990s (Herskind et al., 2005). In addition, no differences were found in studies comparing cognitive development and school performance in IVF and naturally conceived children, controlling for parental education (Mains et al., 2010; Wagenaar et al., 2008).

Missing data was another limitation; however, a similar pattern of differences in test scores for those with missing scores vs those with all scores available was found for SS and OS twins as well as for singletons.

Conclusions

In conclusion, we found no evidence that having a male co-twin has a masculinizing effect on females with regard to academic performance in adolescence. Scores for male twins were not dependent on whether they had a twin sister or brother.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by research Grants from the National Institute on Aging (NIA-PO1-AG08761, NIAP01-AG031719) and from the European Union's Seventh Framework Programme (FP7/2007–2011) under grant agreement no 259679.

References

- Bacete FJG, Remirez JR. Family and personal correlates of academic achievement. Psychol Rep. 2001; 88:533–547. [PubMed: 11351904]
- Baker JH, Lichtenstein P, Kendler KS. Intrauterine testosterone exposure and risk for disordered eating. Br J Psychiatry. 2009; 194:375–376. [PubMed: 19336794]
- Bartels M, Rietveld MJ, Van Baal GC, Boomsma DI. Heritability of educational achievement in 12year-olds and the overlap with cognitive ability. Twin Res. 2002; 5:544–553. [PubMed: 12573186]
- Benetos A, Dalgard C, Labat C, Kark JD, Verhulst S, Christensen K, Kimura M, Horvath K, Kyvik KO, Aviv A. Sex difference in leukocyte telomere length is ablated in opposite-sex co-twins. Int J Epidemiol. 2014; 43:1799–1805. [PubMed: 25056338]
- Berenbaum SA, Bryk KL, Beltz AM. Early androgen effects on spatial and mechanical abilities: evidence from congenital adrenal hyperplasia. Behav Neurosci. 2012; 126:86–96. [PubMed: 22289044]
- Bharadwaj, P.; De Giorgi, G.; Hansen, D.; Neilson, C. The Gender Gap In Mathematics: Evidence From Low- And Middle-Income Countries. National Bureau of Economic Research; Cambridge, Mass: 2012.
- Blondel B, Kaminski M. Trends in the occurrence, determinants, and consequences of multiple births. Semin Perinatol. 2002; 26:239–249. [PubMed: 12211614]
- Christensen K, Petersen I, Skytthe A, Herskind AM, McGue M, Bingley P. Comparison of academic performance of twins and singletons in adolescence: follow-up study. BMJ. 2006; 333:1095. [PubMed: 17012267]
- Christiansen L, Frederiksen H, Schousboe K, Skytthe A, von Wurmb-Schwark N, Christensen K, Kyvik K. Age- and sex-differences in the validity of questionnaire-based zygosity in twins. Twin Res. 2003; 6:275–278. [PubMed: 14511432]
- Cohen-Bendahan CC, van de Beek C, Berenbaum SA. Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings. Neurosci Biobehav Rev. 2005; 29:353–384. [PubMed: 15811504]
- Constantinescu M, Hines M. Relating prenatal testosterone exposure to postnatal behavior in typically developing children: methods and findings. Child Dev Perspect. 2012; 6:407–413.
- Culbert KM, Breedlove SM, Burt SA, Klump KL. Prenatal hormone exposure and risk for eating disorders: a comparison of opposite-sex and same-sex twins. Arch Gen Psychiatry. 2008; 65:329– 336. [PubMed: 18316679]
- de Zeeuw EL, van Beijsterveldt CE, de Geus EJ, Boomsma DI. Twin specific risk factors in primary school achievements. Twin Res Hum Genet. 2012; 15:107–115. [PubMed: 22784460]
- Dempsey PJ, Townsend GC, Richards LC. Increased tooth crown size in females with twin brothers: evidence for hormonal diffusion between human twins *in utero*. Am J Hum Biol. 1999; 11:577–586. [PubMed: 11533976]
- Education, D.M.o. Law Of Public School [In Danish]. Danish Ministry of Education; Copenhagen: 1993.
- Education, D.M.o. Tests, Assessment And Teaching: An Overall Evaluation Of Compulsory School Completion Assessment And Test Scores May–June 2004 [In Danish]. Danish Ministry of Education; Copenhagen: 2000.
- Edwards RD, Roff J. Negative effects of paternal age on children's neurocognitive outcomes can be explained by maternal education and number of siblings. PLoS One. 2010; 5:e12157. [PubMed: 20856853]
- Else-Quest NM, Hyde JS, Linn MC. Cross-national patterns of gender differences in mathematics: a meta-analysis. Psychol Bull. 2010; 136:103–127. [PubMed: 20063928]

- Even MD, vom Saal FS. Seminal vesicle and preputial gland response to steroids in adult male mice is influenced by prior intrauterine position. Physiol Behav. 1992; 51:11–16. [PubMed: 1741435]
- Gaist D, Bathum L, Skytthe A, Jensen TK, McGue M, Vaupel JW, Christensen K. Strength and anthropometric measures in identical and fraternal twins: no evidence of masculinization of females with male co-twins. Epidemiology. 2000; 11:340–343. [PubMed: 10784255]
- Glinianaia SV, Magnus P, Harris JR, Tambs K. Is there a consequence for fetal growth of having an unlike-sexed cohabitant *in utero*? Int J Epidemiol. 1998; 27:657–659. [PubMed: 9758121]
- Halpern DF, Benbow CP, Geary DC, Gur RC, Hyde JS, Gernsbacher MA. The science of sex differences in science and mathematics. Psychol Sci Public Interest. 2007; 8:1–51. [PubMed: 25530726]
- Haworth CM, Dale PS, Plomin R. Sex differences in school science performance from middle childhood to early adolescence. Int J Educ Res. 2010; 49:92–101. [PubMed: 21499451]
- Hedges LV, Nowell A. Sex differences in mental test scores, variability, and numbers of high-scoring individuals. Science. 1995; 269:41–45. [PubMed: 7604277]
- Heil M, Kavsek M, Rolke B, Beste C, Jansen P. Mental rotation in female fraternal twins: evidence for intra-uterine hormone transfer? Biol Psychol. 2011; 86:90–93. [PubMed: 21094200]
- Henderson BA, Berenbaum SA. Sex-typed play in opposite-sex twins. Dev Psychobiol. 1997; 31:115– 123. [PubMed: 9298637]
- Hernandez-Diaz S, Schisterman EF, Hernan MA. The birth weight "paradox" uncovered? Am J Epidemiol. 2006; 164:1115–1120. [PubMed: 16931543]
- Herskind AM, Basso O, Olsen J, Skytthe A, Christensen K. Is the natural twinning rate still declining? Epidemiology. 2005; 16:591–592. [PubMed: 15951683]
- Hines M. Sex-related variation in human behavior and the brain. Trends Cogn Sci. 2010; 14:448–456. [PubMed: 20724210]
- Hines M. Gender development and the human brain. Annu Rev Neurosci. 2011; 34:69–88. [PubMed: 21438685]
- Hjern A, Ekeus C, Rasmussen F, Lindblad F. Educational achievement and vocational career in twins — a Swedish national cohort study. Acta Paediatr. 2012; 101:591–596. [PubMed: 22353254]
- Ho A, Todd RD, Constantino JN. Brief report: autistic traits in twins vs. non-twins—a preliminary study. J Autism Dev Disord. 2005; 35:129–133. [PubMed: 15796128]
- Hyde JS. The gender similarities hypothesis. Am Psychol. 2005; 60:581-592. [PubMed: 16173891]
- Hyde JS, Fennema E, Lamon SJ. Gender differences in mathematics performance: a meta-analysis. Psychol Bull. 1990; 107:139–155. [PubMed: 2138794]
- Korsoff P, Bogl LH, Korhonen P, Kangas AJ, Soininen P, Ala-Korpela M, Rose RJ, Kaaja R, Kaprio J. A comparison of anthropometric, metabolic, and reproductive characteristics of young adult women from opposite-sex and same-sex twin pairs. Front Endocrinol. 2014; 5:28.
- Lærerforening, D. Undervisning Skolestruktur og lovændringer [in Danish]. 2014. http://www.dlf.org
- Laffey-Ardley S, Thorpe K. Being opposite: is there advantage for social competence and friendships in being an opposite-sex twin? Twin Res Hum Genet. 2006; 9:131–140. [PubMed: 16611478]
- Lindberg SM, Hyde JS, Petersen JL, Linn MC. New trends in gender and mathematics performance: a meta-analysis. Psychol Bull. 2010; 136:1123–1135. [PubMed: 21038941]
- Luke B, Hediger M, Min SJ, Brown MB, Misiunas RB, Gonzalez-Quintero VH, Nugent C, Witter FR, Newman RB, Hankins GD, Grainger DA, Macones GA. Gender mix in twins and fetal growth, length of gestation and adult cancer risk. Paediatr Perinat Epidemiol. 2005; 19 (Suppl 1):41–47. [PubMed: 15670121]
- Lummaa V, Pettay JE, Russell AF. Male twins reduce fitness of female co-twins in humans. Proc Natl Acad Sci U S A. 2007; 104:10915–10920. [PubMed: 17576931]
- Lydecker JA, Pisetsky EM, Mitchell KS, Thornton LM, Kendler KS, Reichborn-Kjennerud T, Lichtenstein P, Bulik CM, Mazzeo SE. Association between co-twin sex and eating disorders in opposite sex twin pairs: evaluations in North American, Norwegian, and Swedish samples. J Psychosom Res. 2012; 72:73–77. [PubMed: 22200526]

- Mains L, Zimmerman M, Blaine J, Stegmann B, Sparks A, Ansley T, Van Voorhis B. Achievement test performance in children conceived by IVF. Hum Reprod. 2010; 25:2605–2611. [PubMed: 20716561]
- Malaspina D, Reichenberg A, Weiser M, Fennig S, Davidson M, Harlap S, Wolitzky R, Rabinowitz J, Susser E, Knobler HY. Paternal age and intelligence: implications for age-related genomic changes in male germ cells. Psychiatr Genet. 2005; 15:117–125. [PubMed: 15900226]
- Matte TD, Bresnahan M, Begg MD, Susser E. Influence of variation in birth weight within normalrange and within sibships on IQ at age 7 years: cohort study. BMJ. 2001; 323:310–314. [PubMed: 11498487]
- McFadden D. A masculinizing effect on the auditory systems of human females having male co-twins. Proc Natl Acad Sci U S A. 1993; 90:11900–11904. [PubMed: 8265645]
- McFadden D, Loehlin JC, Pasanen EG. Additional findings on heritability and prenatal masculinization of cochlear mechanisms: click-evoked otoacoustic emissions. Hear Res. 1996; 97:102–119. [PubMed: 8844191]
- McHale SM, Updegraff KA, Helms-Erikson H, Crouter AC. Sibling influences on gender development in middle childhood and early adolescence: a longitudinal study. Dev Psychol. 2001; 37:115–125. [PubMed: 11206426]
- Medland SE, Loehlin JC, Martin NG. No effects of prenatal hormone transfer on digit ratio in a large sample of same- and opposite-sex dizygotic twins. Personal Individ Differ. 2008a; 44:1225–1234.
- Medland SE, Loehlin JC, Willemsen G, Hatemi PK, Keller MC, Boomsma DI, Eaves LJ, Martin NG. Males do not reduce the fitness of their female co-twins in contemporary samples. Twin Res Hum Genet. 2008b; 11:481–487. [PubMed: 18828730]
- Miller EM. Prenatal sex hormone transfer: a reason to study opposite-sex twins. Personal Individ Differ. 1994; 17:511–529.
- Miller DI, Halpern DF. The new science of cognitive sex differences. Trends Cogn Sci. 2014; 18:37– 45. [PubMed: 24246136]
- Naglieri JA, Bornstein BT. Intelligence and achievement: just how correlated are they? J Psychoeduc Assess. 2003; 21:244–260.
- Pedersen CB. The Danish civil registration system. Scand J Public Health. 2011; 39:22–25. [PubMed: 21775345]
- Peper JS, Brouwer RM, Van Baal GC, Schnack HG, van LM, Boomsma DI, Kahn RS, Hulshoff Pol HE. Does having a twin brother make for a bigger brain? Eur J Endocrinol. 2009; 160:739–746. [PubMed: 19218283]
- Petersen, JK. The Danish Demographic Database: Longitudinal Data For Advanced Demographic Methods. Odense, Denmark: 2000.
- Petersen I, Jensen VM, McGue M, Bingley P, Christensen K. No evidence of genetic mediation in the association between birthweight and academic performance in 2,413 Danish adolescent twin pairs. Twin Res Hum Genet. 2009; 12:564–572. [PubMed: 19943719]
- Petersson F, Baadsgaard M, Thygesen LC. Danish registers on personal labour market affiliation. Scand J Public Health. 2011; 39:95–98. [PubMed: 21775363]
- Phoenix CH. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Horm Behav. 2009; 55:566. [PubMed: 19302826]
- Phoenix CH, Goy RW, Gerall AA, Young WC. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology. 1959; 65:369–382. [PubMed: 14432658]
- Pulkkinen L, Vaalamo I, Hietala R, Kaprio J, Rose RJ. Peer reports of adaptive behavior in twins and singletons: is twinship a risk or an advantage? Twin Res. 2003; 6:106–118. [PubMed: 12723997]
- Puts DA, McDaniel MA, Jordan CL, Breedlove SM. Spatial ability and prenatal androgens: metaanalyses of congenital adrenal hyperplasia and digit ratio (2D:4D) studies. Arch Sex Behav. 2008; 37:100–111. [PubMed: 18074217]
- Raevuori A, Kaprio J, Hoek HW, Sihvola E, Rissanen A, Keski-Rahkonen A. Anorexia and bulimia nervosa in same-sex and opposite-sex twins: lack of association with twin type in a nationwide study of Finnish twins. Am J Psychiatry. 2008; 165:1604–1610. [PubMed: 18981064]

- Reilly D. Gender, culture, and sex-typed cognitive abilities. PLoS One. 2012; 7:e39904. [PubMed: 22808072]
- Resnick SM, Gottesman II, McGue M. Sensation seeking in opposite-sex twins: an effect of prenatal hormones? Behav Genet. 1993; 23:323–329. [PubMed: 8240211]
- Ribeiro DC, Brook AH, Hughes TE, Sampson WJ, Townsend GC. Intrauterine hormone effects on tooth dimensions. J Dent Res. 2013; 92:425–431. [PubMed: 23549090]
- Richards M, Hardy R, Kuh D, Wadsworth ME. Birth weight and cognitive function in the British 1946 birth cohort: longitudinal population based study. BMJ. 2001; 322:199–203. [PubMed: 11159613]
- Rodgers C, Fagot B, Winebarger A. Gender-typed toy play in dizygotic twin pairs: a test of hormone transfer theory. Sex Roles. 1998; 39:173–184.
- Ryan BC, Vandenbergh JG. Intrauterine position effects. Neurosci Biobehav Rev. 2002; 26:665–678. [PubMed: 12479841]
- Saha S, Barnett AG, Foldi C, Burne TH, Eyles DW, Buka SL, McGrath JJ. Advanced paternal age is associated with impaired neurocognitive outcomes during infancy and childhood. PLoS Med. 2009; 6:e40. [PubMed: 19278291]
- Sirin SR. Socioeconomic status and academic achievement: a meta-analytic review of research. Rev Educ Res. 2005; 75:417–453.
- Skytthe A, Kyvik K, Holm NV, Vaupel JW, Christensen K. The Danish twin registry: 127 birth cohorts of twins. Twin Res. 2002; 5:352–357. [PubMed: 12537858]
- Skytthe A, Ohm KK, Vilstrup HN, Christensen K. The Danish twin registry. Scand J Public Health. 2011; 39:75–78. [PubMed: 21775358]
- Slutske WS, Bascom EN, Meier MH, Medland SE, Martin NG. Sensation seeking in females from opposite- versus same-sex twin pairs: hormone transfer or sibling imitation? Behav Genet. 2011; 41:533–542. [PubMed: 21140202]
- Sorensen HT, Sabroe S, Olsen J, Rothman KJ, Gillman MW, Fischer P. Birth weight and cognitive function in young adult life: historical cohort study. BMJ. 1997; 315:401–403. [PubMed: 9277604]
- Sorensen K, Juul A, Christensen K, Skytthe A, Scheike T, Kold Jensen T. Birth size and age at menarche: a twin perspective. Hum Reprod. 2013; 28:2865–2871. [PubMed: 23925395]
- Stoet G, Geary DC. Sex differences in mathematics and reading achievement are inversely related: within- and across-nation assessment of 10 years of PISA data. PLoS One. 2013; 8:e57988. [PubMed: 23516422]
- Strand S, Deary IJ, Smith P. Sex differences in cognitive abilities test scores: a UK national picture. Br J Educ Psychol. 2006; 76:463–480. [PubMed: 16953957]
- Tapp AL, Maybery MT, Whitehouse AJO. Evaluating the twin testosterone transfer hypothesis: a review of the empirical evidence. Horm Behav. 2011; 60:713–722. [PubMed: 21893061]
- Tul N, Lucovnik M, Novak Z, Verdenik I, Blickstein I. No "masculinization" effect of a male on birth weight of its female co-twin. J Perinat Med. 2012; 40:255–257. [PubMed: 22505503]
- van Anders SM, Vernon PA, Wilbur CJ. Finger-length ratios show evidence of prenatal hormonetransfer between opposite-sex twins. Horm Behav. 2006; 49:315–319. [PubMed: 16143332]
- vom Saal FS. Sexual differentiation in litter-bearing mammals: influence of sex of adjacent fetuses *in utero*. J Anim Sci. 1989; 67:1824–1840. [PubMed: 2670873]
- Voracek M, Haubner T. Twin-singleton differences in intelligence: a meta-analysis. Psychol Rep. 2008; 102:951–962. [PubMed: 18763469]
- Vuoksimaa E, Kaprio J, Kremen WS, Hokkanen L, Viken RJ, Tuulio-Henriksson A, Rose RJ. Having a male co-twin masculinizes mental rotation performance in females. Psychol Sci. 2010; 21:1069– 1071. [PubMed: 20581340]
- Wagenaar K, Ceelen M, van Weissenbruch MM, Knol DL, Delemarre-van de Waal HA, Huisman J. School functioning in 8- to 18-year-old children born after in vitro fertilization. Eur J Pediatr. 2008; 167:1289–1295. [PubMed: 18270734]

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yhbeh. 2015.01.007.

Author Manuscript

Characteristics and summary statistics for the academic performance scores of twins and singletons born in Denmark during 1986–1990. Values are numbers (percentages) unless otherwise stated.

Ahrenfeldt et al.

| Characteristics | Opposite-sex females | Same-sex females | Female twins | Singleton ^d females | Opposite-sex males | Same-sex males | Male twins | Singleton ^a males | Totals |
|---|-----------------------------|-------------------------|-------------------------|--------------------------------|---------------------------|-------------------------|-------------------------|------------------------------|---------------------------|
| Number of live births in Denmark | 968 (9.5) | 2198 (21.6) | 3166 (31.1) | 7010 (68.9) | 966 (9.2) | 2194 (20.8) | 3160 (30.0) | 7391 (70.1) | 20,727 (100.0) |
| Deaths before age $17b$ | 23 (2.4) | 76 (3.5) | 99 (3.1) | 58 (0.8) | 19 (2.0) | 78 (3.6) | 97 (3.1) | 92 (1.2) | 346 (1.7) |
| 1-28 days after birth | 17 (1.8) | 58 (2.7) | 75 (2.4) | 17 (0.2) | $14\ (1.5)$ | 54 (2.5) | 68 (2.2) | 36 (0.5) | 196 (1.0) |
| 29-365 days after birth | 3 (0.3) | 12 (0.6) | 15 (0.5) | 18 (0.3) | 3 (0.3) | 18 (0.8) | 21 (0.7) | 28 (0.4) | 82 (0.4) |
| Death of co-twin before age 17 | 8 (0.9) | 38 (1.8) | 46 (1.5) | I | 12 (1.3) | 48 (2.3) | 60 (2.0) | Ι | 106 (1.7) |
| $\operatorname{Emigrated} b$ | 30 (3.2) | 50 (2.4) | 80 (2.7) | 181 (2.6) | 30 (3.2) | 48 (2.3) | 77 (2.6) | 170 (2.3) | 508 (2.5) |
| Study base (% of live born) ^c | 907 (93.7) | 2034 (92.5) | 2941 (92.9) | 6771 (96.6) | 905 (93.7) | 2020 (92.1) | 2926 (92.6) | 7129 (96.5) | 19,766 (95.4) |
| Mean (SD) birth weight | 2562 (521) n = 905 | 2491 (495) n = 2029 | 2513 (504) n = 2934 | 3389 (517) n = 6754 | 2648 (542) n = 904 | 2586 (557) n = 2017 | 2605 (553) n = 2921 | 3514 (558) n = 7104 | 3188 (676) n = 19,713 |
| Mean (SD) maternal age | 29.8 (4.5); n = 908 | 28.9 (4.6); n = 2035 | 29.2 (4.6); n = 2943 | 28.0 (4.8); n = 6769 | 29.8 (4.5); n = 907 | 28.9 (4.5); n = 2023 | 29.2 (4.5); n = 2930 | 28.0 (4.8); n = 7122 | 28.38 (4.8) n = 19,757 |
| Mean (SD) paternal age | 32.7 (5.9); n = 906 | 31.9 (5.7); n = 2025 | 32.1 (5.8); n = 2931 | 31.0 (5.9); n = 6712 | 32.7 (5.8); n = 905 | 31.4 (5.7); n = 2015 | 31.8 (5.8); n = 2920 | 30.9 (5.7); n = 7072 | 31.3 (5.8) n = 19,628 |
| Maternal education | | | | | | | | | |
| Basic school | 225 (25.8) | 574 (28.9) | 799 (28.0) | 1823 (27.6) | 224 (25.8) | 558 (28.0) | 782 (27.3) | 1900 (27.3) | 5304 (27.5) |
| Vocational school | 260 (29.9) | 644 (32.4) | 904 (31.6) | 2192 (33.1) | 259 (29.8) | 670 (33.6) | 929 (32.5) | 2394 (34.4) | 6419 (33.3) |
| Secondary education | (6.1) (6.2) | 152 (7.7) | 221 (7.7) | 603 (9.1) | (6.1) 69 | 182 (9.1) | 251 (8.8) | 606 (8.7) | 1681 (8.7) |
| Short higher education | 37 (4.3) | 100(5.0) | 137 (4.8) | 269 (4.1) | 37 (4.3) | 80 (4.0) | 117 (4.1) | 304 (4.4) | 827 (4.3) |
| Medium higher education and Bachelor's degree | 223 (25.6) | 430 (21.6) | 653 (22.8) | 1442 (21.8) | 223 (25.7) | 422 (21.2) | 645 (22.5) | 1466 (21.1) | 4206 (21.8) |
| Higher academic or professional degree | 57 (6.5) | 88 (4.4) | 145 (5.1) | 286 (4.3) | 57 (6.6) | 80 (4.0) | 137 (4.8) | 292 (4.2) | 860 (4.5) |
| Paternal education | | | | | | | | | |
| Basic school | 219 (25.3) | 490 (25.4) | 709 (25.4) | 1659 (26.1) | 219 (25.4) | 488 (25.8) | 707 (25.6) | 1818 (27.9) | 4893 (26.2) |
| Vocational school | 341 (39.4) | 818 (42.4) | 1159 (41.5) | 2772 (43.6) | 339 (39.2) | 839 (44.3) | 1178 (42.7) | 2824 (41.9) | 7933 (43.0) |
| Secondary education | 64 (7.4) | 146 (7.6) | 210 (7.5) | 394 (6.2) | 64 (7.4) | 102 (5.4) | 166~(6.0) | 441 (6.6) | 1211 (6.5) |
| Short higher education | 44 (5.1) | 84 (4.4) | 128 (4.6) | 307 (4.8) | 44 (5.1) | 83 (4.4) | 127 (4.6) | 325 (4.8) | 887 (4.8) |

| | • | • | | | | | | | |
|---|----------------------------------|------------------|---------------|--------------------------------|---------------------------|----------------|-------------|------------------------------|----------------------------|
| Unaracteristics | Upposite-sex remates | Same-sex remales | F emale twins | Singleton ^d females | Upposite-sex males | Same-sex males | Male twins | Singleton ^a males | 1 OTAIS |
| Medium higher education and Bachelor's degree | 108 (12.5) | 228 (11.8) | 336 (12.0) | 730 (11.5) | 108 (12.5) | 214 (11.3) | 322 (11.7) | 767 (11.4) | 2155 (11.6) 2155 (11.6) |
| Higher academic or professional degree | 90 (10.4) | 164 (8.5) | 254 (9.1) | 501 (7.9) | 90 (10.4) | 168 (8.9) | 258 (9.4) | 558 (8.3) | 1571 (8.4) 1571 (8.4) |
| Mathematics — oral and | written average ^d ,e | | | | | | | | 1. |
| Test score available | 787 (86.8) | 1749 (86.0) | 2536 (86.2) | 5907 (87.2) | 744 (82.2) | 1655 (81.9) | 2399 (82.0) | 5819 (81.6) | 16,661 (84.3) |
| Mean (SD) test score | 7.84 (1.4) | 7.98 (1.4) | 7.94 (1.4) | 7.96 (1.4) | 8.14(1.4) | 8.13 (1.4) | 8.13 (1.4) | 8.15 (1.5) | 8.05 (1.4) |
| Teacher rating available | 794 (87.5) | 1771 (87.1) | 2565 (87.2) | 6046 (89.3) | 761 (84.1) | 1688 (83.5) | 2449 (83.7) | 5953 (83.5) | 17,013 (86.1) |
| Mean (SD) teacher rating | 7.97 (1.4) | 8.08 (1.4) | 8.05 (1.4) | 8.07 (1.4) | 8.08 (1.4) | 8.06 (1.4) | 8.07 (1.4) | 8.03 (1.4) | 8.05 (1.4) |
| Physics/chemistry — oral | | | | | | | | | |
| Test score available | 613 (67.6) | 1424 (70.0) | 2037 (69.3) | 4701 (69.4) | 582 (64.3) | 1360 (67.3) | 1942 (66.4) | 4700 (65.9) | 13,380 (67.7) |
| Mean (SD) test score | 7.90 (1.6) | 8.01 (1.6) | 7.98 (1.6) | 7.98 (1.6) | 8.09 (1.6) | 8.05 (1.7) | 8.06 (1.7) | 7.94 (1.7) | 7.98 (1.7) |
| Teacher rating available | 771 (85.0) | 1715 (84.3) | 2486 (84.5) | 5786 (85.5) | 715 (79.0) | 1632 (80.8) | 2347 (80.2) | 5648 (79.2) | 16,267 (82.3) |
| Mean (SD) teacher rating | 7.94 (1.4) | 8.03 (1.3) | 8.00 (1.4) | 7.94 (1.3) | 8.05 (1.4) | 7.99 (1.4) | 8.01 (1.4) | 7.91 (1.4) | 7.95 (1.4) |
| Danish — oral, written ai | nd spelling average ^f | | | | | | | | |
| Test score available | 797 (87.9) | 1774 (87.2) | 2571 (87.4) | 5981 (88.3) | 747 (82.5) | 1655 (81.9) | 2402 (82.1) | 5819 (81.6) | 16,773 (84.9) |
| Mean (SD) test score | 8.38 (1.2) | 8.36 (1.2) | 8.37 (1.2) | 8.41 (1.2) | 7.88 (1.3) | 7.83 (1.3) | 7.85 (1.3) | 7.84 (1.3) | 8.13 (1.3) |
| Teacher rating available | 798 (88.0) | 1779 (87.5) | 2577 (87.6) | 6055 (89.4) | 754 (83.3) | 1677 (83.0) | 2431 (83.1) | 5936 (83.3) | 16,999 (86.0) |
| Mean (SD) teacher rating | 8.47 (1.2) | 8.47 (1.2) | 8.47 (1.2) | 8.49 (1.1) | 7.90 (1.3) | 7.84 (1.2) | 7.86 (1.25) | 7.81 (1.2) | 8.16 (1.2) |
| English — oral | | | | | | | | | |
| Test score available | 765 (84.3) | 1720 (84.6) | 2485 (84.5) | 5809 (85.8) | 721 (79.7) | 1607 (79.5) | 2328 79.6 | 5664 (79.5) | 16,286 (82.4) |
| Mean (SD) test score | 8.54 (1.8) | 8.48 (1.8) | 8.50 (1.8) | 8.52 (1.8) | 8.18 (1.7) | 8.10 (1.8) | 8.13 (1.8) | 8.22 (1.8) | 8.36 (1.8) |
| Teacher rating available | 792 (87.3) | 1758 (86.4) | 2550 (86.7) | 6007 (88.7) | 744 (82.2) | 1665 (82.4) | 2409 (82.3) | 5885 (82.6) | 16,851 (85.3) |
| Mean (SD) teacher rating | 8.27 (1.4) | 8.23 (1.4) | 8.24 (1.4) | 8.21 (1.4) | 7.96 (1.4) | 7.87 (1.4) | 7.90 (1.4) | 7.85 (1.5) | 8.05 (1.5) |
| 1 | | | | | | | | | |

¹5% random sample of Danish birth cohorts 1986–1990.

bBefore January 1, 2003.

 $^{\ensuremath{\mathcal{C}}}$ Rest of table gives percentage of study base.

 d Average performance was rated as 8.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

 $^{e}\!\mathrm{Average}$ scores in oral and written mathematics for individuals with both scores available.

 $f_{
m A}$ verage scores in oral, written and spelling Danish for individuals with all three scores available.

Author Manuscript

Author Manuscript

Table 2

Risk of test score non-attainment (odds ratios and 95% confidence intervals), raw and adjusted for parental age and education, separated by sex and cotwin sex for Danish twins and singletons born 1986–1990.

Ahrenfeldt et al.

| Characteristic | Male/females (ref. female) | Opposite-sex/ same- sex females (ref. same-sex) | Opposite-sex/ singleton females (ref. singletons) | Same-sex/ singleton females (ref. singletons) | Opposite-sex/ same- sex males (ref. same- sex) | Opposite-sex/ singleton males (ref. singletons) | Same-sex/ singleton males (ref. singletons) |
|--------------------------------------|--------------------------------|---|---|---|--|---|---|
| Risk of test score non-attainment | 1.65 (1.51; 1.80) ^a | 0.98 (0.74; 1.29) | 1.15 (0.92; 1.44) | 1.18 (0.97; 1.43) | 0.98 (0.77; 1.25) | 0.98 (0.81; 1.19) | 1.00 (0.85; 1.18) |
| Adjusted risk | $1.70 \ (1.54; 1.88)^{d}$ | 1.05 (0.76; 1.47) | 1.25 (0.96; 1.63) | 1.16 (0.92; 1.45) | 1.10 (0.83; 1.46) | 1.14 (0.91; 1.43) | 1.02 (0.84; 1.24) |
| a p < 0.001. | | | | | | | |

Table 3

higher scores for males compared with females and/or opposite-sex twins compared with same-sex twins and/or opposite-/same-sex twins compared with physics/chemistry, Danish and English by sex and co-twin sex for Danish twin and singleton females born 1986–1990. Positive values of d represent Standardised mean differences, d, in ninth grade test scores and teacher ratings, crude and adjusted for parental age and education, in mathematics, singletons.

Ahrenfeldt et al.

| 0 | | | | | | | | |
|--|----------------------------|-------------------------|--|---------------|---|---------------|---|-----------|
| Characteristic | Male/females (ref. female) | Adjusted | Opposite-sex/ same- sex females (ref. same- sex) | Adjusted | Opposite-sex/ singleton females (ref. singletons) | Adjusted | Same-sex/ singleton females (ref. singletons) | Adjusted |
| Mathematics — oral | | | | | | | | |
| Test scores available | 16,727 | 15,589 | 2540 | 2377 | 6726 | 6243 | 7688 | 7158 |
| Effect size difference ^{c} | .07 | .06 | 09 | 13 | 07 | 13 | .01 | 00 |
| 95% CI <i>d</i> | $(.04; .10)^{b}$ | $(.03; .09)^{b}$ | (18; .00) | $(21;04)^{d}$ | $(15;.00)^{a}$ | $(20;06)^{b}$ | (05; .07) | (06; .06) |
| Teacher ratings available | 17,014 | 15,814 | 2565 | 2391 | 6840 | 6325 | 7817 | 7248 |
| Effect size difference c | 02 | 04 | 08 | 12 | 06 | 11 | .02 | .01 |
| 95% CI <i>d</i> | (05; .01) | $(07;01)^d$ | (17; .01) | $(20;03)^{d}$ | (13; .01) | $(18;03)^{d}$ | (05; .08) | (05; .07) |
| Mathematics — written | | | | | | | | |
| Test scores available | 16,777 | 15,627 | 2549 | 2383 | 6747 | 6254 | 7710 | 7171 |
| Effect size difference ^{c} | .17 | .15 | 10 | 14 | 07 | 14 | .02 | 00 |
| 95% CI <i>d</i> | $(.14; .20)^b$ | (.12; .18) ^b | $(19;01)^{d}$ | $(23;05)^{d}$ | $(15;.00)^{a}$ | $(21;07)^{b}$ | (04; .09) | (06; .06) |
| Teacher ratings available | 17,031 | 15,830 | 2570 | 2395 | 6849 | 6333 | 7827 | 7258 |
| Effect size difference ^{c} | 01 | 03 | 08 | 12 | 07 | 14 | .01 | 01 |
| 95% CI <i>d</i> | (05; .02) | $(06; .00)^{a}$ | (17; .01) | $(21;03)^{d}$ | (15; .00) | $(21;06)^{b}$ | (06; .07) | (07; .05) |
| Mathematics — neatness | | | | | | | | |
| Test scores available | 13,086 | 12,168 | 1986 | 1857 | 5239 | 4835 | 6045 | 5600 |
| Effect size difference c | 63 | 64 | 01 | 02 | 05 | 08 | 04 | 05 |
| 95% CI <i>d</i> | (67;60)b | $(67;60)^{b}$ | (11; .09) | (12; .08) | (13; .04) | (17; .01) | (10; .03) | (12; .02) |
| Teacher ratings available | 13,270 | 12,309 | 2002 | 1867 | 5307 | 4885 | 6129 | 5662 |
| Effect size difference c | 71 | 72 | 08 | 12 | 05 | 12 | .03 | 00. |
| 95% CI <i>d</i> | $(74; -68)^{b}$ | $(75;68)^{b}$ | (19;.02) | $(22;01)^{d}$ | (14; .03) | $(21;03)^{d}$ | (04; .10) | (07; .07) |
| Physics/chemistry — Oral | | | | | | | | |

| Characteristic | Male/females (ref. female) | Adjusted | Opposite-sex/ same- sex females (ref. same- sex) | Adjusted | Opposite-sex/ singleton females (ref. singletons) | Adjusted | Same-sex/ singleton females (ref. singletons) | Adjusted |
|--|----------------------------|------------------------|--|---------------|---|-----------------|---|----------------------|
| Test scores available | 13,381 | 12,483 | 2037 | 1915 | 5314 | 4922 | 6125 | 5695 |
| Effect size difference ^{c} | 00. | 01 | 07 | 11 | 05 | 12 | .02 | 00 |
| 95% CId | (04; .03) | (05; .02) | (18; .03) | $(22;01)^{a}$ | (14; .03) | $(20;04)^{a}$ | (05; .09) | (07; .07) |
| Teacher ratings available | 16,267 | 15,149 | 2486 | 2320 | 6557 | 6909 | 7501 | 6965 |
| Effect size difference ^{c} | 01 | 04 | 06 | 12 | 00. | 06 | .06 | .05 |
| 95% CId | (04; .02) | $(07;00)^{a}$ | (16; .03) | $(21;03)^{d}$ | (07; .08) | (13; .01) | (00; .13) | (01; .11) |
| Danish — Oral | | | | | | | | |
| Test scores available | 16,889 | 15,722 | 2581 | 2412 | 6825 | 6319 | 7810 | 7253 |
| Effect size difference ^{c} | 33 | 35 | .02 | 03 | 01 | 08 | 03 | 04 |
| 95% CId | (36; -30)b | $(38; -32)^{b}$ | (07; .11) | (12; .06) | (08; .06) | $(15;01)^{d}$ | (09; .03) | (10; .02) |
| Teacher ratings available | 17,034 | 15,833 | 2581 | 2407 | 6861 | 6345 | 7844 | 7276 |
| Effect size difference c | 44 | 46 | .03 | 01 | .03 | 04 | 00 | 02 |
| 95% CId | (47; -41)b | $(49; -43)^{b}$ | (06; .13) | (10; .08) | (04; .10) | (11; .03) | (07; .06) | (08; .04) |
| Danish — Written | | | | | | | | |
| Test scores available | 16,887 | 15,720 | 2583 | 2413 | 6829 | 6323 | 7806 | 7250 |
| Effect size difference ^{c} | 48 | 49 | .04 | 02 | 02 | -00 | 06 | 07 |
| 95% CId | (51; -45)b | $(52; -46)^{b}$ | (05; .13) | (10; .07) | (10; .05) | $(17;02)^{d}$ | (12; .00) | $(-13;01)^{a}$ |
| Teacher ratings available | 17,033 | 15,831 | 2581 | 2407 | 6861 | 6345 | 7844 | 7276 |
| Effect size difference ^{c} | 58 | 60 | 02 | 06 | 06 | 13 | 05 | 07 |
| 95% CId | (61; -55)b | (63; -57) ^b | (11; .08) | (15; .03) | (14; .01) | $(20;06)^{b}$ | (11; .02) | (13;01) ^a |
| Danish — spelling | | | | | | | | |
| Test scores available | 16,930 | 15,758 | 2591 | 2420 | 6846 | 6335 | 7827 | 7267 |
| Effect size difference ^{c} | 31 | 33 | 02 | 05 | 03 | 08 | 02 | 04 |
| 95% CId | $(35; -28)^{b}$ | $(36; -30)^{b}$ | (11; .08) | (14; .04) | (11; .04) | $(16;01)^{d}$ | (08; .04) | (10; .02) |
| Teacher ratings available | 17,023 | 15,827 | 2577 | 2405 | 6857 | 6342 | 7838 | 7273 |
| Effect size difference ^{c} | 42 | 43 | 02 | 06 | 03 | 08 | 01 | 03 |
| 95% CId | $(45; -39)^b$ | $(46; -40)^{b}$ | (11; .08) | (15; .03) | (11; .04) | $(15; -01)^{a}$ | (08; .05) | (09; .03) |

Horm Behav. Author manuscript; available in PMC 2015 May 27.

Ahrenfeldt et al.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Page 23

| Characteristic | Male/females (ref. female) | Adjusted | Opposite-sex/ same- sex females (ref. same- sex) | Adjusted | Opposite-sex/ singleton females (ref. singletons) | Adjusted | Same-sex/ singleton females (ref. singletons) | Adjusted |
|--|----------------------------|----------------------|--|-----------|---|-----------------|---|-----------|
| Danish — Neatness | | | | | | | | |
| Test scores available | 16,883 | 15,716 | 2582 | 2412 | 6828 | 6322 | 7804 | 7248 |
| Effect size difference c | 44 | 45 | 01 | 03 | 04 | 07 | 03 | 04 |
| 95% CId | $(47; -41)^{b}$ | (48;41) ^b | (10; .08) | (12; .06) | (12; .03) | (15;.00) | (09; .03) | (10; .02) |
| Teacher ratings available | 17,019 | 15,819 | 2580 | 2406 | 6854 | 6338 | 7836 | 7268 |
| Effect size difference c | 66 | 67 | 04 | 07 | 07 | 11 | 03 | 04 |
| 95% CId | $(69; -63)^{b}$ | $(70; -64)^{b}$ | (13; .05) | (16; .02) | (14; .01) | $(18; -03)^{a}$ | (09; .04) | (10; .03) |
| English — oral | | | | | | | | |
| Test scores available | 16,286 | 15,197 | 2485 | 2328 | 6574 | 6100 | 7529 | 7008 |
| Effect size difference ^{c} | 18 | 20 | .03 | 03 | .01 | 07 | 02 | 03 |
| 95% CId | $(21; -15)^{b}$ | $(23; -17)^{b}$ | (06; .13) | (12; .06) | (06; .09) | (15; .00) | (08; .04) | (09; .03) |
| Teacher ratings available | 16,851 | 15,682 | 2550 | 2380 | 6469 | 6291 | 7765 | 7209 |
| Effect size difference ^{c} | 25 | 27 | .03 | 03 | .04 | 05 | .01 | 01 |
| 95% CId | $(28; -22)^{b}$ | (30;24) ^b | (07; .12) | (11; .06) | (04; .11) | (12; .03) | (05; .08) | (07; .05) |
| a = 0.05. | | | | | | | | |
| $b_{n < 0.001}$ | | | | | | | | |
| $\mathbf{p} \sim 0.001$ | | | | | | | | |

^c In units of SD.
^d Confidence intervals.

Horm Behav. Author manuscript; available in PMC 2015 May 27.

Ahrenfeldt et al.

Author Manuscript