

# Comparison of serum FSH and Inhibin B levels between adult male dizygotic and monozygotic twins

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**BACKGROUND:** FSH hypersecretion occurs in mothers of dizygotic (DZ) twins. Twinning is inherited via both sexes and transmitted through the female. FSH hypersecretion may thus occur in male DZ twins. **METHODS:** We assayed FSH and its counter-regulatory hormone, Inhibin B, in 108 adult male DZ and 100 monozygotic (MZ) twins (as controls) and compared our results to published norms. **RESULTS:** Inhibin B was elevated and higher in DZ compared with MZ twins with similar FSH. **CONCLUSION:** The normal FSH: Inhibin B endocrine feedback axis is different in adult male DZ twins. This contributes to the theory that the answer to human DZ twinning lies in the actions of FSH and Inhibin, and in their mutual interaction.

*Key words:* FSH hypersecretion/inhibin B/twins

## Introduction

FSH stimulates spermatogenesis in the male. Infertility and testicular germ cell cancer are associated with an elevated FSH but a diminished level of Inhibin B as a marker of atrophy (Jensen *et al.*, 1997; Byrd *et al.*, 1998). Reports that mothers who produce familial dizygotic (DZ) twins have an elevated FSH compared with normal controls—possibly genetically controlled (Martin *et al.*, 1991; Lambalk *et al.*, 1998)—and that male DZ twins have a two (Braun *et al.*, 1995) to threefold increased incidence of testicular cancers—particularly seminoma (Swerdlow *et al.*, 1997a)—provided important confirmatory information to support the hypothesis that FSH may play a role in the aetiology of both conditions (Oliver, 1990). This led us to investigate FSH and Inhibin levels in twins to test the hypothesis that gonadotropin gonadal interaction differs between MZ and DZ twins.

## Materials and methods

Serum samples were obtained from 208 individuals (54 DZ and 50 MZ twin pairs) in two voluntary twin registries (Twins UK at St Thomas's Hospital, London, UK, and The Sydney Adult Twin Registry, Australia). The St Thomas's Registry consists of nearly 10000 monozygous and dizygous adult caucasian twins aged 18 to 80 from all over the UK and was started in 1993. It has an average age of around 45, who are predominantly female and same sex, because the diseases initially focussed on were more common in women. The

ratio of identical to non-identical twins is ~50:50. Zygosity is ascertained using the standard 'peas in the pod' questionnaire and, if there is uncertainty, checked by genotyping. The Sydney Twin Study consists of over 2000 monozygous and dizygous adult caucasian twins aged 18 to 80 from all over Australia and was started in 1996. This is a volunteer sample recruited by successive media campaigns without selecting for particular diseases or traits. Zygosity is ascertained by genotyping.

The male twins in this study varied from 20 to 68 years and all MZ and DZ samples were age matched. No patient in either group was IVF conceived. All twins joining the registry are asked to indicate their conception status. Most twins in this study were anyway too old to have been conceived by IVF.

Ethical approval for participation in the study was given by both twin registries. All samples were taken by venesection between 10 and 11 am and cryopreserved for future analyses.

## Assays

Assay of samples was performed in one laboratory using the following protocol. LH and FSH were measured by immunometric (sandwich) method on Roche E170 (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's recommendations. Both testosterone and dehydroepiandrosterone sulphate (DHEA-S) were measured by a competitive immunometric assay on Roche E170 following the manufacturer's recommendations. Sex hormone-binding globulin (SHBG) was measured on DPC Immulite (EURO/DPC Ltd, Glyn Rhonwy, Llanberis, Gwynedd, UK) using an immunometric assay. Inhibin B was measured by an enzymatically

amplified two-site two-step sandwich-type immunoassay using a DSL-10-84100 Active Inhibin B enzyme-linked immunosorbent assay (ELISA) kit (Diagnostic Systems Laboratories Inc., Webster, TX, USA.).

DHEA-S and SHBG were determined using a solid-phase, competitive chemiluminescent immunoassay with DPC Immulite.

Testosterone, LH and FSH were measured using a two-step sandwich electro chemi luminescent immunoassay (ECLIA).

### Analysis of results and statistics

Linear regression models were developed to determine the effect of MZ versus DZ twins on the response variables of interest, FSH and Inhibin B levels. Stepwise selection methods were used to identify possible confounding variables from the dataset and adjusted for those found to have a significant effect on the response variables.

For FSH level, the model was adjusted for LH, DHEA-S and testosterone levels, and an analysis of variance revealed no significant difference between MZ and DZ twins, ( $F$ -ratio = 0.60,  $P$  = 0.440). For Inhibin B level, the model was adjusted for LH, SHBG and testosterone levels, and evidence in the data suggested a significant difference for Inhibin B levels ( $F$ -ratio = 7.72,  $P$  = 0.006) (Fig. 1). Interactions between variables were investigated but did not appear to alter the results significantly.

The appropriateness of the selected models was tested by ensuring the data were: (i) normally distributed with a constant variance; (ii) independent; and (iii) robust (removal of outliers did not significantly influence results). These requirements were satisfied. However because each group contained twin pairs, there was potential correlation of values between them and a sensitivity analysis—using the mean values of each twin pair and thus halving the sample size—was conducted. Analysis of variance once more failed to uncover a significant difference between FSH levels in MZ and DZ twins ( $F$ -ratio = 0.08,  $P$  = 0.772), but suggested a significant difference for Inhibin B levels ( $F$ -ratio = 4.22,  $P$  = 0.043).

## Results

### FSH: Inhibin B ratio

Linear regression was conducted on a  $\log_e$  transformation of the ratio FSH/Inhibin B. Investigation of the data revealed that

a transformation was needed in order to normalize the data. An unadjusted analysis revealed no significant difference between MZ and DZ twins, ( $F$ -ratio = 1.97,  $P$  = 0.162). Using a stepwise selection procedure to select potentially confounding variables, the analysis was adjusted accordingly. Hormone levels of LH and testosterone were found to significantly affect the results, but age was not found to be a confounder. The adjusted analysis found no difference between MZ and DZ twins ( $F$ -ratio = 1.68,  $P$  = 0.196.) With the addition of age in the model, this remained exactly the same. Excluding outliers with Inhibin B levels <20 pg/ml, there was even less evidence of a difference in ratio between MZ and DZ twins ( $F$ -ratio = 0.61,  $P$  = 0.435). Models were checked and found to meet the requirements of normality, homogeneity of variance and independence.

Observed means and standard deviations can be found in Table I, together with  $P$  values from two-tailed  $t$ -tests in order to compare differences in the two sets of twins before adjustment.

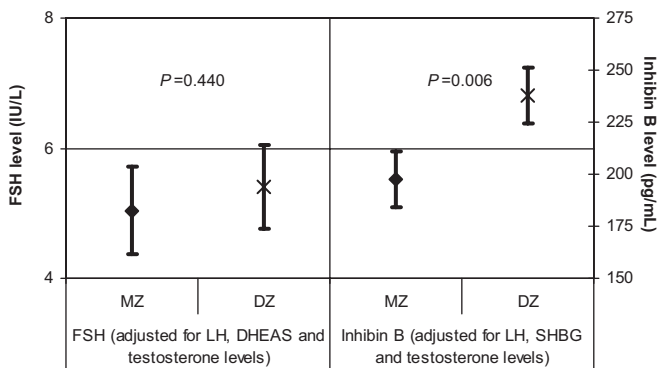
We compared hormone biochemistry between twin groups (MZ versus DZ) (Table I) overall and across defined age categories. The effects of age and LH, SHBG, testosterone and DHEA-S levels on FSH and Inhibin B levels were investigated to identify potential confounders via a stepwise selection method. After adjustment for LH, DHEA-S and testosterone, FSH levels were not significantly different in DZ compared with MZ twins, [ANOVA  $F$  = 0.60,  $P$  = 0.440 (Fig. 1)]. However, levels of Inhibin B were significantly higher in the DZ group ( $F$  = 7.72,  $P$  = 0.006) after adjustment for LH, SHBG and testosterone.

## Discussion

The peak incidence of testicular tumours occurs relatively early at 20–40 years of age—suggesting an age-specific aetiological factor—and has been increasing (Bergstrom *et al.*, 1996; Power *et al.*, 2001; Richiardi *et al.*, 2004a) in parallel with the increase in male subfertility rates (Carlsen *et al.*, 1992; Auger *et al.*, 1995; Irvine *et al.*, 1996) over the past four decades. The latter has led authors to postulate common aetiologies which include:

- (i) toxic insults to germinal epithelium from environmental or endogenous overexposure to transplacental estrogen (Swerdlow *et al.*, 1997a; Skakkebaek *et al.*, 2001);
- (ii) testicular maldevelopment (Skakkebaek *et al.*, 2001; Slowikowska-Hilczler *et al.*, 2001) and maldescent (Swerdlow *et al.*, 1997b; Moller *et al.*, 1998);
- (iii) heredity (Swerdlow *et al.*, 1997a);
- (iv) dizygotic twinning (Lambalk and Boomsma, 1998), with which it shares a geographical distribution;
- (v) subfertility which is related both to testicular cancers (Moller and Skakkebaek, 1999; Jacobsen *et al.*, 2000; Richiardi *et al.*, 2004b) and cryptorchidism (Swerdlow *et al.*, 1997a; de Gouveia Brazao *et al.*, 2003).

The syndrome of testicular dysgenesis may comprise a spectrum from mild subfertility to severe gonadal malformation, often with partial sex reversal, and neoplasia (Skakkebaek *et al.*, 2001). There is growing evidence to suggest tumours



**Figure 1.** Hormone levels in monozygotic (MZ) and dizygotic (DZ) twins (data shown as adjusted means and 95% confidence intervals). Although still within the normal range (<400 pg/ml), DZ twins have significantly higher Inhibin B levels than MZ twins but similar FSH levels.  $P < 0.05$  indicates significance of difference between MZ and DZ groups.

**Table 1.** Observed means and SDs of hormone levels in monozygotic (MZ) and dizygotic (DZ) twins by 10-year age band

Mean (SD)	Age group (years)						Overall
	<20	21–30	31–40	41–50	51–60	>60	
No. of individuals	28	26	38	24	30	32	208
FSH (IU/l)	MZ 3.5 (2.3)	4.9 (2.0)	6 (2.4)	6 (5.9)	5.3 (2.5)	4.8 (4.9)	5.2 (4.0)
	DZ 6.3 (7.9)	4.8 (2.6)	7.1 (8.6)	4.3 (2.6)	4.3 (3.6)	5 (3.5)	5.2 (5.0)
Inhibin B (pg/ml) NR <400	MZ 225.9 (66.9)	175.3 (41.9)	207.0 (61.7)	210.4 (86.7)	188.7 (45.8)	178.7 (98.3)	196.8 (73.4)
	DZ 184.7 (81.8)	193.4 (42.2)	224.9 (112.0)	236.9 (75.2)	254.4 (107.5)	236.4 (86.2)	225.1 (87.7)
LH (IU/l)	MZ 3.7 (1.2)	4.7 (2.0)	3.3 (1.1)	3.9 (1.7)	3.3 (1.5)	4.8 (1.8)	4.0 (1.7)
	DZ 5.1 (4.7)	4.1 (1.9)	5.2 (3.0)	3.5 (1.4)	3.6 (1.5)	3.6 (1.8)	4.0 (2.6)
Testosterone (nmol/l)	MZ 17.7 (6.8)	19.4 (5.9)	17.5 (5.0)	17.2 (6.3)	15.1 (7.0)	20.9 (8.6)	18.1 (6.7)
	DZ 20.6 (6.4)	16.9 (9.3)	19.6 (5.1)	19.6 (4.6)	19.5 (8.4)	19.3 (5.8)	19.4 (6.4)
DHEAS (μmol/l)	MZ 7.3 (3.3)	12.5 (4.7)	9.5 (3.3)	6.5 (3.2)	6.3 (2.4)	8.9 (3.3)	8.6 (4.1)
	DZ 9.3 (3.4)	10.4 (4.3)	10.1 (6.6)	8.0 (3.1)	8.4 (3.3)	8.5 (3.6)	8.9 (3.9)
SHBG (nmol/l)	MZ 27.3 (7.2)	29.8 (15.3)	29.3 (7.3)	31.2 (11.3)	22.5 (12.6)	31.6 (9.5)	29.3 (11.3)
	DZ 27.7 (9.7)	25.7 (9.6)	32.3 (25.4)	26.0 (8.9)	31.8 (15.3)	30.9 (13.2)	29.5 (13.6)

NR = normal range. The elevated Inhibin B levels in DZ compared with MZ twins appear to be due to a failure to undergo the expected age-related decline.

arise from persistent fetal gonocytes or carcinoma-in-situ (CIS) (Slowikowska-Hilczer *et al.*, 2001; (Rajpert-De Meyts *et al.*, 2004), whose impaired or delayed ability to differentiate into spermatogonia—most probably caused by disturbed down-regulation of transcription factors maintaining totipotency (Rajpert-De Meyts *et al.*, 2004)—are implicated in the subsequent sex cord tumours (Slowikowska-Hilczer *et al.*, 2001; Skakkebaek *et al.*, 2003). Potential hormonal hypersecretion, in particular that of FSH causing over-expression of mitogenic cyclin D2, (Sicinsky *et al.*, 1996) has been anticipated to play a role in the aetiology of testicular carcinoma (Lambalk and Boomsma, 1998; Slowikowska-Hilczer *et al.*, 2001). In testicular subfertility or testicular dysgenesis, a defect in Sertoli cell function with consequent demise of spermatogenesis could cause both the observed decline in Inhibin B secretion and the FSH hypersecretion (Jensen *et al.*, 1997; Andersen *et al.*, 2000). In male DZ twinning, it was hypothesized that the boys/men may have inherited from their mother the autosomal genetic factor causing her multiple follicular growth and ovulation with raised early follicular FSH levels and normal Inhibin A and B levels (Lambalk *et al.*, 1998). We found no such FSH hypersecretion in the male DZ twins we studied but, to our surprise, noted elevations in Inhibin B compared with male MZ twins—even when the youngest group was excluded.

There are two possible interpretations of this finding. The first is that the normal testicular Inhibin B feedback response to endogenous pituitary FSH secretion (Hayes *et al.*, 2001) is in some way amplified or 'over sensitive' in male DZ twins. It is unlikely that polymorphisms of the FSH receptor associated with up-regulation of expression account for this over-sensitivity since a large study of 183 sister pairs and trios (Montgomery *et al.*, 2001), who had all given birth to spontaneous DZ twins, significantly excluded linkage to the region of chromosome 2 where the FSH receptor is located rather precluding FSH receptor gene mutations as a common cause of familial DZ twinning. It is more possible that intragonadal factors such as BMP15 and GDF9, which increase gonadal response to FSH in animal studies, are involved. Alternatively, since in the adult, Inhibin B is primarily a marker of Sertoli cell function and

positively correlated with sperm count (Jensen *et al.*, 1997; Byrd *et al.*, 1998), the raised Inhibin B levels in DZ twins may rather mirror greater numbers of Sertoli cells and/or better spermatogenesis. The normal infant pituitary gonadal axis is far from quiescent (Andersson *et al.*, 1998), so that relative FSH hyperstimulation at this time might further stimulate Sertoli cell proliferation which, in turn, would result in higher Inhibin B levels in later life. This explanation of maternal (and/or fetal) FSH hyperstimulation would accord with the theory that environmental factors operating perinatally influence male reproductive health (Swerdlow *et al.*, 1997a; Skakkebaek *et al.*, 2001). Since DZ twinning has been related to both male and female fecundity (Tong and Short, 1998; Richiardi *et al.*, 2004), it may also be speculated that the male DZ twins' offspring have inherited good sperm quality from their fathers—manifest as a higher adult Inhibin B level.

Our results indicate for the first time that, in male DZ offspring, the endocrine activity of the pituitary-gonadal axis is different from male MZ controls. This contributes to the theory that the answer to human DZ twinning lies in the actions and mutual interactions of FSH and Inhibin B. It remains open to question whether this mechanism plays a role in the development of testicular seminoma.

### Contributors

Alastair Sutcliffe, Pierre Bouloux and Helen Spoudeas designed the study. Samples were obtained from the St Thomas's Adult Twin Registry and the Sydney Adult Twin Registry by Tim Spector and Philip Sambrook, respectively. Assays were performed by Devaki Nair. Alastair Sutcliffe, Pierre Bouloux, Cornelis B. (Nils) Lambalk, Tim Oliver and Helen Spoudeas contributed to the interpretation of the data. Wendy Bannister performed statistical analyses. All authors contributed to writing of the paper.

### Conflict of interest statement

We have no conflicts of interest to declare.

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## References

- Auger J, Kuntsman JM, Czyglik F and Jouannet P (1995) Decline in semen quality among fertile men in Paris during the last 20 years. *N Engl J Med* 332,281–288.
- Bergstrom R, Adami H-O, Mohner M, Zatonski W, Storm H, Ekblom A, Tretli S, Teppo L, Akre O and Hakulinen T (1996) Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon. *J Natl Cancer Inst* 88,727–733.
- Braun MM, Ahlbom A, Floderus B, Brinton LA and Hoover RN (1995) Effect of twinning on incidence of cancer of the testis, breast, and other sites (Sweden). *Cancer Causes Control* 6,519–524.
- Byrd W, Bennett MJ, Carr BR, Dong Y, Wiens F and Rainey W (1998) Regulation of biologically active dimeric inhibin A and B from infancy to adulthood in the male. *J Clin Endocrinol Metab* 83,2849–2854.
- Carlsen E, Giwercman A, Keidig N and Skakkebaek NE (1992) Evidence for decreasing quality of sperm during past 50 years. *Br Med J* 305,609–613.
- de Gouveia Brazao CA, Pierik FH, Ernepreiss Y, de Jong FH, and Dohle Weber RF (2003) The effect of cryptorchidism on inhibin B in a subfertile population. *Clin Endocrinol* 59,136–141.
- Hayes FJ, Pitteloud N, DeCruz D, Crowley WF Jr and Boepple PA (2001) Importance of inhibin B in the regulation of FSH secretion in the human male. *J Clin Endocrinol Metab* 86,5541–5546.
- Irvine S, Cawood E, Richardson D, MacDonal E and Aitken J (1996) Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. *Br Med J* 312,467–471.
- Jacobsen J, Bostofte E, Engholm G, Hansen J, Olsen JH, Skakkebaek NE and Moller H (2000) Risk of testicular cancer in men with abnormal semen characteristics: cohort study. *Br Med J* 321,789–792.
- Jensen TK, Andersson A, Hjollund NHI, Scheike T, Kolstad H, Giwercman A, Henriksen TB, Ernst E, Bonde JP, Olsen J *et al.* (1997) Inhibin B as a serum marker of spermatogenesis: correlation to differences in sperm concentration and follicle-stimulating hormone levels. A study of 349 Danish men. *J Clin Endocrinol Metab* 82,4059–4063.
- Lambalk CB and Boomsma DI (1998) Twinning, cancer, and genetics. *Lancet*, 351,909–910.
- Lambalk CB, Boomsma DI, De Boer L *et al.* (1998) Increased levels and pulsatility of follicle-stimulating hormone in mothers of hereditary dizygotic twins. *J Clin Endocrinol Metab* 83,481–486.
- Martin NG, Robertson DM, Chenevix-Trench G, de Kretser DM, Osborne J and Burger HG (1991) Elevation of follicular phase inhibin and luteinizing hormone levels in mothers of dizygotic twins suggests nonovarian control of human multiple ovulation. *Fertil Steril* 56,469–474.
- Moller H and Skakkebaek NE (1999) Risk of testicular cancer in subfertile men: case-control study. *Br Med J* 318,559–562.
- Moller H, Cortes D, Engholm G and Thorup J (1998) Risk of testicular cancer with cryptorchidism and with testicular biopsy: cohort study. *Br Med J* 317,729–792.
- Montgomery GW, Duffy DL, Hall J *et al.* (2001) Mutations in the follicle-stimulating hormone receptor and familial dizygotic twinning. *Lancet* 357, 773–774.
- Oliver RT (1990) Atrophy, hormones, genes and viruses in aetiology of germ cell tumours. *Cancer Surv* 9,263–286.
- Power DA, Brown RSD, Brock CS, Payne HA, Majeed A and Babb P (2001) Trends in testicular carcinoma in England and Wales, 1971–99. *BJU Int* 87,361–365.
- Rajpert-De Meyts E, Hanstein R, Jorgensen N, Graem N, Vogt PH and Skakkebaek NE (2004) Developmental expression of POU5F1 (OCT-3/4) in normal and dysgenetic human gonads. *Hum Reprod* 19,1338–1344.
- Richiardi L, Belloc R, Adami HO *et al.* (2004a) Testicular cancer incidence in eight northern European countries: secular and recent trends. *Cancer Epidemiol Biomarkers Prev* 13,2157–2166.
- Richiardi L, Akre O, Montgomery SM, Lambe M, Kvist U and Ekblom A (2004b) Fecundity and twinning rates as measures of fertility before diagnosis of germ-cell testicular cancer. *J Natl Cancer Inst* 96,145–147.
- Skakkebaek NE, Rajpert-De Meyts E and Main KM (2001) Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 16,972–978.
- Skakkebaek NE, Hoei-Hansen CE, Holm M, Jorgensen N and Rajpert-De Meyts E (2003) Association between testicular dysgenesis syndrome (TDS) and testicular neoplasia: evidence from 20 adult with maldevelopment of the testis. *APMIS* 111,1–11.
- Slowikowska-Hilczner J, Szarra-Czapnik M and Kula K (2001) Testicular pathology in 46XY dysgenetic male pseudohermaphroditism: an approach to pathogenesis of testis cancer. *J Androl* 22,781–792.
- Swerdlow AJ, De Stavola BL, Swanwick MA and Maconochie NE (1997a) Risks of breast and testicular cancers in young adult twins in England and Wales: evidence on prenatal and genetic aetiology. *Lancet*, 350,1723–1728.
- Swerdlow AJ, Higgins CD and Pike MC (1997b) Risk of testicular cancer in cohort of boys with cryptorchidism. *Br Med J* 314,1507–1511.
- Tong S and Short RV (1998) Dizygotic twinning as a measure of human fertility. *Hum Reprod* 13,95–98.

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