Early life events predict adult testicular function; data derived from the Western Australian (Raine) birth cohort.

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Abstract

Context The impact of early life events on testicular function in adulthood is not well understood.

Objective To study the early influences of fetal growth, exposures to cigarette smoke *in-utero* and cord blood estrogens, and the influences of growth and adiposity in childhood through adolescence; on testicular function in adulthood.

Design Male members of the Western Australian Pregnancy Cohort (Raine) were contacted at 20-22 years of age. Of 913 contacted 423 (56%) agreed to participate; 404 underwent a testicular ultrasound, 365 provided a semen sample and reproductive hormones were measured (384). Fetal growth measurements (n=137), umbilical cord estrogen concentrations (n=128), cord testosterone (n=125), and child-adulthood growth charts (n=395) were available.

Results Median sperm output for the 18.6% of men exposed *in-utero* to smoking was lower than non-exposed ($82.4x10^6$ *vs* $123.1x10^6$, p=0.029).

Sperm output in adulthood was inversely correlated with cord serum oestradiol (p=0.019) and estrone (p=0.018). The sperm output of men whose cord blood estradiol and estrone were $<50^{th}$ centile *vs* $>50^{th}$ centile was $191.1x10^6$ *vs* $100.5x10^6$ (p=0.002) and $190.0x10^6$ *vs* $106.0x10^6$ (p=0.012) respectively.

Men with favorable fetal growth patterns *in-utero* were less likely to have total motile sperm counts (TMS) within the lowest quartile (p=0.011), and men born prematurely had reduced serum testosterone levels in adulthood, (13.4 *vs* 16.6nmol/L, p=0.024).

Consistent height above the 50^{th} centile for age through childhood was associated with larger adult mean testicular volume (TV) (p<0.001). Optimal BMI trajectory through childhood and adolescence was associated with larger TV (p=0.009), and higher serum inhibin B (p=0.010) and testosterone (p=0.003) in adulthood.

Conclusions. Exposures to maternal smoking and higher cord blood estrogens at delivery were associated with a reduced sperm output in adulthood. Optimal adult testicular function depends on being born at or above average weight, and maintaining optimal growth and adiposity into adulthood.

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Introduction

The influence of maternal health and early life events upon the subsequent growth and development of a child and its lifelong predisposition to disease is well established (1). Apart from exposure to maternal smoking leading to a reduction in semen parameters (2-5), and hormonal markers of testicular function in adulthood (4, 5), early life influences upon testicular function in adulthood are poorly understood. No association has been identified between birthweight or body mass index (BMI) in childhood with semen quality in adulthood (6). Although the association of reduced semen parameters with smoking (7) and drinking alcohol (8) in adulthood is established, the association with obesity remains uncertain (9-12).

It is unclear whether sperm densities have been diminishing across the Western World (13-17). Although animal studies demonstrate that exposure to exogenous estrogens (18, 19), androgen receptor blockers (18), and sufficient doses of environmental xenoestrogens (19) can damage testicular function. Furthermore, despite the increasing prevalence of xenoestrogens in the environment, claims of adverse effects of global estrogen pollution on testicular function are largely refuted by evidence that domestic animal sperm production has not changed during the last century (20), and that men exposed prenatally to a high doses of diethylstilboestrol have normal adult testicular function and fertility (21). Indeed the human testis appears less susceptible to exogenous estrogens than some animal models (22), hence caution must be exercised when extrapolating data from animal studies.

Our aim was to study the effects of early influences such as prenatal smoking, growth restraint, prematurity, and cord blood estrogens as well as childhood and adolescent growth trajectories and adiposity upon adult testicular function, in a birth cohort featuring reduced selection and participation bias relative to previous studies (23).

Materials and Methods

The Raine study

The Western Australian Pregnancy Cohort (Raine) Study was formed from a pregnancy cohort study of 2,900 women enrolled in a controlled trial by the 18th week of gestation in Perth between 1989-91 and who delivered 2,868 live-born children (24). Participants were equally randomized to either standard antenatal care or to an intensive investigation where maternal blood samples were collected and fetal ultrasound measurements were made at 18 weeks, 24 weeks, 28 weeks, and 34/36 weeks, and again at 38 weeks of gestation. Mixed arterial and venous umbilical cord blood was collected at birth and serum stored unthawed at -80°C until hormone assay. Smoking was defined by responses at interview at 18-20 weeks of gestation. Anthropometric measurements of children were repeated at ages 1, 2, 3, 6, 8, 10, 13-14, 16-17, 20, and 22.

At 20 years of age cohort members were invited to attend follow-up, which involved dual energy X-ray absorptiometry (DEXA) measurement for lean and total fat mass [percentage body fat calculated (25)], and fasting morning serum obtained for measurement of serum testosterone, luteinizing hormone (LH), follicular stimulating hormone (FSH), and inhibin B (inhB), a testicular ultrasound examination, and providing at least one semen sample as reported previously (23). Cohort members could participate in some or all the follow-up assessments. This study was approved by the Human Research Ethics Committee of the University of Western Australia and written consent was provided.

The current cohort of men aged 20-22 years included 913 contactable men of whom 423 (46.3%) participated in the testicular function study previously described (Figure 1) (23).

In-utero growth patterns

Ultrasound fetal growth assessments were performed using serial measurements of head circumference, abdominal circumference, femur length, and estimated fetal weight during

pregnancy (24). Fetal growth patterns growth were classified as: consistently small for gestational age (SGA) (below the 10th centile, "SGA"); showing signs of growth restriction by being marked on a lower centile with increasing gestation ("drifting towards SGA"); fetal growth consistently within the 10th and 90th centiles ("normal growth"); consistently large for gestational age (LGA) (above the 90th centile, "LGA"); and showing signs of excessive growth by being marked on a higher centile with increasing gestation ("drifting towards LGA").

Cord blood steroid measurement

Concentrations of total testosterone (TT) and estrogens (estrone, E_1; oestradiol, E_2; estriol, E_3; estetrol, E_4) in cord serum were measured after solvent extraction by highly specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays performed by CPR Pharma Services Pty Ltd, Thebarton, South Australia. The TT assay has previously been described in detail; the limit of quantitation was 0.08 nmol/L (26). Free testosterone (FT) was calculated using the empirical method as described by Sartorius *et al.* (27), which has been validated for use in samples with low albumin and SHBG concentrations. The LC-MS/MS estrogen assays have been reported previously (28).

Growth in childhood and adolescence

Age and gender specific height z-scores were calculated using Center for Disease Control and Prevention (CDC) growth chart norms using a SAS Program for the 2000 CDC Growth Charts (ages 0 to 20 years) accessed on 1 Mar 2015 (29). BMI measurements between ages 2 and 20 were derived from recorded height and weight measurements. Corresponding sex-adjusted and age-adjusted BMI z-scores and BMI percentiles (see below) were derived using CDC z-scores (ages 2-20 years) (29) categorized at all ages between 2 and 20 years as indicative of being underweight (below 5th percentile), being of normal weight (5-85th percentile), being overweight (>85th and ≤95th percentile), and being obese (>95th percentile). These BMI categories based on the percentiles between up to 20 years of age were also extended until the maximal age participants (22.1 years).

Testicular assessment at 20 years of age

Semen samples were provided and analyzed according to World Health Organization (WHO) semen manual guidelines (30). Sperm output was characterized as both sperm concentrations (million sperm per ml of ejaculate) and total sperm output (million per ejaculate). Total motile sperm (TMS) was derived from calculation of the seminal volume x concentration x motility (%A grade + %B grade motility). The sperm chromatin structural assay (SCSA) was performed as described (31) with slight modifications. The DNA fragmentation index (DFI) represents the percentage of sperm within the sample with fragmented or damaged DNA. The percentage of sperm with high DNA stainability (HDS) represent the percentage of sperm within the sample with incomplete chromatin condensation. Serum concentrations of inhB, LH, FSH, testosterone, estradiol (E1), and estrone (E2) were measured and a testicular ultrasound performed to estimate the volume of each testis with the mean testis volume (TV) calculated using the formula for a prolate ellipsoid (length x width x height x 0.52) (32, 33).

Statistical Analysis

The primary end-points were sperm output and testicular volume at 20 years of age. The total number of 350-420 participants allows to detect the significant bivariate (and partial) linear correlations between each of the primary endpoints and other continuous characteristics with >80% and >95% power to detect respective correlations of 0.15 and 0.20. Statistical power of around 35% is achieved to detect bivariate correlations in hypothesis tests that include cord blood steroid measurements available on 110-130 participants. Evaluation of factors associated with the semen parameters above and below their 25th percentiles conducted using Mann-Whitney tests on 350-420 participants attains >80 % power to detect a difference in 0.4 standard deviations between the groups for the characteristics considered (and >25% on 110-130 participants) (PASS 2008 Kaysville, Utah USA www.ncss.com).

Construction of BMI and height z-score trajectories was conducted independently before the statistical analysis of the early life influence on testicular function. The group-based trajectory modelling technique to approximate the number of trajectory groups or latent classes (Latent Class Group Analysis) was used to construct the BMI and growth trajectories on all Raine cohort male participants with at least 3 measurements available over time (34). The trajectory modelling was implemented using the SAS statistical software macro (proc TRAJ) (29, 35, 36). Construction of the BMI trajectories extended the adiposity trajectories on Raine cohort participants developed up to 14 years of age by Huang *et al*. (37) and by Smith *et al.* (38). Reports by Smith *et al. and* Huang *et al*. concluded 6 and 7 adiposity groups as optimal; hence, models including between 5 and 8 trajectories were considered. For each model individual participants were assigned to the trajectory group with the highest posterior probability of membership. All trajectory models included the exact age of participants at time of BMI measurement as covariates to account for the irregular spacing of the BMI z-scores collected during the follow up visits. No other participant's characteristics were modelled. Initially, each trajectory model considered all trajectories with cubic shape over time, and the order was subsequently reduced according to the statistical significance of highest order parameter beyond the intercept. Models that included a different number of trajectories and linear or quadratic terms reflecting the shape of trajectories over time were compared using Bayesian Information Criterion (BIC) and examination of the posterior probability diagnostics. The optimal, BMI and height, trajectories were examined for their association with testicular function. The BMI trajectories were compared relative to the 'healthy BMI' trajectory. Height trajectories were considered with the objective to dichotomize the participants with 'favorable' and 'unfavorable' growth pattern. Up to 8 height trajectories were examined; 7 trajectory groups were retained and unfavorable growth was defined by a pattern of z-scores below age-specific average z-score.

Continuous data variables were summarized using means and standard deviations (SD) if Gaussian, otherwise by medians, inter-quartile ranges (IQR) (reported as Q1-Q3) and ranges. Categorical data were summarized using frequency distributions. Mann-Whitney or Kruskal-Wallis tests (for non-Gaussian data) for two groups or analysis of variance for more than two groups were used to compare subgroups of the cohort. Association between the testicular function parameters and hormone concentrations were estimated using Pearson correlation coefficient. Where data had a non-Gaussian distribution, power transformation was performed with cube-root transformation and other optimal power transforms according to Box-Cox analysis using Shapiro-Wilks λ as an omnibus estimate to transform to a Gaussian distribution as required. Hypothesis tests were two-sided with p-values<0.05 considered statistically significant. To adjust for multiple comparisons, for *k* pairwise contrast comparisons, p-values <0.05/k for each contrast were interpreted as statistically significant to maintain an overall level of significance of 0.05. SPSS (version 20.0, IBM SPSS Statistics for Windows, Version 20.0 Armonk, NY: IBM Corp) or NCSS (version 10, Kaysville, Utah, USA www.ncss.com) statistical software were used for data analysis.

Results

Description of study population

Among 423 cohort members who participated in the testicular function study, 365 underwent semen assessments, and 404 underwent testicular ultrasound examination (Figure 1). The reproductive hormone measurements, semen parameters, and TV measurements have been previously reported (23). The demographic, anthropometric, reproductive hormones and DEXA indices of the participants and the non-participating Raine cohort members are provided in Table 1. Men who did not participate in the testicular evaluation study (n=282) did not differ from those who did with regard to age, weight, BMI, smoking, or circulating levels of reproductive hormones (LH, FSH, inhB, T), but were significantly shorter (p=0.008), had lower alcohol consumption (p=0.019), had less lean mass (p=0.022), and a greater total fat mass (p=0.042). As previously reported, alcohol

consumption and cigarette smoking in adulthood had no significant influence on TV, semen variables or concentrations of circulating reproductive hormones (23).

Intrauterine growth and associations with testicular volume, sperm output, and reproductive hormones in adulthood

While no direct linear relationship between growth in *utero* and average adult TV and sperm output was determined (data not shown), men with *in-utero* growth patterns described as consistently LGA or drifting LGA, when compared to the fetal growth patterns of the other men *in-utero,* were less likely to have TMS <25th percentile (p=0.011, Supplementary Figure 1). The median TMS for SGA/normal growth/LGA were 433./74.0/90.4 million motile sperm, respectively. The proportions of men with a sperm output <25th percentile were 38.9%, 25.0%, 13.0% for SGA, AGA, LGA groups respectively (p=0.166). No significant relationships associations were found for fetal growth trajectory and adult serum inhB, FSH, LH or testosterone levels (Table 2).

Maternal smoking and association with sperm output in adulthood

Median sperm output for men born to mothers who were or were not smoking in mid-pregnancy was 82.4x10^6 *vs* 123.1x10^6 sperm (p=0.029), and the TMS was reduced (43.4x10^6 *vs* 67.3 x10^6 sperm, p=0.046, Table 2).

Gestational age at delivery and association with serum testosterone in adulthood

Thirty seven participants were born preterm (less than 37 weeks of gestation). Preterm delivery was associated with a reduction in median serum testosterone in adulthood (13.4 nmol/L *vs* 16.6 nmol/L, p=0.024), and remained significant after adjustment for current overweight/obese BMI (Table 2). No other significant associations of testicular function were associated with premature delivery (tables 2 and 4), and gestational age at delivery as a continuous variable was not associated with any marker of testicular function (table 4).

Cord blood testosterone concentration and association with markers of testicular function in adulthood

No correlations between cord total testosterone or free testosterone and markers of adult testicular function were determined (Table 4), other than a sperm morphology below WHO standard of 4% was associated with a higher cord blood free testosterone table 4.

Cord blood estrogens and association with sperm output in adulthood

Mixed cord blood serum estrogen levels were available for 128 men (Tables 4 and 5). Sperm output and TMS were associated with lower cord blood serum E_2 (p=0.019, p=0.035) and E_1 concentrations (p=0.018, p=0.030 respectively, Table 4). Cord E_2 and E_1 concentrations >50th percentile were associated with a reduced sperm output (p=0.002, p=0.012 respectively), TMS (p=0.002, p=0.021 respectively) and sperm concentration in adulthood (E_1 only, p=0.033, Table 2). Higher cord E_2 concentration was associated with reduced sperm concentration and serum LH in adulthood (Table 4).

Mixed cord blood serum testosterone and estradiol levels were both available for 111 men. The cord blood total T:E_2 ratio was positively related to the sperm output, TMS and sperm concentration in adulthood (r=0.237, r=0.207 and r=0.226 respectively) (Table 4). The range in T:E_2 ratio was from 0.0038 – 0.3303 (5th and 95th centiles 0.0064 and 0.0666 respectively).

Childhood growth into adulthood, and associations with testicular volume ~~TV~~

There was a positive association for TV with a favorable childhood growth pattern (p=0.001, Table 2 and Supplementary Figure 3), and a linear association of poor growth with an increased chance of mean TV <25th percentile (12.6 mls, p=0.006). There were no other significant associations of childhood and adolescent growth patterns detected (Table 2).

Final adult height was associated with TV such that the shortest men (lowest tertile) had a lower TV compared to the men in the middle and upper height tertiles (median TV 13.8, 15.1 and 15.9 mls, respectively, p<0.001). There was no relationship between final adult height with other markers of testicular function (Table 2).

Adiposity through childhood into adulthood, and associations with testicular volume and reproductive hormones in adulthood

BMI trajectories were associated with TV (p=0.009), serum inhB (p=0.010) and serum TT concentrations (p=0.003) (Supplementary Figure 2, Table 2). Compared to the optimal normal BMI trajectory, 'very low stable' (p=0.005) and 'low stable' adiposity trajectories (p=0.003) were associated with reduced mean testicular volume (p=0.005 and p=0.003 respectively, Table 3); 'rising to high' adiposity was associated with reduced serum inhB (p<0.001), and reduced TT concentrations in adulthood (p<0.001). BMI at 20-21 years was associated with serum inhB (p=0.001) and TT levels (p<0.001) (table 2), with obesity being associated with reduced serum inhB and TT concentration relative to normal BMI (p<0.001 for both, Table 3).

Adult adiposity assessed by DEXA scan and association with testicular volume and reproductive hormones in adulthood

DEXA total soft tissue positively correlated with average TV (r=0.231, p<0.001), and total lean mass (r= 0.291, p<0.001), but total soft tissue was inversely correlated with serum inhB and TT at 20 years of age (r=-0.113 and -0.146 respectively, p<0.001 for both, Table 4). Total lean mass was positively associated with mean TV (r=0.291, p<0.001), and total fat mass was inversely correlated with adult serum TT concentration (r=-0.122, p=0.022).

Discussion

Summary of results

This study shows that optimal adult testicular function is associated with good intrauterine growth, without exposure to maternal smoking, or higher concentrations of endogenous cord serum estrogens and being delivered at term. Optimal testicular function is also associated with good growth through childhood, with a normal BMI trajectory and a lean tissue mass, to become a tall adult. As previously reported (23), and in agreement with a recent study (39), but contrary to other reports (8, 40), we did not demonstrate an adverse influence of personal history of smoking or heavy drinking with reduced testicular function in adulthood. Over a quarter of our participants were overweight, and in line with previous studies, we confirmed that overweight or obese men and those with a higher fat mass had poorer testicular function (41).

The prenatal influence of growth restriction

We demonstrated that a normal fetal growth trajectory (consistently between the 10^{th} and 90^{th} centiles) was associated with better adult testicular function. A study of growth restricted fetuses demonstrated that in adulthood these men had lower testicular volume and serum testosterone levels with higher serum LH levels, but no difference in the serum FSH or inhB values compared with non-growth restricted men (42). Other studies have failed to demonstrate an influence of birth weight on subsequent semen parameters (6, 43); however it is recognized that birth weight is only a summary measure of fetal growth patterns (44).

The prenatal influence of exposure to cigarette smoke

Almost 20% of men within Raine cohort were exposed to maternal cigarette smoke in pregnancy, and in agreement with previous studies (2, 3, 45), we demonstrated that cigarette exposure *in-utero* leads to a reduction in sperm production. Other researchers have demonstrated that the sons of mothers who smoked had lower sperm counts, an earlier onset of puberty and reductions in serum inhB in adulthood (5). The reason proposed for this association is the dose-dependent reduction in both human fetal germ and somatic cells numbers associated with maternal smoking (46). A study of

male fetuses who underwent termination of pregnancy in the second trimester demonstrated no significant differences in fetal size, the testis weight, their cell number or the diameter of the seminiferous tubule diameter whether the mother was a smoker or not (47). Further there were no difference in the circulating LH and testosterone, however the Sertoli cell-specific gene; desert hedgehog, was significantly altered by maternal smoking (47).

The prenatal influence of cord blood estrogens

Human studies of exposure to pesticides with estrogenic potential in adulthood suggest that exposure to exogenous xenoestrogens may have a detrimental effect on spermatogenesis (48). While antenatal exposure to trace levels of ~~estrogenic~~ phthalates have been purported to be estrogenic and associated with subtle differences in the genital development of male off-spring (49), this has not been confirmed in populations with even lower levels of phthalate exposure (50). Evidence derived from study of male infants exposed *in-utero* to high doses of diethylstilbestrol demonstrated normal male fertility and reproductive tract development (21, 51), and recent evidence would suggest that the extrapolation of data from animal models to humans may not be valid (22, 52). The major period of Sertoli cell proliferation is believed to occur during fetal or early postnatal development, when testosterone levels are maximal (53). It is proposed that exogenous estrogenic environmental exposures at this vulnerable stage of development may influence testicular function in adulthood (54). We demonstrated that elevated endogenous estrogens at the time of delivery were associated with apparently worse adult testicular function, and a suppressive effect on serum LH independent of BMI. Furthermore we demonstrated a positive effect of a higher testosterone to estradiol ratio on sperm output in adulthood, with a substantial range in order of magnitude in the differential levels of these hormones. This apparent adult effect of early life endogenous estrogen exposure is consistent with evidence that early life exogenous xenoestrogenic chemical exposure can potentially lead to latent effects on the mature male reproductive system (55, 56). A further possible mechanism is that endogenous estrogens may inhibit the hypothalamic-

pituitary-gonadal axis via steroid negative feedback to reduce LH secretion, which may lead to reduction in intratesticular testosterone at a crucial stage of development.

Sensitive periods for fetal exposure likely occur early in gestation and in the early neonatal period, which may not be reflected in term cord blood levels, although limited human data exist (57). Furthermore, it is possible that the sex steroids levels measured immediately after birth may be affected by the onset and stress associated with delivery (28).

The postnatal influence of adiposity

Our study suggests that growth through childhood and adolescence with a normal BMI is associated with better adult testicular function than men with adverse BMI trajectories through childhood and adolescence. The association between adult adiposity and a reduction in semen parameters (58), and serum testosterone and inhB levels (59) is consistent with previous findings.

The limitations of the study

There is some potential for bias that could have occurred due the participants' attrition over time and self-selection not to participate in the testicular function aspect of the study by some Raine cohort male participants. While the participants (n=423) were similar to the non-participants (n=282) with respect to BMI, weight and reproductive hormones, statistically significant differences were evident between heights, DEXA measures of adiposity and alcohol consumption. The clinical significance of the height and DEXA adiposity differences is difficult to determine, and the difference in DEXA measurements was not related to height differences. The higher alcohol consumption reported by the participants rather than those who opted against participation may have resulted in underestimation of the testicular function in the study. Another possible limitation is the number of cord blood samples available for measurement, a consequence of the original study design.

Conclusion

Early life exposures to maternal smoking and to higher concentrations of maternal cord estrogens are associated with reduced sperm output in adulthood. Furthermore, a positive association with adult testicular function was determined for a child that is born at an appropriate weight for gestational age and maintains good growth during childhood and adolescence, without being under or over-weight.

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Authors' contributions

RJH conceived the study, secured grant funding, performed the study and was primarily responsible for writing the manuscript.

DAD assisted with grant writing, and was primarily responsible for data collation, management and statistical analysis, and assisted with writing the manuscript.

JAK was primarily responsible for the coordination of the adult reproductive hormone samples analysis.

RML assisted with grant writing and writing the manuscript.

NES assisted with grant writing and writing the manuscript.

RJN assisted with grant writing and writing the manuscript.

JED was primarily responsible for the testicular ultrasound examinations and for assisting with writing the manuscript

CEP was primarily responsible for the fetal growth trajectory analyses and for assisting with writing the manuscript

JPN was instrumental in the establishment the Raine cohort, and was responsible for the cord blood collection and for assisting with writing the manuscript

MH assisted with writing the manuscript

DJH assisted with grant writing, data interpretation and writing the manuscript.

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Table 1. Comparison of study participants with those who did not participate.

Characteristic	Participants N=423		Non-participants N=282		p-value
	N	Median (IQR,R) or N (%)	N	Median (IQR,R) or N (%)	
Age (yrs)	423	20 (19.7-20.3; 19.3-22.1)	282	19.9 (19.7-20.3; 19.4-22.0)	0.493
Height (cm)	410	180 (175-185; 162-199)	277	178 (174-183; 156-198)	**0.008**
Height tertile†					
lowest< 1.76 m	410	123 (29.1%)	277	101 (35.8%)	**0.044**
middle		137 (32.4%)		99 (35.1%)	
highest> 1.82m		150 (35.5%)		77 (27.3%)	
Weight (kg)	410	76.6 (68.4-86.1; 52.2-137.5)	277	75 (68.3-86.3; 50.2-176.5)	0.833
BMI (kg/m^2)	410	23.4 (21.4-26.2; 16.7-39.3)	277	24.0 (21.5-26.4; 16.9-48.9)	0.160
BMI category†					
- underweight	410	17 (4.0%)	277	13 (4.6%)	0.300
- normal		319 (75.4%)		202 (71.6%)	
- overweight		41 (9.7%)		28 (9.9%)	
- obese		33 (7.8%)		34 (12.1%)	
Favourable growth†	395	265 (62.6%)	260	156 (58.5%)	0.064
Smoking	324	50 (15.4%)	208	34 (16.3%)	0.778
Alcohol†	323		206		
Nil		44 (10.4%)		47 (16.7%)	**0.019**
Moderate		168 (39.7%)		101 (35.8%)	
Binge		111 (26.2%)		58 (20.6%)	
Adiposity (DEXA)					
Total soft tissue (g)	381	73334 (65515-81785; 50687-118655)	253	71910 (65553-82326; 49489-168839)	0.684
Total lean mass (g)	381	57239 (52648-62086; 40141-83318)	253	55931 (50489-61031; 35281-89622)	**0.022**
Total fat mass (g)	381	14516 (10244-21354; 5428-62673)	253	15885 (11177-23472; 5220-105957)	**0.042**
Soft tissue percentage#	381	20 (15-27; 9-54)	253	22 (17-30; 8-63)	**0.007**
Total fat percentage##	381	20 (15-26; 8-52)	253	21 (16-29; 7-61)	**0.008**
Serum hormones					
LH (IU/l)	384	10.5 (8.3-13.0; 2.3-28.4)	224	10.4 (8.2-13.1; 4.3-21.3)	0.681
FSH (IU/l)	384	4.4 (3.0-6.2; 0.6-39.5)	224	4.2 (3.0-6.2; 0.8-25.8)	0.837
Inhibin B (pg/ml)	384	215.0 (166.4-264.1; 4.5-431.2)	225	221.2 (177.4-269.0; 28.9-543.9)	0.195
Testosterone (ng/ml)	382	4.68 (3.69-5.79; 1.07-9.75)	225	4.43 (3.53-5.86; 1.29-10.3)	0.355

†percentages calculated using 423 and 282 as denominators, and may not add to 100% when data are missing.

Total soft tissue fat percentage = fat mass*100/(fat mass + lean mass); ## Total fat percentage = fat mass*100/(fat mass + lean mass + bone mineral content)

Table 2. The influence of early life and adolescent to adult lifestyle factors on seminal parameters and serum hormone concentrations in adulthood.

			Total motile sperm	Sperm output	Sperm concentration	Mean testicular volume	FSH	LH	Inhibin B	Testosterone
Early life influence			**(million sperm)**	**(million/ejaculate)**	**(million/ml)**	**(ml)**	**(IU/l)**	**(IU/l)**	**(pg/ml)**	**nmol/l**
Smoking at 18wk gestation	No	Med (Q1,Q3)	67.3 (25.3,128.6)	123.1 (50.5,207.0)	45.0 (23.0-73.8)	14.8 (12.6,17.1)	4.4 (2.8,6.1)	10.5 (8.3,12.9)	215.3 (167.5,262.2)	16.3 (12.5,20.0)
		Min-Max	0.0-649.3	0.0-927.5	0.0-220.0	8.4-28.4	0.7-14.9	4.2-22.9	73.4-416.2	5.7-31.5
		N	284	284	284	313	295	295	295	294
	Yes	Med (Q1,Q3)	43.4 (21.0,89.2)	82.4 (44.6,171.5)	37.0 (15.2,64.8)	15.5 (12.8,17.8)	4.2 (3.2,6.4)	10.6 (8.7,13.1)	209.7 (163.4,277.7)	16.1 (13.9,19.7)
		Min-Max	0.0-391.6	0.0-529.2	0.0-136.0	7.6-26.1	1.1-39.5	4.4-28.4	5.2-431.2	5.6-33.4
		N	64	64	64	69	69	69	69	68
		p-value	**0.046**	**0.029**	0.141	0.650	0.762	0.504	0.921	0.642
Preterm delivery <37wk	No	Med (Q1,Q3)	62.0 (24.9,125.7)	106.4 (50.4,202.4)	44.0 (21.5,72.0)	14.8 (12.6,17.3)	4.4 (3.0,6.2)	10.5 (8.3,13.0)	215.0 (166.7,264.8)	16.6 (13.1,20.2)
		Min-Max	0.0-649.3	0.0-927.5	0.0-220.0	8.3-28.4	1.1-39.5	4.2-28.4	55.0-431.2	5.7-33.9
		N	335	335	335	367	348	348	348	346
	Yes	Med (Q1,Q3)	84.3 (25.2,166.0)	122.2 (46.6,257.6)	50.0 (24.0,63.5)	15.1 (12.3,16.8)	4.4 (2.5,5.6)	11.1 (8.3,13.7)	210.2 (164.1,259.9)	13.4 (11.1,17.9)
		Min-Max	1.6-420.5	5.4-592.2	8.0-121.0	9.0-22.1	0.6-14.7	5.7-17.8	73.8-387.4	6.6-29.2
		N	29	29	29	34	35	35	35	35
		p-value	0.297	0.395	0.785	0.729	0.402	0.551	0.853	**0.024**
Cord E_1 >50th percentile (>68.85 nmol/L)	No	Med (Q1,Q3)	119.5 (29.8,204.4)	190.0 (76.5,303.8)	51.0 (37.0,96.0)	15.3 (13.0,18.3)	4.4 (2.8,6.9)	10.8 (8.4,13.2)	208.7 (157.8,269.0)	15.2 (12.3,20.3)
		Min-Max	0.1-420.5	0.7-693.2	0.5-160.0	9.2-25.9	0.6-11.6	4.6-19.3	105.2-416.3	6.3-33.9
		N	55	55	55	64	61	61	61	60
	Yes	Med (Q1,Q3)	65.4 (22.2,111.1)	106.0 (48.2,188.1)	47.5 (21.5,74.0)	14.6 (13.2,16.7)	4.5 (2.8,6.8)	11.7 (7.4,15.8)	214.8 (172.1,236.8)	14.9 (12.5,20.2)
		Min-Max	0.0-547.5	0.0-693.0	0.0-210.0	9.1-26.1	0.6-15.9	2.4-18.3	4.5-387.4	5.6-28.2
		N	56	56	56	62	57	57	57	56
		p-value	**0.021**	**0.012**	0.193	0.365	0.848	0.575	0.968	0.969
Cord E_2 >50th percentile (>25.86 nmol/L)	No	Med (Q1,Q3)	119.3 (45.0,204.8)	191.0 (93.7,286.0)	53.8 (38.3,102.0)	15.1 (12.6,18.4)	4.4 (3.0,7.3)	12.1 (9.1,14.0)	216.4 (160.7,271.4)	15.2 (11.8,21.1)
		Min-Max	0.0-420.5	0.4-639.2	0.2-160.0	9.2-25.9	0.6-11.9	4.6-19.3	105.2-416.2	6.3-33.9
		N	54	54	54	63	60	60	60	59
	Yes	Med (Q1,Q3)	45.5 (17.9,100.1)	100.5 (37.4,188.1)	41.0 (15.0,74.0)	14.8 (13.3,16.7)	4.5 (2.7,6.7)	10.1 (7.3,14.1)	206.5 (165.0,233.5)	15.0 (13.1,20.1)
		Min-Max	0.0-547.5	0.0-693.0	0.0-210.0	9.1-26.1	0.6-15.9	2.3-18.2	4.5-387.4	5.6-29.2
		N	57	57	57	63	58	58	58	57
		p-value	**0.002**	**0.002**	**0.033**	0.664	0.821	0.125	0.296	0.659
Adolescence to adulthood										
Adult height	Short	Med (Q1,Q3)	61.5 (29.8,138.3)	117.5 (51.4,210.9)	51.0 (25.8,86.5)	13.8 (12.0,16.1)	4.0 (2.6,6.1)	10.3 (8.2,12.9)	218.2 (167.8,270.7)	16.4 (12.8,20.1)
		Min-Max	0.02-649.3	0.05-927.5	0.04-206.0	8.2-26.1	0.7-14.9	3.5-22.6	14.1-426.9	5.6-29.3
		N	106	106	106	123	115	115	115	115
	Normal	Med (Q1,Q3)	61.5 (20.4,115.9)	101.5 (45.9,186.6)	41.5 (17.1,63.8)	15.1 (12.3,17.0)	4.6 (3.1,5.9)	10.6 (8.8,13.1)	209.4 (159.4,262.1)	16.5 (12.9,20.7)
		Min-Max	0.1-547.5	1.0-693.0	0.4-220.0	8.1-27.8	0.9-16.7	2.8-18.9	66.2-431.2	6.2-33.4
		N	116	116	116	137	122	122	122	121
	Tall	Med (Q1,Q3)	68.0 (23.6,129.3)	112.8 (48.3,206.4)	42.0 (21.6,67.5)	16.0 (14.0,18.7)	4.4 (3.1,6.2)	10.5 (8.1,13.2)	215.2 (165.7,266.2)	15.7 (12.5,19.6)
		Min-Max	0.0-494.1	0.0-810.0	0.0-180.0	7.9-28.4	0.9-39.5	4.3-28.4	22.6-410.3	4.0-33.9
		N	136	136	136	145	141	141	141	140
		p-value	0.601	0.592	0.110	**<0.001**	0.323	0.841	0.707	0.451
Favourable growth 2-20y	No	Med (Q1,Q3)	64.3 (29.8,144.0)	114.0 (48.0,224.0)	49.0 (23.0,84.0)	13.9 (11.9,16.1)	4.3 (2.7,6.7)	10.4 (8.3,13.5)	219.3 (164.8,272.3)	16.0 (12.7,20.2)
		Min-Max	0.0-649.3	0.1-927.5	0.1-206.0	7.9-24.0	0.7-14.9	2.6-22.6	17.6-416.2	6.1-29.2
		N	111	111	111	126	121	121	121	121
	Yes	Med (Q1,Q3)	63.5 (23.0,124.8)	108.5 (47.9,200.3)	43.5 (19.9,68.0)	15.5 (13.5,17.8)	4.4 (3.1,6.1)	10.5 (8.3,13.0)	213.4 (166.9,259.3)	16.3 (12.8,20.0)
		Min-Max	0.0-547.5	0.0-810.0	0.0-200.0	8.5-28.4	0.9-39.5	4.5-28.4	53.6-426.9	4.9-33.9
		N	230	230	230	249	240	240	240	239
		p-value	0.541	0.657	0.251	**<0.001**	0.920	0.849	0.380	0.641

			Total motile sperm	Sperm output	Sperm concentration	Mean testicular volume	FSH	LH	Inhibin B	Testosterone
Adolescence to adulthood			**(million sperm)**	**(million/ejaculate)**	**(million/ml)**	**(ml)**	**(IU/l)**	**(IU/l)**	**(pg/ml)**	**nmol/l**
Adult height	Short	Med (Q1,Q3)	61.5 (29.8,138.3)	117.5 (51.4,210.9)	51.0 (25.8,86.5)	13.8 (12.0,16.1)	4.0 (2.6,6.1)	10.3 (8.2,12.9)	218.2 (167.8,270.7)	16.4 (12.8,20.1)
		Min-Max	0.02-649.3	0.05-927.5	0.04-206.0	8.2-26.1	0.7-14.9	3.5-22.6	14.1-426.9	5.6-29.3
		N	106	106	106	123	115	115	115	115
	Normal	Med (Q1,Q3)	61.5 (20.4,115.9)	101.5 (45.9,186.6)	41.5 (17.1,63.8)	15.1 (12.3,17.0)	4.6 (3.1,5.9)	10.6 (8.8,13.1)	209.4 (159.4,262.1)	16.5 (12.9,20.7)
		Min-Max	0.1-547.5	1.0-693.0	0.4-220.0	8.1-27.8	0.9-16.7	2.8-18.9	66.2-431.2	6.2-33.4
		N	116	116	116	137	122	122	122	121
	Tall	Med (Q1,Q3)	68.0 (23.6,129.3)	112.8 (48.3,206.4)	42.0 (21.6,67.5)	16.0 (14.0,18.7)	4.4 (3.1,6.2)	10.5 (8.1,13.2)	215.2 (165.7,266.2)	15.7 (12.5,19.6)
		Min-Max	0.0-494.1	0.0-810.0	0.0-180.0	7.9-28.4	0.9-39.5	4.3-28.4	22.6-410.3	4.0-33.9
		N	136	136	136	145	141	141	141	140
		p-value	0.601	0.592	0.110	**<0.001**	0.323	0.841	0.707	0.451
BMI trajectory 2-20 y	1: very low stable	Med (Q1,Q3)	67.8 (12.6,82.7)	113.4 (30.0,156.0)	31.0 (15.0,47.0)	12.5 (10.6,14.6)	2.7 (2.0,6.7)	9.7 (7.6,15.4)	197.7 (159.5,362.6)	16.6 (9.1,21.1)
		Min-Max	0.0-234.0	0.0-360.0	0.0-200.0	9.4-21.0	1.1-11.9	2.3-17.4	4.5-408.8	8.2-26.7
		N	15	15	15	15	11	11	11	11
	2: low stable	Med (Q1,Q3)	53.4 (18.9,139.3)	98.0 (40.7,217.6)	39.0 (16.3,74.0)	14.2 (11.5,16.4)	5.1 (3.3,6.9)	11.7 (9.1,14.9)	206.4 (167.5,248.4)	17.8 (13.3,21.8)
		Min-Max	0.07-432.0	0.0-720.0	0.0-206.0	7.6-25.3	0.6-39.5	3.3-28.4	5.2-416.2	8.5-33.9
		N	63	63	63	74	68	68	68	68
	3: rising to moderate	Med (Q1,Q3)	77.1 (19.0,153.3)	116.1 (40.2,262.5)	45.0 (15.3,66.0)	14.8 (13.3,17.7)	4.0 (2.8,5.7)	9.3 (8.4,11.9)	212.7 (171.8,243.4)	15.2 (11.7,17.3)
		Min-Max	1.6-413.3	5.4-558.0	3.0-180.0	8.6-25.0	1.2-14.9	5.5-19.1	64.1-384.5	5.8-23.6
		N	29	29	29	36	34	34	34	34
	4: rising to high	Med (Q1,Q3)	36.2 (20.7,85.2)	84.0 (42.5,130.6)	33.5 (15.8,73.5)	14.4 (11.8,17.0)	4.4 (3.0,6.1)	10.2 (7.5,13.1)	165.5 (133.9,237.3)	11.8 (9.8,15.2)
		Min-Max	0.0-380.7	0.0-551.8	0.0-136.0	9.0-23.2	1.4-14.3	5.7-19.3	70.4-329.2	3.7-28.3
		N	25	25	25	31	29	29	29	29
	5: optimal normal	Med (Q1,Q3)	87.6 (33.9,141.0)	141.0 (64.0,227.0)	50.0 (25.5,74.0)	16.0 (13.7,17.7)	4.2 (2.9,6.3)	10.1 (8.1,12.2)	236.2 (188.6,271.8)	16.9 (13.6,20.8)
		Min-Max	0.1-649.3	0.9-927.5	1.0-220.0	7.9-28.4	1.2-16.7	4.2-18.8	102.6-389.3	7.4-33.4
		N	101	101	101	109	107	107	107	107
	6: moderately high	Med (Q1,Q3)	57.4 (26.9,126.8)	101.5 (47.0,201.5)	45.5 (21.9,74.0)	15.1 (13.1,17.5)	4.3 (3.4,5.9)	10.8 (8.6,13.1)	226.7 (161.8,267.9)	17.3 (12.9,20.4)
		Min-Max	0.0-547.5	0.4-693.0	0.1-210.0	8.9-27.8	1.1-11.3	4.3-22.9	63.9-426.9	4.4-30.5
		N	82	82	82	86	85	85	85	84
	7: stable high	Med (Q1,Q3)	71.3 (26.8,139.9)	120.5 (53.1,204.3)	56.0 (30.0,76.5)	14.9 (13.9,16.5)	3.8 (2.8,5.5)	11.1 (8.8,12.6)	197.5 (166.5,221.9)	16.0 (13.4,18.6)
		Min-Max	0.0-205.9	0.4-361.2	0.2-129.0	11.2-23.8	0.6-10.1	7.0-16.2	93.3-350.8	5.6-24.3
		N	26	26	26	24	27	27	27	27
		p-value	0.436	0.532	0.333	**0.009**	0.509	0.138	**0.010**	**0.003**
BMI category 20 y	Underweight	Med (Q1,Q3)	55.3 (12.3,126.7)	130.2 (30.0,198.0)	39.0 (15.0,55.0)	13.3 (11.4,14.6)	6.3 (3.4,7.0)	9.3 (8.2,14.7)	203.5 (158.4,297.0)	15.3 (12.3,20.6)
		Min-Max	2.8-227.8	7.2-438.0	7.0-146.0	10.1-16.1	2.1-11.9	5.8-18.0	92.7-416.2	8.2-25.5
		N	15	15	15	15	14	14	14	14
	Normal	Med (Q1,Q3)	66.6 (27.3,129.3)	119.7 (52.5,211.4)	47.0 (22.4,73.3)	15.1 (12.6,17.3)	4.3 (2.8,6.2)	10.5 (8.3,13.0)	220.5 (177.8,269.5)	17.2 (13.4,20.4)
		Min-Max	0.0-649.3	0.0-927.5	0.0-220.0	8.1-28.4	0.7-39.5	4.2-28.4	44.0-431.2	6.6-33.9
		N	282	282	282	305	293	293	293	292
	Overweight	Med (Q1,Q3)	38.9 (17.6,114.7)	82.7 (42.3,199.9)	37.8 (15.1,74.0)	15.3 (13.3,18.7)	4.5 (3.0,6.3)	11.1 (9.6,13.4)	200.7 (158.6,270.8)	14.4 (10.8,18.9)
		Min-Max	0.0-415.5	0.0-639.2	0.0-136.0	9.1-25.0	1.2-14.3	5.2-19.3	84.5-384.5	5.8-29.2
		N	36	36	36	39	40	40	40	39
	Obese	Med (Q1,Q3)	35.1 (17.2,83.4)	68.0 (32.5,152.4)	30.0 (15.3,59.5)	14.8 (13.0,16.5)	3.6 (3.1,5.1)	9.3 (7.7,11.8)	166.8 (136.7,211.7)	12.8 (10.3,15.8)
		Min-Max	0.2-380.7	0.0-551.8	0.0-89.0	8.6-23.8	1.2-8.9	5.7-19.3	70.4-269.5	3.7-22.5
		N	25	25	25	31	31	31	31	31
		p-value	0.121	0.124	0.257	0.055	0.266	0.127	**0.001**	**<0.001**

Table 3. The influence of adiposity during childhood and adolescence on markers of testicular function in adulthood.

Pairwise comparisons of BMI trajectory groupings and BMI at 20-21 years of age with mean testicular volume, Inhibin B and testosterone, at the overall significance level 0.05 (individual contrasts compared a significance levels of 0.0083 and 0.0167 for BMI trajectory contrasts and BMI at 20-21 years of age respectively). Statistically significant differences (reductions **↓)** are highlighted in bold. Pairwise tests between mean testicular volume and BMI at 20-21 years of age were not performed due to no difference overall (p=0.055).

Pairwise comparisons	**Testicular volume**		**Inhibin B**		**Testosterone**	
BMI trajectory groupings						
'very low stable' vs 'optimal normal'	**↓**	**0.005**	-	0.531	-	0.409
'low stable' vs 'optimal normal'	**↓**	**0.003**	-	0.050	-	0.646
'rising to moderate' vs 'optimal normal'	-	0.437	-	0.160	-	0.011
'rising to high' vs 'optimal normal'	-	0.060	**↓**	**<0.001**	**↓**	**<0.001**
'moderately high' vs 'optimal normal'	-	0.284	-	0.125	-	0.381
'stable high' vs 'optimal normal'	-	0.550	-	0.030	-	0.211
BMI at 20-21 years of age						
underweight vs normal	-		-	0.957	-	0.541
overweight vs normal	-		-	0.606	-	0.022
obese vs normal	-		**↓**	**<0.001**	**↓**	**<0.001**

Table 4. Pearson correlation coefficients between semen sample parameters and early life influences and adult DEXA adiposity measurements.

		Total motile sperm	Sperm output	Sperm concentration	SCSA	Mean testis volume	Inhibin B	FSH	LH	Total testosterone
		(million sperm)	(million/ejaculate)	(million/ml)		(ml)	(IU/l)	(IU/l)	(pg/ml)	nmol/l
Gestation at delivery (wk)	r	-0.033	-0.034	-0.019	-0.030	-0.006	0.026	0.071	-0.046	0.061
	N	363	364	364	357	401	383	383	383	381
Cord testosterone (nmol/L)										
Total testosterone	r	0.056	0.075	0.098	0.145	-0.001	-0.072	-0.023	-0.117	-0.019
	N	111	111	111	109	126	118	118	118	116
Free testosterone	r	0.017	0.037	0.073	0.090	-0.042	-0.058	-0.079	-0.106	-0.029
	N	111	111	111	109	126	118	118	118	116
Cord estrogens (nmol/L)										
E1	r	**-0.206***	**-0.222***	-0.184	-0.065	0.018	0.033	-0.007	-0.142	0.056
	N	111	111	111	109	126	118	118	118	116
E2	r	**-0.201***	**-0.224***	**-0.199***	-0.010	0.084	0.018	0.002	**-0.202***	0.041
	N	111	111	111	109	126	118	118	118	116
E3	r	0.009	-0.036	-0.041	0.009	0.031	0.004	-0.006	-0.058	0.158
	N	111	111	111	109	126	118	118	118	116
E4	r	-0.009	-0.049	-0.045	0.094	-0.060	-0.026	0.067	-0.027	0.029
	N	110	110	110	108	125	117	117	117	115
Ratio cord Total T:E2	r	**0.207***	**0.237***	**0.226***	0.102	-0.053	-0.040	-0.021	0.056	-0.036
	N	111	111	111	109	126	118	118	118	116
Adiposity at 20-22 years										
Total soft tissue (g)	r	-0.055	-0.073	-0.075	0.018	**0.231****	**-0.113***	-0.007	-0.036	**-0.146****
	N	332	333	333	326	362	352	352	352	350
Total lean mass (g)	r	-0.004	-0.014	-0.057	0.022	**0.291****	-0.062	0.053	0.013	-0.051
	N	332	333	333	326	362	352	352	352	350
Total fat mass (g)	r	-0.062	-0.072	-0.041	0.018	0.069	-0.089	-0.042	-0.054	**-0.122***
	N	332	333	333	326	362	352	352	352	350
Soft tissue percentage[#]	r	-0.057	-0.061	-0.021	0.022	-0.014	-0.070	-0.048	-0.054	-0.098
	N	332	333	333	326	362	352	352	352	350
Total fat percentage[##]	r	-0.057	-0.062	-0.020	0.015	-0.010	-0.063	-0.052	-0.055	-0.097
	N	332	333	333	326	362	352	352	352	350

** p<0.001; * p<0.05; [#] Total soft tissue fat percentage = fat mass*100/(fat mass + lean mass); [##] Total fat percentage = fat mass*100/(fat mass + lean mass + bone mineral content)

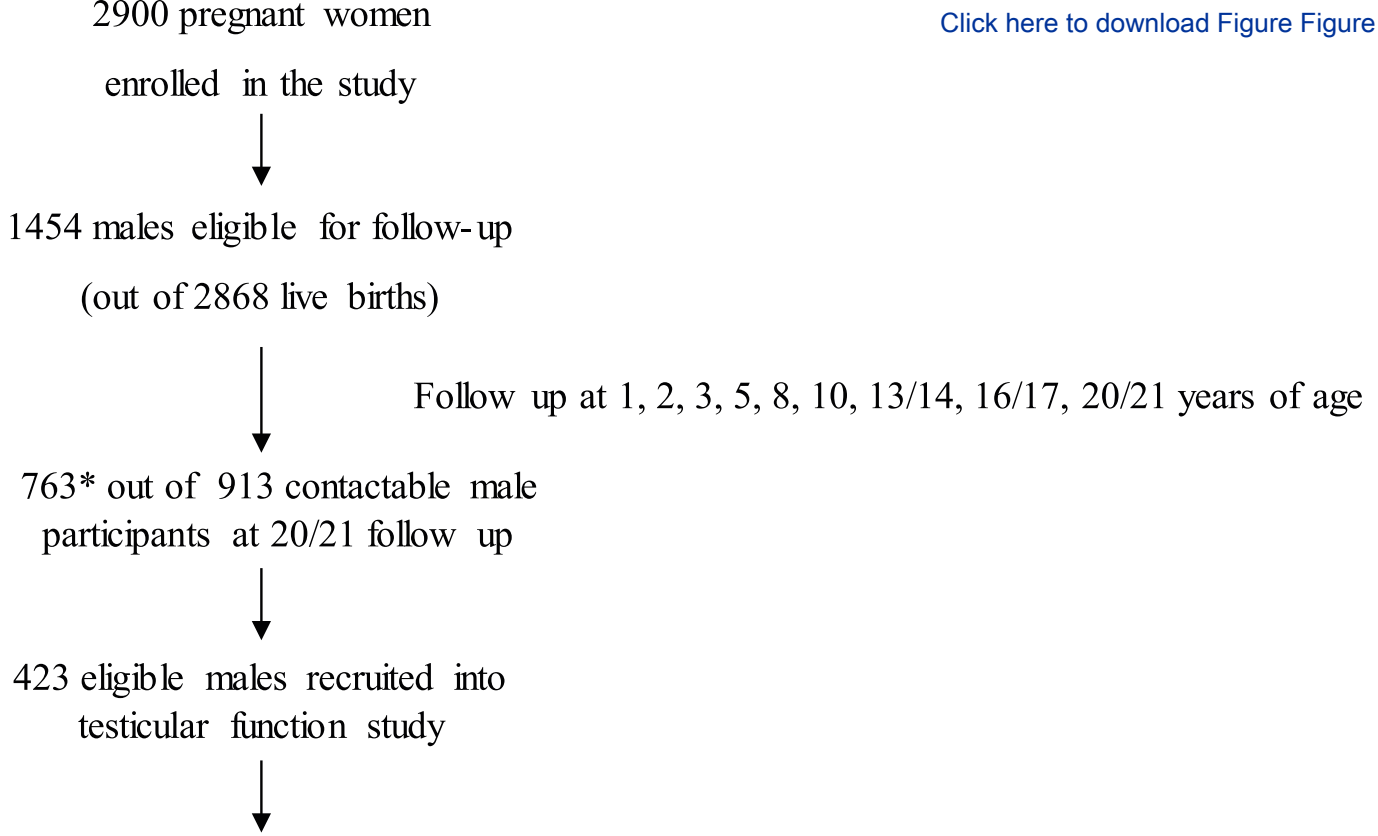
DEXA- Dual energy X-ray absorptiometry

SCSA-Sperm chromatin structural assay

Table 5. Testicular volume and semen parameters and their association with steroid hormone concentrations in mixed cord blood, unless otherwise specified stratified above and below their 25th percentiles. (*Morphology above and below the 4% WHO morphology criteria, [representing the 27th centile for morphology])

Early life influence			Cord total T ng/ml	Cord free T pmol/l	Cord A4 ng/ml	Cord E1 nM	Cord E2 nM	Cord E3 nM	Cord E4 nM
Testicular & semen parameters									
Mean testicular volume (ml)	<12.6	Med (Q1,Q3)	0.1 (0.1,0.2)	6.7 (5.6,10.2)	0.6 (0.5-0.7)	68.8 (35.3,78.8)	25.1 (12.3,31.3)	433.1 (182.0,669.0)	17.8 (9.9,22.5)
		Min-Max	0.0-0.4	2.3-84.1	0.2-1.2	5.3-171.9	3.6-90.8	22.3-1728.9	3.2-54.9
		N	27	27	27	27	27	27	27
	≥12.6	Med (Q1,Q3)	0.1 (0.1,0.2)	8.1 (6.0,11.4)	0.6 (0.4,0.8)	69.2 (45.8,102.7)	26.4 (17.6,40.4)	369.7 (272.2,545.8)	15.5 (11.6,21.4)
		Min-Max	0.0-0.5	2.6-25.3	0.2-4.6	16.3-257.3	5.7-69.1	62.8-1626.8	4.5-57.2
		N	99	99	99	99	99	99	99
		p-value	0.415	0.570	0.847	0.306	0.077	0.924	0.895
Sperm output (million/ejaculate)	<50.39	Med (Q1,Q3)	0.1 (0.1,0.2)	7.5 (5.6,11.6)	0.6 (0.4,0.8)	69.2 (45.8,112.3)	31.0 (20.6,46.6	355.6 (190.5,630.3)	13.3 (11.2,22.7)
		Min-Max	0.0-0.5	2.3-84.1	0.2-2.1	13.3-191.2	8.2-64.0	94.7-1077.5	4.0-57.2
		N	27	27	27	27	27	27	27
	≥50.39	Med (Q1,Q3)	0.1 (0.1,0.2)	7.5 (5.7,10.3)	0.6 (0.5,0.7)	68.1 (45.2,92.0)	23.9 (17.1,33.9)	375.0 (273.1,537.0)	15.8 (12.2,21.3)
		Min-Max	0.0-0.4	2.8-33.3	0.2-4.6	5.3-13.3	3.6-90.8	22.3-1728.9	3.2-51.6
		N	84	84	84	84	84	84	83
		p-value	0.573	0.630	0.383	0.291	0.069	0.736	0.347
Total motile sperm (million sperm)	<25.1	Med (Q1,Q3)	0.1 (0.1,0.2)	7.5 (5.7,11.3)	0.6 (0.5-0.8)	76.3 (58.0,112.1)	31.3 (21.9,46.5)	362.7 (248.9,680.5)	14.4 (12.1,22.3)
		Min-Max	0.0-0.5	2.3-84.1	0.2-1.2	5.3-191.2	3.6-64.0	22.3-1077.5	3.2-57.2
		N	28	28	28	28	28	28	28
	≥25.1	Med (Q1,Q3)	0.1 (0.1,0.2)	7.5 (5.7,10.8)	0.6 (0.5-0.7)	66.9 (44.6,92.3)	23.3 (16.9,33.9)	369.7 (272.2,528.2)	14.4 (12.1,22.3)
		Min-Max	0.0-0.4	2.6-34.3	0.2-4.6	12.5-171.9	5.7-90.8	62.7-1728.9	3.2-57.2
		N	83	83	83	83	83	83	83
		p-value	0.831	0.704	0.203	0.108	**0.015**	0.871	0.612
SCSA (%)	<5.2	Med (Q1,Q3)	0.1 (0.1,0.2)	8.4 (4.9,12.4)	0.6 (0.4-0.8)	69.2(43.1,105.2)	27.7 (18.2,38.1)	333.5(251.7,548.4)	15.2 (12.0,23.2)
		Min-Max	0.0-0.5	2.6-33.3	0.3-2.1	12.5-123.5	11.9-50.0	134.5-968.3	7.2-48.7
		N	26	26	26	26	26	26	26
	≥5.2	Med (Q1,Q3)	0.1 (0.1,0.2)	7.3 (6.0,10.8)	0.6 (0.5-0.7)	68.8 (45.8,98.8)	25.7 (17.4,37.9)	369.7(270.1,531.7)	15.5 (12.0,20.4)
		Min-Max	0.0-0.4	2.3-84.1	0.2-4.6	5.3-191.2	3.6-90.8	22.3-1728.9	3.2-57.2
		N	83	83	83	83	83	83	82
		p-value	0.288	0.594	0.664	0.955	0.798	0.612	0.602
SCSA HDS (%)	<2.9	Med (Q1,Q3)	0.1 (0.1,0.2)	7.6 (5.5,11.7)	0.7 (0.5-0.8)	81.0 (64.3,109.4)	31.3 (20.5,48.1)	493.0 (252.6,707.7)	17.5 (12.5,25.7)
		Min-Max	0.0-0.5	3.8-34.3	0.3-1.2	12.5-160.4	10.4-57.764.0	117.6-1077.5	7.8-57.2
		N	26	26	26	26	26	26	26
	≥2.9	Med (Q1,Q3)	0.1 (0.1,0.2)	7.5 (6.0,10.8)	0.6 (0.4-0.7)	60.4 (44.2,90.4)	23.7 (17.4,33.6)	359.2 (264.8,468.3)	15.0 (11.4,20.2)
		Min-Max	0.0-0.4	2.3-84.1	0.2-4.6	5.3-191.2	3.6-90.8	22.3-1728.9	3.2-51.6
		N	83	83	83	83	83	83	82
		p-value	0.557	0.649	**0.038**	**0.045**	0.061	0.113	0.075
Morphology (%) *	<4	Med (Q1,Q3)	0.1 (0.1,0.2)	8.7 (6.3,15.5)	0.6 (0.5-0.8)	69.2 (45.0,107.3)	25.0 (18.7,43.7)	436.6 (189.8,697.2)	16.0 (12.0,22.5)
		Min-Max	0.1-0.5	3.5-84.1	0.2-4.6	5.3-161.9	3.6-54.8	22.3-1077.5	3.2-57.2
		N	34	34	34	34	34	34	34
	≥4	Med (Q1,Q3)	0.1 (0.1,0.2)	6.9 (5.5,10.2)	0.6 (0.4-0.7)	66.7 (44.9,95.4)	26.4 (17.3,37.9)	364.4 (286.5,510.6)	15.4 (11.8,22.6)
		Min-Max	0.0-0.4	2.3-19.8	0.2-2.1	16.3-191.2	5.7-90.8	62.7-1728.9	4.4-51.6
		N	74	74	74	74	74	74	74
		p-value	0.083	**0.032**	0.259	0.578	0.753	0.876	0.495

Figure



* 753 males participated in the core Raine follow up and 10 males participated in the testicular study alone

Supplemental Figure 1

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