

Prepubertal and Pubertal Predictors of Semen Quality in a Prospective Cohort Study of Russian Young Men: Focus on Endocrine Disrupting

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Background

We have a unique longitudinal study following a cohort of boys with prepubertal assessment of exposures to endocrine disrupting chemicals (EDCs) and annual term follow-up of growth and puberty to evaluate semen quality

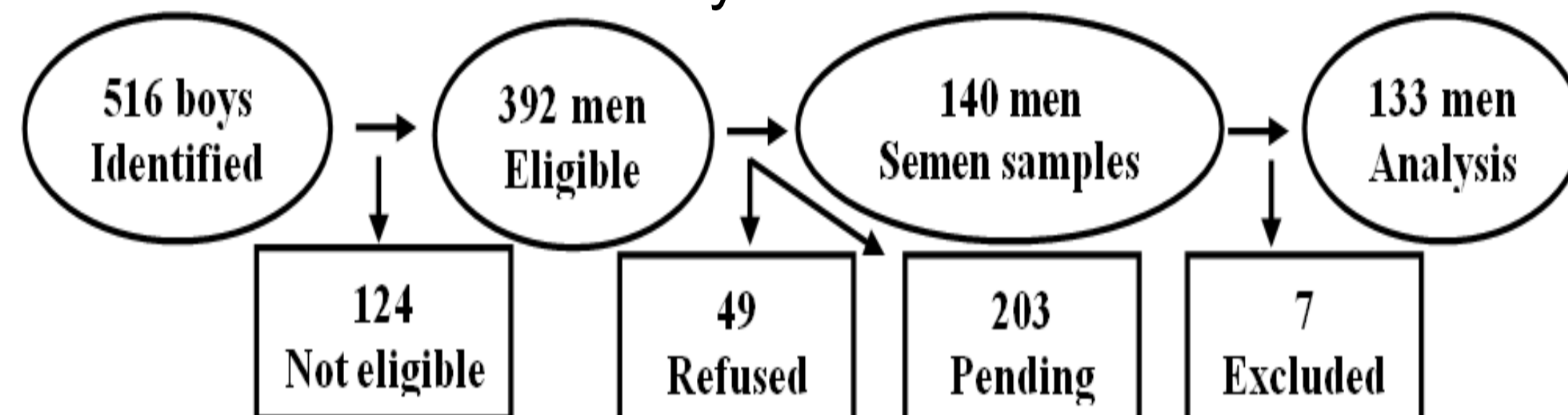
Objective

To describe semen quality and explore associations of prepubertal serum 2,3,7,8-tetrachlorodibenzodioxin (TCDD) levels and pubertal measures with semen parameters in a longitudinal cohort of Russian boys

Study Population

- 516 8-9-year-old boys were enrolled from 2003 to 2005 and underwent annual growth and sexual development assessments (Tanner staging and measurement of testicular volume) for ten years (total 4697 visits).
- At age 18 years, 392 subjects were eligible for semen sample collection.

Figure 1. Recruitment flow diagram for boys/men in Russian Children's Study



Footnote: Identified – using town records during 2003-2005; Not eligible – deceased, moved, location is unknown, refused to participate in follow-up; Pending – not responded yet, temporarily relocated, less than 18 years old, physically immature, postponed; Excluded – from analysis because no baseline serum EDCs measurements (n=4) and severe chronic disease (n=3)

Methods

- The study was approved by the Human Studies Institutional Review Boards of the Chapaevsk Medical Association (Chapaevsk, Russia); HSPH and BWH (Boston, MA, USA), and UMass Medical School (Worcester, MA, USA).

Semen Collection and Analysis:

- At age 18, the subjects were asked to provide two semen samples one week apart (October 2012 – February 2015).
- 133 men contributed 257 semen samples.
- Semen evaluation (SE) includes measurement of volume, sperm concentration and motility (a+b+c categories) by one technician (LS) according to the NAFA-ESHRE manual^{1,2}.

Organochlorine Exposure Assessment:

- Baseline serum samples were analyzed at the NCEH, CDC, Atlanta, USA for TCDD and 57 other organochlorine compounds (described in Burns et al³).

Pubertal Measures at Semen Evaluation:

- Testicular volume was measured using the Australian orchidometer (which extends the Prader orchidometer to 35 mL)

Statistical Analysis:

Prepubertal TCDD and Semen at 18 years

- Sperm concentration, total sperm count and total motile sperm count were log transformed
- Linear mixed models with random intercepts were used to examine the relation between quartiles of TCDD serum concentration with semen parameters
- Final models were adjusted for:
 - body mass index (BMI) (continuous)
 - season (autumn and winter vs. spring and summer)
 - abstinence time (<2days, 2-5 days, ≥5days)

Testicular Volume and Semen at 18 years

- Volume of left and right testicles was averaged
- Semen parameters were log₁₀ transformed
- Linear univariate regression was used

Results

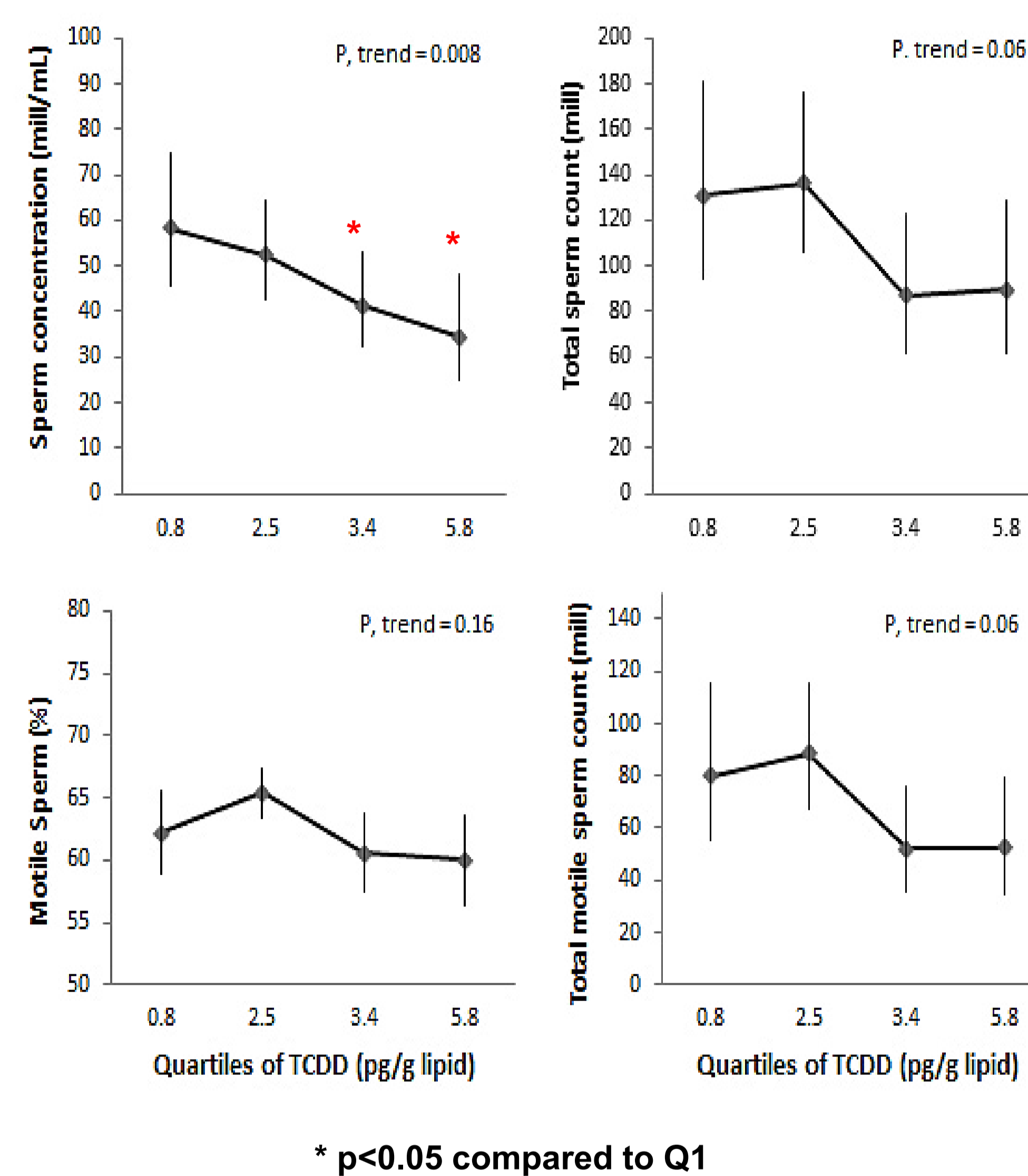
Descriptive Statistics of Cohort

Table 1. Baseline and exposure characteristics of young Russian adults

Baseline characteristics	Median (IQR) or N (%)
Age, years	18.3 (18.1, 18.7)
Body Mass Index, kg/m ²	20.4 (18.8, 22.3)
Men with 2 semen samples	123 (93)
Abstinence time, hrs	70.5 (48.0, 144.0)
Serum organochlorine concentrations (pg/g lipid)	
TCDD	2.9 (1.8, 4.2)
PCDDs	157 (115, 200)
PCDFs	44.5 (29.4, 63.3)
Co-PCBs	188 (131, 273)
ΣPCBs	235 (152, 352)
Total TEQ	21.9 (16.8, 33.3)

Prepubertal TCDD and Semen

Figure 2. Associations between prepubertal TCDD concentration and semen parameters among 133 young Russian adults



Semen Parameters

Table 2. Distribution of semen parameters among 133 young Russian adults, 257 samples

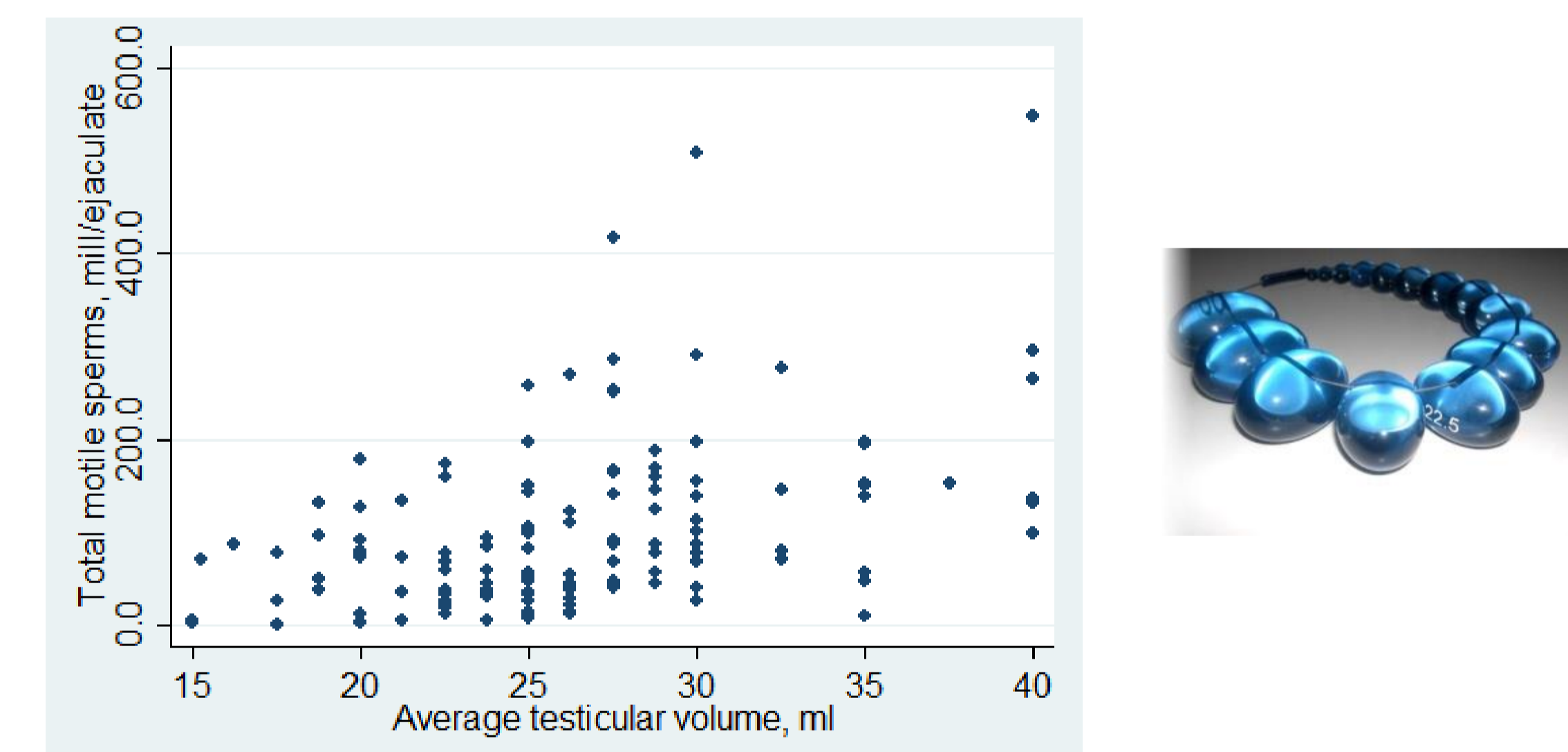
Semen parameters	Median (IQR)	WHO 2010 cutoffs	n (%) < WHO 2010
Semen volume, mL	2.4 (1.8, 3.5)	1.5	46 (18%)
Sperm concentration, mill/mL	51.3 (26.6, 78.8)	15	23 (9%)
Total sperm count, mill	127 (61.0, 222)	39	38 (15%)
Sperm motility, %	64.0 (57.0, 68.0)	40	10 (4%)
Total motile sperm count, mill	80.5 (35.8, 141)	-	-

Testicular Volume and Semen

Table 3. Average testicular volume (ml) as predictor of log semen parameters among young Russian adults

Parameters	Estimate	95% CI	P-value
Semen volume, ml	0.020	(0.002 - 0.038)	0.029
Motility, %	0.009	(0.002 - 0.015)	0.008
Sperm count, mill/ml	0.064	(0.038 - 0.091)	<0.001
Total sperm, mill	0.084	(0.054 - 0.114)	<0.001
Total motile sperm, mill	0.092	(0.059 - 0.125)	<0.001

Figure 3. Association between average testicular volume and total motile sperm among young Russian adults



- For every 1 ml increase in average TV, we observed a mean increase of 9.6% in total motile sperm count per ejaculate, p<0.001

Conclusion

In a prospective cohort of 18 year old Russian men:

- *higher* prepubertal serum TCDD levels are associated with *lower* semen parameters: sperm concentration, total sperm count, and total motile sperm count
- *higher* testicular volume is associated with *higher* semen parameters

In Summary

- This is one of the first prospectively designed studies to follow a large cohort of boys annually from prepuberty until young adulthood, including collection of semen samples at 18 years
- Consistent with the Seveso study, our results suggest that the prepubertal period is a sensitive window of exposure to dioxin for adult sperm quality⁴
- Collection of semen samples is ongoing with anticipated participation of 250 young men
- We are studying EDCs effects on sperm epigenetic programming among this Russian male cohort.

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