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Association of pubertal trajectories with molecular markers and health outcomes in young men: prospective cohort study

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Motivation and Aim: Puberty is a hormone-dependent period in which spermatogenesis begins in the testicles. The age of pubertal onset, the pubertal progression and the age of sexual maturation varies in healthy children/adolescents. There is evidence that spermatogenesis is accompanied by dramatic changes in the epigenetic marks of sperm [1]. Particularly, in the profile of small non-coding RNAs that are less than 200 nucleotides and play a role in RNA silencing and gene expression [2]. The aim of our study was to prospectively investigate the relationship of pubertal trajectories with the reproductive outcomes, semen quality, serum hormone level and sperm sncRNA profile in healthy young adults. The secondary aim was to investigate the sperm sncRNA profile by semen quality and serum hormones.

Methods and Algorithms: Physical examinations including measurement of testicular volume (TV) by Prader orchidometer were performed at enrollment of the prospective cohort Russian Children Study (ages 8–9 years), and annually up to 19 years of age. We used group-based trajectory models (GBTMs) to identify subgroups of boys who followed similar pubertal trajectories from ages 8 till 19 years based on annual TV [3]. At 18–19 years semen samples were collected from 225 young adults. Semen was fractionated using 90 % and 45 % density gradients to isolate spermatozoa by density and maturity and to remove somatic cell contamination. Basic semen quality parameters, including semen volume, sperm concentration, motility and total progressive motile sperm count (TPMSC) were analyzed in accordance with the updated criteria by the Nordic Association for Andrology (NAFA) and European Society of Human Reproduction and Embryology–Special Interest Group in Andrology (ESHRE-SIGA) [5]. Hormone levels were analyzed using the Architect i1000SR and chemiluminescent microparticle immunoassay (Abbott Laboratories). Kruskal-Wallis rank test was used for comparison of different factors between pubertal subgroups/trajectories.

Fifty one subjects were randomly selected for analysis of molecular markers. RNA isolation from sperm was performed as described earlier [6]. RNA was purified using the RNeasy Mini Kit (Qiagen). Libraries of small RNA were constructed using NEBNext Multiplex Small RNA Library Prep Set (New England BioLabs) per manufacturer's guidelines followed by gel-size selection of 145–165 nucleotides

fragments. Libraries were sequenced on NextSeq 500 (Illumina) using one multiplexed single-end run for 75 nt.
We used the previously constructed pipeline for small RNA seq data analysis [8]. It is based on mapping reads on the reference genome and assigning them to RNA species using ITAS – integrated annotation of microRNA, piRNA and mature tRNA [7]. Reads attributed to mature tRNA were assigned to different tRNA fragments (tsRNA) using the pseudo-alignment method. Expression data matrices were filtered and differential expression analysis by DESeq2 was used separately for less mature (n=20) and more mature (n=27) sperm.
Results: Three different pubertal trajectories were identified among 225 subjects: slower, 37 %, moderate, 41 %, and faster, 18 % pubertal progression (3 % missing). Further, groups with faster and moderate trajectories were joined in one group. For slower trajectory group the birth weight, BMI and TV at semen sampling, sperm concentration, TPMSC were significantly lower and serum FSH level was significantly higher compared with faster combined with moderate one (Table 1).

Table 1. Significant different reproductive outcomes, serum hormone, and anthropometric parameters by slower vs faster pubertal trajectories

Parameters\ Trajectory	Slower				Moderate and Faster				p-value
	N	**median**	p25	p75	N	**median**	p25	p75	
Mean testicular volume, ml	77	**21.3**	20.0	23.8	116	**28.8**	26.3	31.3	0.0001
Sperm concentration, mill/ml	84	**39.9**	21.7	70.6	132	**62.7**	37.5	106.4	0.0001
TPMSC, mill	84	**55.7**	19.6	93.4	132	**88.1**	42.0	152.8	0.0002
FSH, U/l	84	**3.4**	2.4	5.1	133	**2.6**	1.7	3.4	0.0001
BMI, kg/m^2	84	**19.9**	18.6	22.3	133	**21.8**	19.7	23.6	0.0001
Birth weight, kg	82	**3.3**	3.0	3.5	132	**3.5**	3.2	3.8	0.0001

Sequencing was completed with median (25–75 percentile) 7.9 (5.1-10.7) million reads per sample and 63.56 (48.8-73.54) % of alignment rate. 153 miRNA, 362 piRNA and 59 tsRNA (a total of 574 sncRNA) were identified with strict criteria of mean count > 5 and no zero value of counts in any samples. We found 11 differentially expressed (DE) sncRNA with pubertal trajectories (1 microRNA, 3 piRNA, 7 tsRNA). Additionally, 152 sncRNA (41 microRNA, 94 piRNA, 11 tsRNA) were DE with semen quality parameters. Of these, 31 sncRNA were also DE with serum hormone levels. There were 5 microRNA, 5 piRNA and 12 tsRNA differentially expressed with more than one factor (Fig. 1). Particularly, hsa-piR-32951 was DE with pubertal trajectories, TV, sperm concentration and TPMSC. miR-93-5p was DE with semen quality (TV, TPMSC) and serum hormone levels (testosterone, LH, TSH, estradiol).

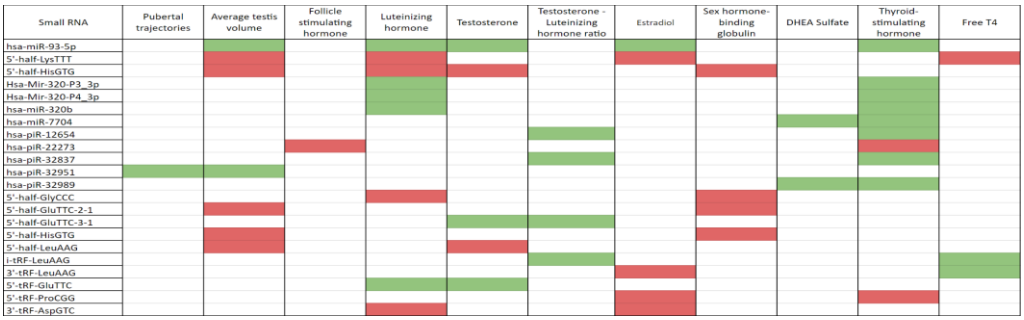

Fig. 1. Differentially expressed small RNA in less mature sperm (green) and more mature sperm (red) by pubertal trajectories, serum hormones and reproductive outcomes

Conclusion: The slower pubertal trajectory was associated with lower birth weight, lower BMI and TV at semen collection, lower TPMSC and sperm concentration and higher serum FSH. We found some sperm sncRNA as promising candidate biomarkers of pubertal trajectories and reproductive outcomes in young adulthood.

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