Gender-Specific Alterations in Salivary Cortisol Levels in Pubertal Intracytoplasmic Sperm Injection Offspring

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Key Words
Intracytoplasmic sperm injection - Cortisol - Pubertal stage - HPA axis - Outcome

Abstract
Background: There is accumulating evidence that in vitro conception in humans may be associated with adverse health outcomes later in life. It has been proposed that suboptimal early life conditions may programme key endocrine systems. A disturbance of the hypothalamic-pituitary-adrenal (HPA) axis leading to alterations in cortisol secretion in the offspring may be such a mechanism. To date, no data on cortisol levels in children conceived by intracytoplasmic sperm injection (ICSI) are available in the literature. Methods: In this cross-sectional study, salivary cortisol was known as a key regulator of metabolism was measured and results were compared between 201 pubertal ICSI children and 190 spontaneously conceived (SC) counterparts. Results: ICSI females had lower mean salivary cortisol levels (9.0 µg/dl; 95% CI 8.1 - 9.0) than SC females (10.6 µg/dl; 95% CI 9.7 - 11.5; p = 0.01). This difference remained after adjusting for current characteristics, early-life factors and maternal characteristics. In ICSI males, no difference in cortisol levels was found in comparison with the SC group. Conclusion: In our study, 14-year-old female but not male ICSI teenagers were found to have lower salivary cortisol concentrations in comparison with SC peers. However, before definite conclusions can be drawn, our results should be completed by longitudinal sampling.

Introduction
There is accumulating evidence that in vitro conception in humans is associated with adverse health outcomes later in life. Emerging evidence has demonstrated altered body composition [1, 2], elevated blood pressure [3, 4], vascular dysfunction [5] and glucose intolerance [3] in offspring conceived by assisted reproductive technologies (ART). These findings broadly support the ‘fetal programming hypothesis’ which states that suboptimal early life conditions can predispose individuals to chronic diseases in adulthood including obesity, hypertension and insulin resistance by permanently re-programming endocrine key systems. A disturbance of the hypothalamic-pituitary-adrenal (HPA) axis leading to alterations in cortisol secretion in the offspring may be such a mechanism. Animal studies have demonstrated that changes in the set point of several hormonal systems, including the HPA axis, may result in increased blood pressure, adiposity and changes in glucose and insulin metabolism [6-8]. Cortisol affects blood pressure and is a well-known regulator of metabolism, including effects on glucose metabolism, body composition and vascular function. Unfortunately, only few studies report on the cortisol levels in children in relation to adiposity, blood pressure and glucose tolerance [9]. Lower mean cortisol concentrations were reported in 5- to 16-year-old obese children in comparison with lean children [10]. In adults, both elevated [11, 12] and low [13] cortisol levels have been described in obese subjects. In a study of 9-year-old children, higher mean morning salivary cortisol concentrations were found in children with higher blood pressure in comparison with those who had lower blood pressure [14]. Recently, we found evidence for increased adiposity among girls in a cohort of 217 adolescents conceived by intracytoplasmic sperm injection (ICSI) [2]. In addition, 8-year-old ICSI children were found to have higher systolic blood pressure in comparison with spontaneously conceived peers [15]. The objective of the study was to investigate if an end-product of HPA axis functioning is altered among children born after assisted conception, since an altered early life condition, in case in vitro conception may re-program endocrine key systems with long-lasting health consequences. Therefore, we compared salivary cortisol levels between 212 pubertal children born after ICSI and 222 pubertal children born after spontaneous conception (SC).

Material and Methods
Subjects
This study is part of a prospective follow-up study on cardio-metabolic risk in a well-described cohort of 16-year-old children who were transferred from fertility-embryo obtained by ICSI using stimulated and non-stimulated cycles. Results regarding adiposity risk, blood pressure and sexual maturation have been reported previously [2, 14, 15]. The study setup has been described in detail previously [2]. Briefly, ICSI and SC children were eligible for inclusion if they were Caucasian, singletons and born after 32 weeks of gestation. ICSI children (n = 217), 116 males, 101 females) were part of the eldest prospectively followed cohort worldwide and were all conceived at the UZ Brussel, after ICSI application predominantly because of male-factor infertility. SC children (n = 223; 115 males, 108 females) were recruited cross-sectionally in schools around the UZ Brussels. Participation rates and reasons for refusal have been described in detail elsewhere [2].

Cortisol in ICSI Offspring

Data from a physical examination were supplemented with data from a parental questionnaire on anthropometric, medical and socio-economic indicators including the child's personal and family history. Maternal and paternal age, current body mass index (BMI), educational level and medical antecedents were recorded. Educational level, as a proxy of socio-economic status, was classified as low (primary school or lower) or high (bachelor degree or higher). Information of pregnancy-induced disorders (hypertension, preeclampsia, gestational diabetes, thyroid) were collected by means of self-report. In addition, instructions to obtain a saliva sample for the measurement of awakening cortisol levels were provided. Saliva samples were obtained in 107 of out 116 (92%) ICSI males and in 99 out of 115 (85%) SC males. In 3 (3%) SC males the saliva sample was insufficient to assess the cortisol concentration. Saliva samples were obtained from 94 out of 101 ICSI females (93%) and from 98 out of 108 SC females (91%). All samples were collected on a weekend between 06:00 and 07:00 h because of the known difference in cortisol levels between weekends and weekdays [18]. In ICSI males, 59% of the saliva samples were collected in spring and summer, while this rate was 83% in SC males due to the scheduled check-up by the school physician in this group. In ICSI females, 59% of the saliva samples were collected in spring and summer, while this rate was 96% in the SC group. All parents gave written informed consent. The study was approved by the ethics committee of the UZ Brussel.

Saliva Collection and Analysis
Saliva samples were collected during weekdays at home, immediately after awakening, using a cotton-based neutral swab with a specific container (Silvotest, Surthord) and were sent by post to the laboratory. Samples collected in this way are stable at room temperature for at least a week. Salivettes were centrifuged at 2200 g for 10 min, and the filtrates were stored at -20°C. Before analysis, the samples were thawed and mixed. Salivary cortisol was measured by a commercial RIA for serum (GammaCoat 125I RIA, Diagnostic Products Corporation), using a slight modification: 200 µl saliva was pipetted into the coated tube and incubated with 125I cortisol for 45 min at 37°C. The modified cortisol assay had a measuring range from 0.5 to 30 pg/ml and within- and between-run coefficients of variation of <5% and <10% respectively.

Statistical Analysis
Cortisol data were stratified according to gender and were logarithmically transformed prior to analysis in order to remove skewness from the data. Continuous data are presented as mean ± SD, or as geometric means and their 95% confidence interval.
out childhood and adolescence [3, 35]. In youngsters, scarce literature data exist on the relation between salivary cortisol levels and blood pressure. Finally, our data could not confirm an inverse association between low birth weight and cortisol levels [37].

Other factors that are known to influence cortisol levels of preterm neonates [38] and stress [39] were also present in our study. For instance, the parents of the children whose salivary cortisol was not related to systolic or diastolic blood pressure in our study. Furthermore, our data could not confirm an inverse association between low birth weight and cortisol levels [37].

Therefore, our study is limited in its ability to detect the associated factors for increase in the HPA axis following physical or psychological stressors. A larger study will be able to confirm the role of stress in the development of cortisol levels in the population. However, our study confirms that children with higher cortisol levels are at increased risk of developing hypertension in adulthood.

The results of the current study suggest that children with higher cortisol levels are at increased risk of developing hypertension in adulthood. Therefore, it is important to identify children with high cortisol levels during early childhood, as they may benefit from interventions aimed at reducing their cortisol levels. This knowledge can be used to develop targeted interventions for children at risk of developing hypertension. In conclusion, the results of the current study highlight the importance of studying the relation between cortisol levels and blood pressure in children, and the potential impact of interventions aimed at reducing cortisol levels in children.


