Association of Breastfeeding With Higher Serum Inhibin B Level at Adolescence

Breastfeeding has a number of beneficial effects on child growth and development. Surprisingly, however, very few data exist about the possible influence of breastfeeding on the development of the male reproductive system, which is known to be particularly vulnerable to hormone dysregulation. A recent study in young adults found no association between breastfeeding and male fertility but most subjects examined in this study had been exposed to maternal smoking during pregnancy, which is an important risk and confounding factor.

Herein, we report observations among school-aged adolescents showing for the first time, to our knowledge, a beneficial effect of breastfeeding on testes development.

Methods | The ethics committee of the Faculty of Medicine of the Catholic University approved the study, which was conducted with the assent of all adolescents and the informed consent of their parents. Study participants were secondary school-aged male adolescents (participation rate, 72%) who were examined in a cross-sectional study evaluating the impact of environmental stressors on adolescent health. Of 361 recruited subjects, we excluded 108 subjects with no information about breastfeeding duration and another 55 subjects who were smokers or who had been exposed to maternal smoking during pregnancy. Because there were only 6 subjects who had been exclusively breastfed, we could only study the influence of the total breastfeeding duration. To consolidate our study, we measured inhibin B with 2 different methods: the Oxford Bio-Innovation (OBI) enzyme-linked immunosorbent assay and the Diagnostic System Laboratories (DSL) enzyme-linked immunosorbent assay. Other hormones were measured by the Immulite assay. We used stepwise regression analyses to assess the influence of breastfeeding while adjusting for potential confounders. When age and time of blood sampling were not retained, they were forced in the models. After adjustment for model covariates, we stratified subjects according to breastfeeding duration and compared inhibin B levels by analysis of variance and the Bonferroni-Dunn post hoc test. Continuous variables were normalized by cubic root (inhibin B and testosterone levels) or log transformation. The threshold for statistical significance was a 2-sided α level of .05.

Results | As shown in the Table, adolescents who had been breastfed as infants were well matched with those who were not with respect to age, birth weight, body mass index, and time of blood sampling. While there were no significant differences in gonadotropin and testosterone levels, inhibin B measured by the OBI or DSL method was significantly higher in breastfed adolescents than in their peers. Multiple regression analyses confirmed this increase of inhibin B associated with breastfeeding, whether testing follicle-stimulating hormone or not among predictors (all \( P < .02 \)). The Figure shows that the covariate-adjusted concentrations of OBI and DSL inhibin B increased dose dependently with the duration of breastfeeding. Associations were the strongest with the DSL inhibin B concentration, with a median increase up to 25% in the group breastfed for more than 4 months. A similar increase was seen in the prevalences of high values of OBI and DSL inhibin B (>90th percentile of values in controls) that were 3 times higher when breastfeeding duration exceeded 4 months (no follicle-stimulating hormone adjustment, \( P \) for trend = .08 and .05; follicle-stimulating hormone adjustment, \( P \) for trend = .02 and .003, respectively).

Table. Characteristics and Reproductive Hormone Levels of Study Participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Breastfeeding, Median (IQR)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>76</td>
<td>122</td>
</tr>
<tr>
<td>Age, y, mean (SD)</td>
<td>15.4 (0.67)</td>
<td>15.5 (0.76)</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>20.6 (3.0)</td>
<td>20.2 (2.49)</td>
</tr>
<tr>
<td>Birth weight, g, mean (SD)</td>
<td>3332 (587)</td>
<td>3462 (594)</td>
</tr>
<tr>
<td>Breastfeeding duration, mo, median (range)</td>
<td>...</td>
<td>4 (1-24)</td>
</tr>
<tr>
<td>Clock time of blood sampling, mean (SD)</td>
<td>11.3 (2.32)</td>
<td>11.1 (2.19)</td>
</tr>
<tr>
<td>Luteinizing hormone level, mIU/mL</td>
<td>1.62 (1.07-2.46)</td>
<td>1.89 (1.14-2.63)</td>
</tr>
<tr>
<td>Follicle-stimulating hormone level, mIU/mL</td>
<td>3.71 (2.42-5.10)</td>
<td>3.80 (2.34-5.57)</td>
</tr>
<tr>
<td>Testosterone level, ng/dL</td>
<td>354.5 (236.3-443.8)</td>
<td>377.5 (263.7-464.0)</td>
</tr>
<tr>
<td>Free testosterone level, pmol/L</td>
<td>178 (98.7-257)</td>
<td>192 (125-266)</td>
</tr>
<tr>
<td>Inhibin B level, ng/L</td>
<td>220 (170-289)</td>
<td>269 (199-335)</td>
</tr>
<tr>
<td>OBI</td>
<td>142 (101-216)</td>
<td>192 (123-243)</td>
</tr>
<tr>
<td>DSL</td>
<td></td>
<td></td>
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</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); DSL, Diagnostic System Laboratories; ellipses, not applicable; IQR, interquartile range; OBI, Oxford Bio-Innovation.

SI conversion factors: To convert follicle-stimulating hormone to international units per liter, multiply by \( l \); luteinizing hormone to international units per liter, multiply by \( l \); and testosterone to nanomoles per liter, multiply by 0.0347.
Figure. Serum Inhibin B Levels in School-Aged Adolescents According to the Total Duration of Breastfeeding

The middle horizontal bars represent the median values; the upper and lower limits of the boxes, the interquartile range; and the whiskers, the 10th and 90th percentiles. Inhibin B level was adjusted for age, time of blood sampling, and the cumulative attendance at indoor chlorinated pools before the age of 10 years. The group of breastfed adolescents was dichotomized at the median split of breastfeeding duration. P values represent the Bonferroni-adjusted P values for the comparison of breastfed groups (n = 61 in each group) with the nonbreastfed group (n = 76). DSL indicates Diagnostic System Laboratories; FSH, follicle-stimulating hormone; and OBI, Oxford Bio-Innovation.

Discussion | Serum inhibin B is a marker of the Sertoli cell number that determines testis size and sperm production in adults. Sertoli cells develop during fetal and neonatal life and also at puberty when their number is definitively set. Our study suggests that breastfeeding is important for neonatal testes development because the inhibin B increase associated with breastfeeding was of the same magnitude as the proportion of Sertoli cells formed during the first year of life.5

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Author Contributions: Drs Bernard and Nickmilder had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Bernard.

Acquisition of data: All authors.

Analysis and interpretation of data: All authors.

Drafting of the manuscript: Bernard.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: All authors.

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Study supervision: Bernard.

Conflict of Interest Disclosures: None reported.

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Effect of Routine Vaccination on Aluminum and Essential Element Levels in Preterm Infants

Parenteral feedings containing more than 4 to 5 μg/kg/d of aluminum have been shown to result in neurodevelopmental delay in preterm infants.1 However, an infant at the 2-month checkup receives multiple aluminum-containing vaccines that in combination may have as high as 1225 μg of intramuscular aluminum; this is a much higher intramuscular aluminum dose than the safely recommended intravenous aluminum dose.2 Our first objective was to measure prevaccine and postvaccine levels of aluminum in preterm infants, a population at higher risk of aluminum neurotoxic effects. Our second objective was to measure prevaccine and postvaccine levels of essential elements (EE). Inflammation from trauma can cause declines in serum levels of specific EE such as zinc and selenium3-5; there may be similar EE perturbations secondary to vaccination-induced inflammation.

Methods | After institutional review board approval and parental consent, 15 preterm infants scheduled for routine 2-month vaccinations while still hospitalized were recruited in the Sparrow Hospital neonatal intensive care unit in East Lansing, Michigan. One day prior to scheduled vaccination, 0.25-mL blood and 12-hour urine collections were obtained. Prevnar 13, PedvaxHIB, and Pediatric vaccines were administered, in total containing 1200 μg of aluminum, as determined by company literature and confirmed by testing a set of these vaccines in our laboratory. One day postvaccination, 0.25-mL blood and 12-hour urine collections were obtained. Aluminum and EE concentrations were quantified by inductively coupled plasma