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# Urinary gonadotropin measurements in neonates; a valuable non-invasive method

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## Abstract

To measure low neonatal gonadotropin levels, a sensitive non-invasive method is optimal. The aim of the current study was to validate the Architect, an automated immunoassay analyser for the measurement of gonadotropins in unextracted neonatal urine samples against serum gonadotropin levels as a gold standard. Blood and urine were sampled from 30 approximately six-week-old male and female neonates undergoing elective paediatric surgery. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) were measured and the urine results were corrected for creatinine. The agreement between neonatal serum and urinary FSH was 0.904 (3-5 hours between samples) and 0.704 (18-20 hours). For LH the correlation coefficients were 0.785 and 0.507 respectively. We conclude that gonadotropins can be reliably measured using the Architect on randomly voided, non-extracted urine samples collected from neonates by an adhesive device. Urinary gonadotropin levels are a proper reflection of the serum levels.

## Introduction

Urine has been the subject of chemical analysis since the late 18th century<sup>1</sup>. The first to measure gonadotropins in urine of children were Fitschen and Kulin in the 1960s<sup>2,3</sup>. The methods used were labor-intensive and often required the urine to be pretreated before analysis<sup>3,4</sup>. Oosterhuis et al. established that follicle stimulating hormone (FSH) in randomly voided unextracted urine samples of adult women could be measured reliably on an automated immunoanalyser<sup>5</sup>. In future research, we intend to measure FSH among other parameters in newborns several weeks after birth. The aims of the study presented here were:

1. to validate gonadotropin measurement in serum and urine by the Architect versus the AxSYM method and
2. to validate the Architect-automated method for the measurement of gonadotropins in unextracted neonatal urine samples against serum gonadotropin levels as the gold standard.

## Subjects and methods

### Study procedure and assessment in adults

Serum and random voided urinary urine samples were obtained from 30 healthy volunteers (12 men and 18 women), aged 24-65 years.

### Study procedure and assessment in neonates

Parents of 30 healthy neonates (9 girls and 21 boys) aged 0-4 months agreed to participate. They were admitted to the hospital for elective, non-genital surgery and divided into two groups based on the time gap between serum and urine sampling. A 2 ml serum sample was taken immediately after insertion of the intravenous infusion system needed to anaesthetize the child before surgery. Urine samples of at least 2 ml were collected at two time points; 3-5 and 18-20 hours before serum sampling. The samples were collected in a paediatric urine collection pouch (Braun Urinocol<sup>®</sup> Pédiatrie, reference number for girls H07556/27556 and for boys H07546/27546, Braun Medical, Boulogne Cedex, France).

### Ethics

The study was approved by the Scientific Research Committee and the Ethical Committee of the institute. All parents signed an informed consent.

### Blood and urine sample processing and analysis

The serum samples were processed according to the standard procedure for FSH and

LH determination. The urine samples were stored at 4°C without preservatives and gonadotropins were determined within one week, with and without correction for creatinine.

### Assays

An AxSYM random access immunoassay analyser (Abbott Laboratories, Abbott Park, IL, USA) with a microparticle enzyme immunoassay (MEIA) reagent kit was used to measure FSH in adult serum and urine. The lower limit of detection for FSH was 0.09 IU/L, both the inter-assay coefficients of variation (CVs) and the intra-assay CVs are both below 6%<sup>5</sup>. The Architect i2000SR (Abbott Laboratories, Abbott Park, IL, USA) using reagents obtained from Abbott Laboratories (Diagnostic Division Abbott Park, IL, USA) has a lower limit of detection for FSH of 0.07 IU/L. Inter-assay CVs for urinary FSH were all below 5% at levels of 0.2, 5, 25 and 75 IU/L and the intra-assay CVs were below 3% (0.5, 5, 25 and 75 IU/L). The lower limit of quantitation for LH in urine was also 0.07 IU/L, the inter-assay CVs were 17% (0.09 IU/L) and 5% (5, 40 and 75 IU/L) and the intra-assay CVs were all below 3% (5, 40 and 75 IU/L).

### Statistics

The data in both groups were positively skewed. In order to use linear regression and the Pearson's correlation coefficient (SPSS version 12), the data were logarithmically transformed. We a priori considered an 80% correlation scientific justification for substitution of urinary gonadotropin values for those found in serum. A correlation below 0.8 was considered to be small, and if found, the method would not be implemented.

## Results

### Adult serum and urinary FSH

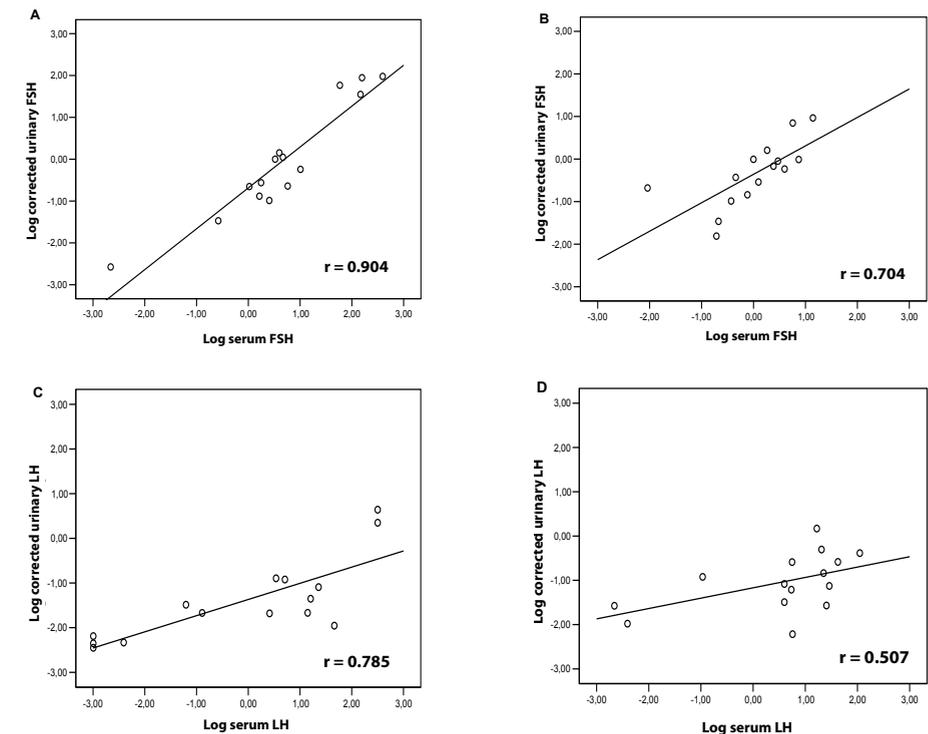
The agreement for serum FSH measured by the Architect versus the AxSYM method was 0.999. For urinary FSH, the coefficients of correlation were, respectively, 0.998 uncorrected for creatinine and 0.997 after correction. The relationship for urinary versus serum FSH measured by the Architect is 0.795 uncorrected for creatinine and 0.979 after correction. The data presented here all showed optimal correlations between serum and urinary FSH levels after creatinine correction. Based on this, we decided to continue with creatinine correction in all neonatal samples.

### Urinary versus serum FSH in neonates

The time between urine and serum sampling was 3-5 hours or 18-20 hours. The agreement between serum and (corrected) urinary FSH were 0.940 (3-5 hours) (Figure 1a) and 0.704 (18-20 hours) (Figure 1b). In all 30 samples, the overall relationship between FSH in serum and urine was 0.873.

### Urinary versus serum LH in neonates

The principal aim of the study was to validate the urinary FSH measurement. However, in a later stage, it became relevant to also evaluate LH levels in urine. As described for the FSH assay, creatinine correction also greatly improved the correlation between serum and urine LH levels, therefore only the corrected urinary LH data are shown. The correlation coefficients for LH in serum and urine were 0.785 (3-5 hours) (figure 1c) and 0.507 (18-20 hours) (Figure 1d). The overall relationship between LH in serum and urine was 0.704.



**Figure 1.** demonstrates corrected urinary versus serum FSH and LH levels in 15 neonates. Time between serum and urine sample was either 3-5 hours (A and C) or 18-20 hours (B and D). (A) Intercept, -0.685 ; slope 0.977 (95% CI 0.765-1.188). (B) Intercept, -0.359 ; slope 0.668 (95% CI 0.264-1.072). (C) Intercept, -1.369, slope ; 0.362 (95% CI 0.191-0.533). (D) intercept, -1.169 ; slope 0.234 (95% CI -0.04-4.72).

## Discussion

This study validates the Architect method for measuring FSH in unextracted urine against an earlier validated procedure in adult women, the AxSYM method<sup>5</sup>. The data indicate that the new Architect technique measures FSH in adult serum and urine in an equally reliable manner as the former established procedure (AxSYM). Creatinine correction significantly improved the correlations and we therefore advise that in all future Architect urinary FSH measurements, creatinine correction should be performed. In neonates we used the Architect method to measure serum and urinary FSH and LH levels. We showed that the complete procedure of measuring FSH by means of the Architect method including the random voided urine sample collected via an adhesive device highly correlates to serum values, in particular, when the time interval between both samples did not exceed 5 hours. We choose two largely different time groups in order to investigate to what extent a time gap between serum and urinary samples influenced the correlation, which is particularly relevant when episodically secreted hormones are measured. The LH measured in urine yielded a less satisfactory relationship to serum values. The striking difference in coefficients of correlation for FSH versus LH is likely to be the result of the difference in serum half-life, resulting in a more pronounced episodic secretion of LH compared to FS. We conclude that gonadotropins measured by the Architect in a randomly voided unextracted urine sample of neonates are a proper reflection of the serum gonadotropin levels.

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