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# Normal amino-terminal pro-brain natriuretic peptide (NT-proBNP) values in amniotic fluid

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ABSTRACT

**Introduction:** Brain natriuretic peptide (BNP) is synthesized by human fetal membranes, both the amnion and chorion. This locally produced BNP inhibits the contraction of the human myometrium, contributing to the maintenance of myometrial quiescence during pregnancy. Reference values for NT-proBNP concentrations in amniotic fluid at different gestational ages have not been completely defined. We aimed to investigate the range of fetal NT-proBNP values in amniotic fluid in normal pregnancy between 17 and 41 weeks of gestation.

**Methods:** Samples of amniotic fluid were obtained from women meeting the following inclusion criteria: gestational age defined by early ultrasound, singleton gestation and not in labor. The exclusion criteria were as follows: multiple gestation, clinically evident chorioamnionitis, laboratory signs of infection in the amniotic fluid sample and fetal conditions that may alter NT Pro-BNP levels (anemia, hydrops, etc.). NT-proBNP concentrations in amniotic fluid were measured using the automated Elecsys® proBNP assay.

**Results:** We analyzed 218 samples of amniotic fluid at various gestational ages. Half of the samples were obtained by amniocentesis (118 samples), and the other half (100 samples) were obtained by direct puncture at the time of cesarean section. We found a significant decline in NT-proBNP concentrations with advancing gestational age.

**Discussion:** Gestational age has to be taken into consideration in the assessment of NT-proBNP values. Our data may be used as reference values in fetal medicine, as a possible predictor of preterm delivery risk using the inferior limit (0.5 multiples of the median (MoM)) of our normal curve.



## 1. Introduction

The key event in controlling the maintenance of pregnancy is the inhibition of myometrial contractions until the appropriate time for delivery, a process termed myometrial quiescence [1]. Myometrial quiescence starts at the beginning of pregnancy and normally ends at approximately 36 weeks, leading to myometrial activation and labor. Myometrial quiescence is characterized by the absence of uterine contractions; the myometrium is actively relaxed, thus refractory to contractile agonists [2].

Initially, investigators focused on the endocrine regulation of myometrial quiescence, primarily the balance between progesterone (pro quiescence) and estrogen (pro activation) [3–5]. Later, paracrine, rather than endocrine, regulation of myometrial contractility was postulated as the main mediator of myometrial quiescence [6–8].

Based on animal models and human experiments, we postulate that the main paracrine mediator of myometrial quiescence is brain natriuretic peptide (BNP), which is produced by the fetal membranes (chorion and amnion) [8–10]. From the fetal membranes, BNP diffuses to the myometrium and inhibits contractions, but at the same time, BNP is released into the amniotic fluid, where it can be measured [9].

The peptide precursor of BNP has 134 amino acids (pre-proBNP) and is rapidly processed by cleavage of its signal peptide (26 aa) producing proBNP (108 aa). During secretion, proBNP is divided into two equimolar products: BNP (the active peptide of 32 aa) and NT-proBNP (amino terminal portion of proBNP, 76 aa) [11]. BNP can be measured by RIA or ELISA [12]; however, the preferred method of estimating BNP concentration is to measure the NT-proBNP concentration [12].

Reference values for NT-proBNP concentrations in amniotic fluid at different gestational ages have not been completely defined. Our aim is to establish the range of NT-proBNP values in normal pregnancies from 17 to 41 weeks gestation.

## 2. Methods

### 2.1. Included women

The Institutional Review Board at the Pontificia Universidad Católica de Chile, where all samples were taken, approved the study protocol, and women were included only after providing informed consent. All

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of the included women were Hispanic. Samples of amniotic fluid were obtained from women meeting the following inclusion criteria:

1. Well-known gestational age, defined by early ultrasound (7–12 weeks)
2. Any gestational age
3. Singleton gestation
4. Not in labor

The exclusion criteria were the following:

1. Multiple gestation
2. Clinically evident chorioamnionitis
3. Laboratory signs of infection in the amniotic fluid sample
4. Fetal conditions that may alter NT Pro-BNP levels (anemia, hydrops, etc.)

### 2.2. Amniotic fluid sampling

A sample of 10 mL was obtained from each included patient and stored at room temperature until being assayed. NT Pro-BNP was measured within 2 h of sampling. The amniotic fluid was obtained by amniocentesis or direct puncture at the time of cesarean section. Amniocentesis was performed when medically indicated, independent of the study, under continuous ultrasound guidance using aseptic technique (Table 1). Direct puncture was performed at the time of cesarean section, after laparotomy and hysterotomy, under direct visualization of the amniotic sac, before membrane rupture. Cesarean sections were indicated by medical reasons independent of the study (Table 1).

### 2.3. NT pro-BNP measurement

At our emergency clinical laboratory, an ISO 15189 accredited medical laboratory, using the automated Elecsys® proBNP assay (Roche Diagnosis), an electrochemiluminescent immunoassay was performed using two polyclonal NT-proBNP-specific antibodies in a sandwich test format [12,13]. The assay measuring range reported by the vendor is 5–35,000 pg/mL; the assay variation within-run is 1.8–2.7% and is 2.2–3.2% (from 175–4962 pg/mL) overall.

### 2.4. Detecting infection in the amniotic fluid samples

All samples of amniotic fluid were analyzed to discard infection by measuring glucose concentration, leukocyte concentration, gram staining, lactate dehydrogenase (LDH) concentration and culture (aerobic and anaerobic bacteria and mycoplasmas) [14]. Samples having positive culture, glucose <0.771 mmol/L, LDH >6.68 μkat/L, leukocytes >50 $mm^3$ or the presence of microorganisms in gram staining were discarded from this study.

**Table 1**
Clinical characteristics of the included women.

| Samples obtained by amniocentesis | | |
|---|---|---|
| Indication | N | Gestational age average (range) |
| Genetic | 53 | 25 + 1 weeks (17–34) |
| Before cerclage | 25 | 20 + 1 weeks (17–23) |
| Membrane rupture | 20 | 28 + 6 weeks (20–34) |
| Suspected intra-amniotic infection | 20 | 29 + 5 weeks (25–35) |
| Total | 118 | 25 + 1 weeks (17–35) |
| *Samples obtained by direct puncture* | | |
| Indication | N | Gestational age average (range) |
| Term pregnancy prior cesarean section | 33 | 38 + 2 weeks (36–41) |
| Fetal growth restriction | 25 | 32 + 3 weeks (25–35) |
| Preeclampsia | 22 | 32 + 1 weeks (30–36) |
| Fetal distress | 8 | 33 + 2 weeks (29–35) |
| Breech | 5 | 38 + 1 weeks (38–39) |
| Membrane rupture | 5 | 32 + 3 weeks (31 − 33) |
| Bleeding | 2 | 25 + 4 weeks (25–26) |
| Total | 100 | 34 + 2 weeks (25–41) |

### 2.5. Statistical analysis

We calculated that 200 women were required to obtain a confidence interval of 95%, based on the means and ranges reported from prior studies of BNP plasma samples [15]. Median values of NT-ProBNP were calculated for each completed gestational week; the medians were smoothed by regression against gestational weeks. An equation was derived to adjust the multiples of the median (MoM) for each gestational age. The data were statistically analyzed using IBM SPSS v. 20.

## 3. Results

We analyzed 218 samples of amniotic fluid at various gestational ages. The clinical characteristics of the included women are given in Table 1. Half of the samples were obtained by amniocentesis (118 samples), most of which were genetic amniocentesis. The other half (100 samples) were obtained by direct puncture at the time of cesarean section. Cesarean sections were performed at term by prior cesarean section or breech presentation or performed preterm by fetal or maternal indication (Table 1).

NT pro-BNP concentrations by gestational age are shown in Table 2 and Fig. 1. The NT pro-BNP concentration is very high at early gestational ages and then decreases as pregnancy progresses. A significant decline in NT-proBNP concentration was detectable with advancing gestational age. In Fig. 1, we depict the median and multiples of the median (0.5–0.75-1.5 and 2 MoM) of NT-proBNP concentrations plotted by gestational age.

## 4. Discussion

Here, we report that the concentration of NT-proBNP in amniotic fluid is very high during the second trimester of pregnancy and that there is a progressive reduction of NT-proBNP concentrations as gestation progresses toward term.

**Table 2**
NT-proBNP values in amniotic fluid.

| Gestational Age | Number of Women | NT-proBNP Concentration | | | |
|---|---|---|---|---|---|
| | | Average pg/mL | Range pg/mL | Median pg/mL | 0.5 MoM |
| 17 | 3 | 4111.0 | 3047–5705 | 3581 | 1790 |
| 18 | 3 | 3910.0 | 2325–5416 | 3989 | 1994 |
| 19 | 2 | 3670.0 | 3220–4120 | 3670 | 1835 |
| 20 | 5 | 3349.6 | 3011–3609 | 3399 | 1699 |
| 21 | 3 | 3281.3 | 2981–3554 | 3309 | 1654 |
| 22 | 4 | 3127.5 | 2557–4343 | 2805 | 1402 |
| 23 | 3 | 2255.7 | 1934–2482 | 2351 | 1175 |
| 24 | 4 | 2109.5 | 1612–2661 | 2082 | 1041 |
| 25 | 6 | 2205.8 | 1607–3951 | 1873 | 936 |
| 26 | 8 | 1970.4 | 1644–2668 | 1829 | 914 |
| 27 | 10 | 1494.7 | 1125–2112 | 1503 | 751 |
| 28 | 12 | 1487.1 | 731–2924 | 1413 | 706 |
| 29 | 15 | 1279.1 | 636–2878 | 958 | 479 |
| 30 | 21 | 1205.1 | 591–2843 | 1182 | 591 |
| 31 | 20 | 922.6 | 614–2587 | 900 | 450 |
| 32 | 21 | 790.9 | 524–1903 | 687 | 343 |
| 33 | 16 | 618.0 | 408–1692 | 555 | 277 |
| 34 | 18 | 634.2 | 401–1414 | 585 | 292 |
| 35 | 7 | 407.0 | 316–780 | 336 | 168 |
| 36 | 5 | 404.6 | 220–890 | 318 | 159 |
| 37 | 5 | 300.6 | 118–680 | 256 | 128 |
| 38 | 12 | 112.7 | 65–260 | 103 | 51 |
| 39 | 5 | 180.6 | 36–510 | 117 | 58 |
| 40 | 5 | 65.4 | 53–80 | 69 | 34 |
| 41 | 5 | 51.0 | 59–49 | 59 | 29 |

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