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# Folic Acid Flour Fortification: Impact on the Frequencies of 52 Congenital Anomaly Types in Three South American Countries

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**The aim of the present investigation was to search for a reduction in birth prevalence estimates of 52 selected types of congenital anomalies, associated with folic acid fortification programs in Chile, Argentina, and Brazil. The material included 3,347,559 total births in 77 hospitals of the three countries during the 1982–2007 period: 596,704 births (17 hospitals) in Chile, 1,643,341 (41 hospitals) in Argentina, and 1,107,514 (19 hospitals) in Brazil. We compared pre- and post-fortification rates within each hospital and the resulting Prevalence Rate Ratios (PRRs) were pooled by country. Statistically significant reductions in birth prevalence estimates after fortification were observed for neural tube defects (NTDs), septal heart defects, transverse limb deficiencies, and subluxation of the hip. However, only the reduction of NTDs appeared to be associated with folic acid fortification and not due to other factors, because of its consistency among the three countries, as well as with previously published reports, and its strong statistical significance. Among the NTDs, the maximum prevalence reduction was observed for isolated cephalic (cervical-thoracic) spina bifida, followed by caudal (lumbo-sacral) spina bifida, anencephaly, and cephalocele. This observation suggests etiologic and pathogenetic heterogeneity among different levels of spina bifida, as well as among different NTD subtypes. We concluded that food fortification with folic acid prevents NTDs but not other types of congenital anomalies.** © 2010 Wiley-Liss, Inc.

**Key words:** folic acid; food fortification; fortified flour; neural tube defects; NTD; anencephaly; spina bifida; cephalocele; congenital anomalies; birth defects monitoring; South America

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## INTRODUCTION

ECLAMC (Spanish acronym for Latin American Collaborative Study of Congenital Malformations) [Castilla and Orioli, 2004] regularly monitors the occurrence of congenital anomalies in South American countries since the late 1960s and early 1970s.

In South America, mandatory folic acid (FA) food fortification, with dosages aimed at the primary prevention of congenital anomalies, has been implemented in 3 of the 10 countries, starting in Chile in 2000, and followed by Argentina in 2003, and Brazil in 2004. However, fortification policies vary among these three countries; the estimated daily dose of FA is around 500 µg in Chile [Hertrampf et al., 2003], and Argentina [Calvo and Biglieri, 2008; Zabala et al., 2008], and half of that dose (264 µg) in Brazil [Ferreira and Giugliani, 2008].

The effectiveness of these FA fortification programs on the prevention of NTDs has already been analyzed by several

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investigators in Chile [Freire et al., 2000; Castilla et al., 2003; Hertrampf et al., 2003; López-Camelo et al., 2005; Corral et al., 2006; Nazer et al., 2007], Argentina [Calvo, 2008; Calvo and Biglieri, 2008; Zabala et al., 2008], and Brazil [Pacheco-Santos and Pereira, 2007; Pacheco et al., 2009]. However, to the authors' knowledge, no data have been published in South America on the effects of fortification for congenital anomalies other than NTDs, except for some subsets of the ECLAMC material presented in the present paper [Castilla et al., 2003; López-Camelo et al., 2005; Nazer et al., 2007].

Botto et al. [2006] evaluated surveillance data on major birth defects of population-based registries from Europe, North America, and Australia. They concluded that FA fortification appears to be effective in reducing NTDs, but the effect on other birth defects remains unclear. In a systematic review of the efficacy of FA fortification to decrease NTD prevalence, Leoncini and Mastroiacovo [2009] found inconclusive results from other parts of the world.

This is the third ECLAMC publication of an ongoing surveillance of FA and birth defects, albeit the first one with data from other countries besides Chile, and with samples large enough to allow the monitoring of birth defects other than those of the neural tube [Castilla et al., 2003; López-Camelo et al., 2005]. The present investigation tested the null hypothesis, assuming no significant reduction in the birth prevalence estimates of 52 selected types of congenital anomalies, between the pre- and post-FA fortification periods in Chile, Argentina, and Brazil.

## METHODS

ECLAMC is a hospital-based, voluntary network dedicated to the research and monitoring of congenital anomalies in South America since 1967. It includes 114 reporting maternity hospitals distributed in all 10 South American countries, except Guianas [Castilla and Orioli, 2004]. Even though the ECLAMC database contains information since 1967 [Castilla and Orioli, 1985; Castilla et al., 1985], complete data on both live- and stillbirths, weighing 500 g or more (of approximately 22 or more gestational weeks), are only available since 1982. Therefore, the study period for this work was restricted to 1982–2007.

According to routine ECLAMC procedures, all consecutive live- and stillbirths occurring in participating hospitals were examined for major and minor congenital anomalies. Each malformed newborn infant was one-to-one matched to a control, defined as the immediately subsequent non-malformed, like-sexed livebirth occurring in the same hospital [Castilla and Orioli, 2004]. Mothers of cases and controls were interviewed postpartum regarding 50 risk factors, including environmental exposures. Case definitions were based on verbatim descriptions given by the reporting pediatricians at birth or within the first week of life. Quality control of data is performed manually for verbatim descriptions and automatically for other data. The proportion of ascertained births is heterogeneous among countries, varying from 1% of all births in Brazil to 10% in Argentina and Chile. Pregnancy terminations are not reported because they are illegal in all South American countries.

Throughout this paper, results from the three countries will be discussed in the chronological order they started FA fortification, namely, Chile, Argentina, and Brazil. The material from the three selected countries included a total of 3,347,559 live- and stillborn infants (weighing 500 g or more), of 77 hospitals: 596,704 from Chile (17 hospitals), 1,643,341 from Argentina (41 hospitals), and 1,107,514 from Brazil (19 hospitals).

Fifty-two of about 300 different congenital anomaly types were selected among the ascertained consecutive birth series, based on available sample size and required statistical power; they are the diagnoses routinely monitored and reported by ECLAMC to the International Clearinghouse for Birth Defects Surveillance and Research and defined according to its norms [ICBDMS, 1991]. A total of 138,778 infants had one or more congenital anomalies (4.15%), and 85,213 of these had one or more of the 52 selected congenital anomaly types (2.68%).

Special attention was given to congenital heart defects because of their potential association with periconceptional FA intake [Robbins et al., 2006; Ionescu-Ittu et al., 2009], and their morphological subtypes were grouped for analysis under two different criteria:

(1) According to the ECLAMC routine [Castilla and Orioli, 2004], five categories were considered: conotruncal (truncus arteriosus, pulmonary artery defect, transposition of great arteries, tetralogy of Fallot, aorta defects, pulmonary valvular defect, and other conotruncal anomalies), septal (atrial septal defect [ASD], ventricular septal defect [VSD], single ventricle, and atrioventricular septal defect), valvular (mitral and tricuspid defects), other severe heart defects (hypoplastic left heart, coarctation of aorta, and total anomalous venous return), and unspecified congenital heart defects.
(2) For comparison with the recently published observations on FA fortification in Quebec, Canada, by Ionescu-Ittu et al. [2009], three categories were considered: severe conotruncal (tetralogy of Fallot, transposition of great arteries, and truncus arteriosus), severe non-conotruncal (atrioventricular septal defect and single ventricle), and severe-total, including the five defects listed above.

In order to preserve diagnostic preciseness, collective categories, such as "other," or "unspecified" were excluded, except for congenital heart defect of unspecified type. Recognized syndromes were excluded as well, except for Down syndrome, which was split into two maternal age groups, 19 years or less and 35 years or older, to increase its etiological specificity [ICBDMS, 1991].

Each of the 52 congenital anomaly types was considered as isolated and total (isolated plus cases with other unrelated anomalies in the same infant), because the isolated forms are expected to be less heterogeneous from an etiopathogenetic standpoint. Even though some isolated cases could have been misclassified as associated because of the presence of minor or pathogenetically related anomalies, we preferred to consider them as such, with the purpose of increasing homogeneity of the isolated group. Infants with more than one anomaly were counted more than once, and therefore, the tables do not present total values.

Two obvious limitations of historical series in hospital-based registries, with ‘‘hospital’’ as the geographic observation unit, are the lack of continuity of each participating hospital, and the unequally biased selection of high-risk pregnancies. Thus, pre- versus post-fortification comparisons were adjusted by hospital of birth, by comparing pre- and post-fortification periods within each hospital, and by pooling the resulting Prevalence Rate Ratios (PRRs) by country, a method already applied in our two previous studies [Castilla et al., 2003; López-Camelo et al., 2005]. A PRR of 0.70 means a birth prevalence reduction of 0.30 (30%) for a specific congenital anomaly between the pre- and post-fortification periods.

To evaluate changes in birth prevalence for each anomaly type between pre-fortification (baseline), and post-fortification periods, we first estimated with a Poisson regression analysis if there was any secular linear trend before fortification began. The regression model was applied to each malformation in each country sub-sample, and the independent variables, temporal changes, both linear and quadratic, were entered into the model. The expected number of cases for the fortified period was then estimated by projection, and these values were compared with actually observed numbers of cases. The resulting estimator was the observed/expected ratio. The observed pre- and post-fortification periods for each country are presented in Table I. The triennium immediately prior to the birth of the first infants periconceptionally exposed to fortification was used for analysis of pre- versus post-fortification rates. The pre-fortification triennium included the 12 months after the date FA fortification started in each country. This 1 year estimate includes 3 months for the newly fortified flour to be made available to the public, plus 9 months of full term gestation. If fortified children were erroneously included in the pre-fortification period, because the time to make fortified flour available to the public was less than 3 months, this would increase the significance of our results, if we disprove the null hypothesis of no difference between pre- and post-fortification periods.

The estimator was the PRR, adjusted by hospital. We used the ‘‘metan’’ routine of Stata, v. 7.0, for a random effects model that assumes heterogeneous fortification among hospitals from the different countries. This routine produced the already mentioned prevalence estimates for each hospital, active during the two consecutive periods; an overall prevalence estimate using the Mantel–Haenszel test, which weights individual hospitals' sample sizes; and a $\chi^2$ heterogeneity test for hospitals' risk ratios with $k-1$ degrees of freedom, where k is the number of active hospitals in both periods. The significance of the Mantel–Haenszel test was evaluated with a $Z$ test, and the prevalence estimate confidence intervals were obtained by the Cornfield method.

According to Bonferroni's correction, the critical value of significance was set at $P < 0.00016$, due to the large number of comparisons, namely, 52 anomalies times two categories (isolated and total), times three country sub-samples (312 comparisons), for which 15 false positive comparisons were to be expected if a $P < 0.05$ limit was chosen.

Statistical power was estimated for rate reduction values between 20% and 50%, and different birth prevalence estimates of the anomalies (1/1,000, 1/2,000, and 1/3,000), taking into consideration the sample sizes of the pre- and post-fortification periods in each of the three countries (Table I).

With the purpose of increasing the precision of the estimated relative risks, the PRR was only calculated for anomalies with 10 or more registered cases in the pre-fortification period (Tables II and III).

TABLE I. Summarized Relevant Information on Folic Acid Fortification (FAF) for Each of the Three South American Countries

| **Characteristics** | **Chile** | **Argentina** | **Brazil** |
|---|---|---|---|
| Date FAF policy regulation | 10/09/99 | 08/22/02 | 12/13/02 |
| Date FAF policy implementation | 01/01/00 | 11/13/03 | 06/13/04 |
| Estimated date of first FAF births | 01/01/01 | 11/13/04 | 06/13/05 |
| Pre-FAF period | 1998–2000 | 2002–2004 | 2003–2006/2005[c] |
| Pre-FAF number of observed births | 69,677 | 193,509 | 102,751 |
| FAF period | 2001–2003 | 2005–2007 | 2007/2005–2007[d] |
| FAF number of observed births | 243,624 | 147,853 | 92,843 |
| Regulation type | Ministry Act | Federal Law | Ministry Act |
| FAF Flour | Wheat | Wheat | Wheat and maize |
| FA concentration in flour (mg/kg) | 2.2[a] | 2.2[b] | 1.5[e] |
| Estimated daily intake of flour per capita (g) | 227[a] | 221[b] | 176[e] |
| FA daily dose ($\mu$g) | 499 | 486 | 264 |
| Population in July 2008 (in millions) | 16 | 42 | 192 |
| Annual births | 244,000 | 685,000 | 3,000,000 |

Dates: mm/dd/yy.
[a]Calvo and Biglieri [2008].
[b]Zabala et al. [2008] for Argentina.
[c]2003–2006/2005: January 2003 to June 2005.
[d]2007/2005–2007: July 2005 to December 2007; data for FA daily dose calculation are from Hertrampf et al. [2003] for Chile.
[e]Ferreira and Giugliani [2008] for Brazil.

TABLE II. Folic Acid Fortification Effect on Birth Prevalence Rates (/10,000) of 52 Specific Types of Congenital Anomalies as Totals (Isolated Plus Associated Forms) in the Three South American Countries, Adjusted by Hospital

| | Chile | | | Argentina | | | Brazil | | |
|---|---|---|---|---|---|---|---|---|---|
| | PRR | 95% CI | *P* | PRR | 95% CI | *P* | PRR | 95% CI | *P* |
| Omphalocele | 0.95 | 0.56–1.62 | 0.856 | 1.34 | 0.92–1.96 | 0.782 | 1.00 | 0.62–1.61 | 0.994 |
| Gastroschisis | 0.80 | 0.40–1.61 | 0.531 | 1.60 | 1.06–2.41 | 0.023 | 1.29 | 0.91–1.82 | 0.156 |
| Anencephaly | 0.54 | 0.36–0.83 | 0.004 | 0.59 | 0.42–0.82 | 0.002 | 0.57 | 0.37–0.87 | 0.010 |
| Spina bifida-cephalic | 0.17 | 0.07–0.42 | <0.0001 | 0.27 | 0.14–0.53 | <0.0001 | 0.49 | 0.26–0.96 | 0.036 |
| Spina bifida-caudal | 0.55 | 0.37–0.81 | 0.002 | 0.75 | 0.57–0.99 | 0.044 | 1.12 | 0.62–2.02 | 0.703 |
| Spina bifida-total | 0.43 | 0.31–0.60 | <0.0001 | 0.59 | 0.46–0.76 | <0.0001 | 0.99 | 0.56–1.74 | 0.973 |
| Hydrocephaly | 1.06 | 0.79–1.47 | 0.715 | 1.15 | 0.93–1.42 | 0.189 | 0.84 | 0.68–1.03 | 0.096 |
| Cephalocele | 0.47 | 0.27–0.83 | 0.009 | 0.83 | 0.53–1.31 | 0.437 | 0.59 | 0.33–1.08 | 0.091 |
| Microcephaly | 1.20 | 0.66–2.20 | 0.541 | 1.25 | 0.83–1.27 | 0.288 | 0.88 | 0.58–1.32 | 0.530 |
| An/microtia | 1.15 | 0.78–1.70 | 0.490 | 0.83 | 0.59–1.17 | 0.291 | 1.14 | 0.81–1.75 | 0.586 |
| CHD-conotruncal | 1.30 | 0.94–1.39 | 0.111 | 0.69 | 0.42–1.07 | 0.063 | 1.03 | 0.71–1.49 | 0.872 |
| CHD-septal | 1.17 | 1.00–1.38 | 0.048 | 0.63 | 0.54–0.75 | 0.002 | 0.72 | 0.59–0.88 | 0.002 |
| CHD-valvular | 0.90 | 0.47–1.72 | 0.756 | 0.83 | 0.46–1.48 | 0.529 | 1.12 | 0.57–2.21 | 0.745 |
| CHD-other severe | 1.07 | 0.66–1.73 | 0.777 | 0.86 | 0.57–1.29 | 0.456 | 1.37 | 0.54–2.73 | 0.311 |
| CHD-unspecified | 1.12 | 0.67–1.88 | 0.658 | 1.60 | 0.78–3.28 | 0.200 | 0.63 | 0.40–0.99 | 0.045 |
| CHD-severe-total (I-I) | 1.28 | 0.95–1.73 | 0.098 | 0.66 | 0.50–96 | 0.028 | 0.77 | 0.51–1.17 | 0.226 |
| CHD-severe-conotruncal (I-I) | 1.46 | 0.99–2.26 | 0.056 | 0.85 | 0.56–1.29 | 0.446 | 1.14 | 0.70–1.83 | 0.601 |
| CHD-severe-non-conotruncal (I-I) | 1.02 | 0.66–1.60 | 0.902 | 0.57 | 0.35–0.92 | 0.021 | 0.27 | 0.11–0.69 | 0.004 |
| Cleft palate only | 1.22 | 0.73–2.06 | 0.441 | 1.43 | 1.04–1.48 | 0.027 | 0.68 | 0.46–1.04 | 0.077 |
| Cleft lip ± cleft palate | 0.97 | 0.74–1.27 | 0.819 | 0.79 | 0.61–1.03 | 0.081 | 1.20 | 0.91–1.57 | 0.196 |
| Cleft lip only | 0.81 | 0.46–1.43 | 0.462 | 0.97 | 0.63–1.51 | 0.906 | 1.15 | 0.70–1.89 | 0.573 |
| Cleft lip and palate | 0.99 | 0.72–1.33 | 0.929 | 0.81 | 0.62–1.07 | 0.129 | 1.21 | 0.87–1.69 | 0.245 |
| Esophageal atresia | 0.67 | 0.34–1.30 | 0.233 | 1.09 | 0.74–1.60 | 0.662 | 0.53 | 0.31–0.90 | 0.020 |
| Duodenal atresia | 1.02 | 0.44–2.32 | 0.959 | 1.06 | 0.53–2.10 | 0.867 | 0.92 | 0.45–1.86 | 0.815 |
| Anal atresia | 0.88 | 0.60–1.31 | 0.537 | 0.94 | 0.69–1.27 | 0.699 | 0.80 | 0.49–1.31 | 0.380 |
| Ambiguous genitalia | — | — | — | 1.07 | 0.63–1.83 | 0.803 | 1.34 | 0.79–2.27 | 0.283 |
| Hypospadias-total | 0.99 | 0.66–1.49 | 0.981 | 0.69 | 0.51–0.93 | 0.016 | 0.90 | 0.73–1.10 | 0.318 |
| Hypospadias-distal | 0.83 | 0.55–1.24 | 0.366 | 0.71 | 0.51–0.99 | 0.046 | 0.84 | 0.67–1.05 | 0.134 |
| Hypospadias-proximal | — | — | — | 0.63 | 0.55–1.25 | 0.314 | 1.06 | 0.56–2.11 | 0.872 |
| Absent kidney/s | 1.04 | 0.51–2.13 | 0.916 | 1.18 | 0.71–1.94 | 0.515 | 0.90 | 0.56–1.46 | 0.687 |
| Polycystic kidneys | 0.88 | 0.59–1.32 | 0.542 | 0.92 | 0.64–1.32 | 0.655 | 0.96 | 0.64–1.44 | 0.856 |
| Hydronephrosis | 1.47 | 0.92–2.34 | 0.106 | 1.30 | 0.95–1.78 | 0.103 | 1.13 | 0.74–1.73 | 0.559 |
| Talipes equinovarus | 1.03 | 0.75–1.43 | 0.840 | 0.96 | 0.80–1.15 | 0.639 | 0.94 | 0.80–1.10 | 0.433 |
| Talipes calcaneovalgus | 0.68 | 0.50–0.93 | 0.015 | 1.02 | 0.67–1.66 | 0.920 | 0.91 | 0.66–1.25 | 0.565 |
| Polydactyly-post-axial | 0.92 | 0.71–1.21 | 0.575 | 0.81 | 0.66–1.02 | 0.071 | 0.87 | 0.67–1.14 | 0.329 |
| Polydactyly-pre-axial | 0.91 | 0.60–1.39 | 0.671 | 1.15 | 0.80–1.66 | 0.451 | 1.21 | 0.59–1.48 | 0.597 |
| Polydactyly-others | — | — | — | 0.77 | 0.32–1.87 | 0.573 | 1.08 | 0.42–1.77 | 0.865 |
| Syndactyly-toes 2–3 | 0.55 | 0.30–0.99 | 0.047 | 1.66 | 0.77–3.59 | 0.197 | 1.53 | 0.78–3.05 | 0.216 |
| Syndactyly-other types | 1.28 | 0.77–2.11 | 0.327 | 1.32 | 0.89–1.95 | 0.169 | 1.12 | 0.71–1.78 | 0.621 |
| LRD-TT: amputation | 0.83 | 0.47–1.49 | 0.544 | 0.61 | 0.39–0.98 | 0.041 | 0.41 | 0.24–0.70 | <0.0001 |
| LRD-TTH: hypoplasia | — | — | — | 0.90 | 0.40–2.02 | 0.803 | 0.67 | 0.26–1.74 | 0.413 |
| LRD-pre-axial | — | — | — | 1.00 | 0.52–1.91 | 0.993 | 0.64 | 0.30–1.35 | 0.241 |
| Hip-subluxation | 0.58 | 0.32–1.06 | 0.077 | 0.75 | 0.46–1.22 | 0.250 | 0.80 | 0.46–1.39 | 0.422 |
| Hip-dislocation | — | — | — | 1.06 | 0.50–1.22 | 0.881 | — | — | — |
| Arthrogryposis | 0.66 | 0.36–1.22 | 0.183 | 0.43 | 0.24–0.78 | 0.005 | 0.46 | 0.22–0.97 | 0.041 |
| Diaphragmatic hernia | 0.75 | 0.49–1.14 | 0.183 | 1.35 | 0.84–2.18 | 0.211 | 0.84 | 0.44–1.59 | 0.596 |
| Abdominal muscle deficiency | — | — | — | 0.72 | 0.31–1.68 | 0.449 | 0.49 | 0.21–1.16 | 0.106 |
| Pectoralis hypoplasia | — | — | — | 1.09 | 0.44–2.72 | 0.850 | — | — | — |
| Skin ring constriction | — | — | — | 0.43 | 0.19–1.00 | 0.049 | 0.82 | 0.37–1.77 | 0.608 |
| Down total | 1.09 | 0.91–1.32 | 0.326 | 1.01 | 0.86–1.20 | 0.859 | 0.81 | 0.65–1.02 | 0.069 |
| Down syndrome ≤19 YMA | 0.55 | 0.29–1.03 | 0.064 | 2.00 | 1.10–3.64 | 0.023 | 0.85 | 0.38–1.84 | 0.666 |
| Down syndrome ≥35 YMA | 1.33 | 1.03–1.73 | 0.027 | 1.05 | 0.82–1.34 | 0.705 | 0.88 | 0.64–1.20 | 0.420 |

PRR, prevalence rate ratio; spina bifida-cephalic, cervical, thoracic; spina bifida-caudal, lumbar, sacral; CHD, congenital heart disease; (I-I), CHD grouped as Ionescu-Ittu et al. [2009]; hypospadias-distal, balanic, balano-prepucial; hypospadias-proximal, penile, scrotal, perineal; LRD, limb reduction defect; TT, transverse terminal; TTH, transverse terminal hypoplasia (includes brachydactyly); YMA, years of maternal age.

TABLE III. Folic Acid Fortification Effect on Birth Prevalence Rates (/10,000) of 52 Specific Types of Congenital Anomalies in Their Isolated Forms in the Three South American Countries, Adjusted by Hospital

| | Chile | | | Argentina | | | Brazil | | |
|---|---|---|---|---|---|---|---|---|---|
| | PRR | 95% CI | *P* | PRR | 95% CI | *P* | PRR | 95% CI | *P* |
| Omphalocele | — | — | — | 0.84 | 0.51–1.40 | 0.517 | 0.80 | 0.48–1.36 | 0.420 |
| Gastroschisis | 0.54 | 0.25–1.170 | 0.117 | 1.40 | 0.89–1.94 | 0.112 | 1.09 | 0.75–1.58 | 0.634 |
| Anencephaly | 0.36 | 0.22–0.61 | <0.0001 | 0.51 | 0.36–0.73 | <0.0001 | 0.47 | 0.32–0.68 | <0.0001 |
| Spina bifida-cephalic | 0.06 | 0.01–0.24 | <0.0001 | 0.21 | 0.10–0.47 | <0.0001 | 0.35 | 0.14–0.85 | <0.0001 |
| Spina bifida-caudal | 0.38 | 0.23–0.62 | <0.0001 | 0.55 | 0.38–0.80 | 0.002 | 0.57 | 0.37–0.88 | 0.012 |
| Spina bifida-total | 0.30 | 0.19–0.46 | <0.0001 | 0.42 | 0.30–0.59 | <0.0001 | 0.52 | 0.35–0.76 | <0.0001 |
| Hydrocephaly | 1.09 | 0.70–1.68 | 0.688 | 0.88 | 0.64–1.19 | 0.416 | 0.64 | 0.47–0.85 | 0.021 |
| Cephalocele | 0.22 | 0.10–0.45 | <0.0001 | 0.59 | 0.32–1.07 | 0.086 | 0.44 | 0.23–0.85 | 0.014 |
| Microcephaly | 1.64 | 0.91–2.95 | 0.098 | 0.91 | 0.46–1.79 | 0.784 | 0.80 | 0.47–1.37 | 0.412 |
| An/microtia | 0.96 | 0.61–1.50 | 0.863 | 0.50 | 0.30–0.84 | 0.046 | 0.84 | 0.39–1.80 | 0.651 |
| CHD-conotruncal | 1.33 | 0.90–1.95 | 0.150 | 0.57 | 0.38–0.84 | 0.006 | 0.80 | 0.51–1.26 | 0.352 |
| CHD-septal | 1.14 | 0.92–1.40 | 0.227 | 0.56 | 0.44–0.70 | 0.003 | 0.69 | 0.57–0.91 | 0.009 |
| CHD-valvular | — | — | — | 1.10 | 0.56–2.15 | 0.783 | 0.94 | 0.41–2.14 | 0.883 |
| CHD-other severe | 0.76 | 0.45–1.31 | 0.333 | 1.01 | 0.65–1.59 | 0.943 | 1.35 | 0.63–2.88 | 0.436 |
| CHD-unspecified | 0.57 | 0.29–1.08 | 0.085 | 1.10 | 0.36–3.39 | 0.871 | 0.62 | 0.34–1.10 | 0.104 |
| CHD-severe-total (I-I) | 1.46 | 0.95–2.23 | 0.081 | 0.43 | 0.27–0.68 | 0.003 | 0.84 | 0.48–1.47 | 0.540 |
| CHD-severe-conotruncal (I-I) | 1.57 | 0.97–2.55 | 0.067 | 0.66 | 0.31–1.01 | 0.059 | 0.83 | 0.47–1.47 | 0.523 |
| CHD-severe-non-conotruncal (I-I) | — | — | — | 0.23 | 0.10–0.55 | 0.004 | — | — | — |
| Cleft palate only | 1.84 | 0.96–3.55 | 0.068 | 0.87 | 0.48–1.54 | 0.614 | 0.42 | 0.20–0.88 | 0.021 |
| Cleft lip ± cleft palate | 0.76 | 0.55–1.04 | 0.087 | 0.67 | 0.52–0.88 | 0.003 | 0.98 | 0.70–1.41 | 0.939 |
| Cleft lip only | 0.70 | 0.37–1.36 | 0.301 | 0.66 | 0.40–1.08 | 0.099 | 1.06 | 0.60–1.88 | 0.841 |
| Cleft lip and palate | 0.74 | 0.52–1.06 | 0.099 | 0.64 | 0.47–0.87 | 0.004 | 0.91 | 0.58–1.44 | 0.698 |
| Esophageal atresia | — | — | — | 0.82 | 0.49–1.39 | 0.470 | 0.36 | 0.16–0.82 | 0.015 |
| Duodenal atresia | — | — | — | — | — | — | — | — | — |
| Anal atresia | 0.76 | 0.39–1.48 | 0.424 | 0.73 | 0.45–1.20 | 0.219 | — | — | — |
| Ambiguous genitalia | — | — | — | — | — | — | 0.40 | 0.15–1.34 | 0.234 |
| Hypospadias-total | 0.78 | 0.56–1.10 | 0.157 | 0.66 | 0.47–0.90 | 0.021 | 0.80 | 0.63–0.99 | 0.040 |
| Hypospadias-distal | 0.73 | 0.52–1.03 | 0.077 | 0.69 | 0.49–0.99 | 0.035 | 0.76 | 0.60–0.99 | 0.026 |
| Hypospadias-proximal | — | — | — | — | — | — | 0.91 | 0.41–2.02 | 0.830 |
| Absent kidney/s | — | — | — | 1.22 | 0.67–2.22 | 0.505 | 0.60 | 0.28–1.29 | 0.191 |
| Polycystic kidneys | 1.01 | 0.72–1.20 | 0.204 | 0.84 | 0.55–1.32 | 0.455 | 0.71 | 0.42–1.20 | 0.204 |
| Hydronephrosis | 0.51 | 0.22–1.16 | 0.109 | 0.77 | 0.50–1.17 | 0.224 | 0.60 | 0.20–1.62 | 0.291 |
| Talipes equinovarus | 1.00 | 0.72–1.25 | 0.700 | 0.99 | 0.68–1.05 | 0.117 | 0.95 | 0.72–1.25 | 0.700 |
| Talipes calcaneovalgus | 0.58 | 0.42–0.80 | 0.003 | 0.75 | 0.45–1.24 | 0.265 | 0.60 | 0.42–0.80 | 0.002 |
| Polydactyly-post-axial | 1.04 | 0.70–1.98 | 0.693 | 0.70 | 0.54–0.90 | 0.005 | 0.94 | 0.70–1.27 | 0.693 |
| Polydactyly-pre-axial | 1.06 | 0.62–1.80 | 0.841 | 0.74 | 0.50–1.12 | 0.161 | 1.06 | 0.62–1.80 | 0.841 |
| Polydactyly-others | — | — | — | 0.50 | 0.20–1.32 | 0.163 | | | |
| Syndactyly-toes 2–3 | — | — | — | — | — | — | 1.41 | 0.73–2.72 | 0.299 |
| Syndactyly-other types | — | — | — | 0.45 | 0.13–1.60 | 0.220 | 3.50 | 0.14–6.03 | 0.442 |
| LRD-TT: amputation | — | — | — | 0.66 | 0.37–1.16 | 0.146 | 0.34 | 0.15–1.62 | 0.432 |
| LRD-TTH: hypoplasia | — | — | — | — | — | — | — | — | — |
| LRD-pre-axial | — | — | — | — | — | — | — | — | — |
| Hip-subluxation | 0.42 | 0.30–0.60 | <0.0001 | 0.40 | 0.23–0.70 | <0.0001 | 0.71 | 0.56–0.90 | 0.005 |
| Hip-dislocation | — | — | — | — | — | — | — | — | — |
| Arthrogryposis | — | — | — | 0.30 | 0.10–1.05 | 0.081 | — | — | — |
| Diaphragmatic hernia | 0.80 | 0.49–1.29 | 0.343 | 1.04 | 0.68–1.60 | 0.850 | 0.54 | 0.30–0.98 | 0.053 |
| Abdominal muscle deficiency | — | — | — | — | — | — | — | — | — |
| Pectoralis hypoplasia | — | — | — | — | — | — | — | — | — |
| Skin ring constriction | — | — | — | — | — | — | — | — | — |

PRR, prevalence rate ratio; spina bifida-cephalic, cervical, thoracic; spina bifida-caudal, lumbar, sacral; CHD, congenital heart disease; (I-I), CHD grouped as Ionescu-Ittu et al. [2009]; hypospadias-distal, balanic, balano-prepucial; hypospadias-proximal, penile, scrotal, perineal; LRD, limb reduction defect; TT, transverse terminal; TTH, transverse terminal hypoplasia (includes brachydactyly); YMA, years of maternal age.

# RESULTS

## Statistical Power

With the available sample sizes for both periods, in Chile, a power greater than 80% was obtained to detect a minimal reduction of 40% for anomalies with birth prevalence estimates of 1/1,000, and of 50% for those with birth prevalence estimates of 1/2,000. In Argentina, a power greater than 80% was obtained to detect a minimal reduction of 30% for anomalies with birth prevalence estimates of 1/1,000, of 40% for 1/2,000, and of 50% for those of approximately 1/3,000. In Brazil, the sample sizes rendered a power greater than 80% to detect a minimal reduction of 40% for anomalies with prevalence estimates of 1/1,000, and of 50% for those of approximately 1/2,000.

## Secular Trends Before Fortification

Secular trends during the pre-fortification period were estimated for each of the 52 congenital anomaly types, grouped as totals (isolated plus associated) (data not shown—available from the corresponding author).

In Chile, significantly rising secular trends ($P < 0.0001$) were observed for gastroschisis, Down syndrome with mothers of 35 years or older, and total Down syndrome; and decreasing trends for congenital heart defects of unspecified type, and subluxation of the hip.

In Argentina, significantly rising secular trends ($P < 0.0001$) were observed for gastroschisis, spina bifida (cephalic, caudal, and total), hydrocephaly, cephalocele, cleft lip and palate, anal atresia, absent kidneys, polycystic kidneys, hydronephrosis, pre-axial polydactyly, arthrogryposis, and diaphragmatic hernia. Decreasing trends were significant for congenital heart defects of unspecified type, subluxation of the hip, and true dislocation of the hip.

In Brazil, significantly rising secular trends ($P < 0.0001$) were observed for omphalocele, gastroschisis, spina bifida (cephalic, caudal, and total), hydrocephaly, cephalocele, microcephaly, an/microtia, cleft palate, esophageal atresia, duodenal atresia, ambiguous genitalia, hypospadias (proximal and total), absent kidneys, polycystic kidneys, hydronephrosis, pes equinovarus, talipes calcaneovalgus, post-axial polydactyly, transverse limb deficiency, pre-axial limb defect, true dislocation of the hip, arthrogryposis, diaphragmatic hernia, abdominal muscle deficiency, skin ring constriction (as seen in the amniotic band sequence), and Down syndrome, with maternal age of 35 years or above and total. No significantly decreasing trend was observed for any anomaly type.

The observed/expected ratios (not shown), with expected values adjusted by projected secular trends, confirmed the results of the intra-hospital comparison approach for a significance level under 0.0001 (Table II).

## Prevalence Rate Ratios (PRRs)

Significant reductions in birth prevalence estimates after fortification were observed for eight of the 52 investigated congenital anomaly types, in their total (Table II), and isolated forms (Table III). The observed numbers by malformation are shown in Appendix A for Chile, in Appendix B for Argentina, and in Appendix C for Brazil.

For isolated anencephaly, significant ($P < 0.0001$) PRRs were registered in all three investigated countries. For isolated cephalic (cervical-thoracic) spina bifida, significant ($P < 0.0001$) PRRs were registered in all three investigated countries, and for the total, in Chile and Argentina. For isolated caudal (lumbo-sacral) spina bifida, significant ($P < 0.0001$) PRRs were registered only in Chile, and for the total, in none of the three country sub-samples. For isolated total spina bifida (cephalic, caudal, and unspecified levels), significant ($P < 0.0001$) PRRs were registered in all three investigated countries, and for the total, in Chile and Argentina. For cephalocele, significant PRRs were only observed for its isolated form in Chile.

For septal heart defects, no significant PRRs were observed. However, marginal significance was registered for their isolated forms in Argentina and Brazil. For transverse limb deficiency, the PRR was significant only for its total form in Brazil. For subluxation of the hip, the PRR was only significant for its isolated form in Chile and Argentina; in Brazil the difference was of marginal significance.

Table IV shows the birth prevalence estimates for isolated and total forms of NTDs, during the pre- and post-fortification periods,

**TABLE IV. Birth Prevalence Estimates for Neural Tube Defects (Isolated and Total) in Pre-Fortification and Post-Fortification Periods**

| | Chile | | | | Argentina | | | | Brazil | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-fortification | | Post-fortification | | Pre-fortification | | Post-fortification | | Pre-fortification | | Post-fortification | |
| | Isolated | Total | Isolated | Total | Isolated | Total | Isolated | Total | Isolated | Total | Isolated | Total |
| Anencephaly | 0.52 | 0.63 | 0.26 | 0.37 | 0.69 | 0.86 | 0.29 | 0.37 | 0.90 | 1.12 | 0.45 | 0.69 |
| Spina bifida-total | 0.73 | 1.02 | 0.24 | 0.46 | 0.82 | 1.27 | 0.33 | 0.66 | 0.86 | 1.45 | 0.69 | 1.42 |
| Spina bifida-cephalic | 0.16 | 0.26 | 0.01 | 0.05 | 0.24 | 0.37 | 0.02 | 0.05 | 0.18 | 0.33 | 0.06 | 0.14 |
| Spina bifida-caudal | 0.55 | 0.72 | 0.21 | 0.38 | 0.57 | 0.88 | 0.30 | 0.60 | 0.62 | 1.04 | 0.56 | 1.23 |
| Cephalocele | 0.26 | 0.33 | 0.09 | 0.18 | 0.21 | 0.32 | 0.10 | 0.20 | 0.31 | 0.57 | 0.12 | 0.32 |

BP, birth prevalence/1,000 births.
Pre-fortification period in Chile 1998–2000, post-fortification period 2001–2007.
Pre-fortification period in Argentina 2002–2004, post-fortification period 2005–2007.
Pre-fortification period in Brazil 2003–2006/2005, post-fortification period 2007/2005–2007.

expressed per 1,000 births, in order to facilitate comparisons with previously published results from Canada [De Wals et al., 2007].

## DISCUSSION

The present study involves three Latin American countries where FA fortification has been implemented, 52 congenital anomalies, and the 1982–2007 period, and it partially overlaps with three previous studies with ECLAMC material: Castilla et al. [2003] who dealt with five Latin American countries (with fortification only in Chile), three types of congenital anomalies (NTDs, oral clefts, and Down syndrome), and the 1999–2001 period; López-Camelo et al. [2005] who dealt only with material from Chile, two types of congenital anomalies (spina bifida and anencephaly), and the 1982–2002 period; Nazer et al. [2007] who analyzed 14 Chilean hospitals, 24 congenital anomalies, and the 1995–1999 period. The present study corroborates the reduction of NTDs after FA fortification observed in the three previous studies, but not the reduction of diaphragmatic hernia observed by Nazer et al. [2007], probably because their data were not corrected by secular trends.

## Limitations and Strengths

As with any ecological study, our study can only suggest cause-effect associations, since interactions with many other uncorrected or partially adjusted factors are very likely to occur, such as the increasing number of pregnancy terminations for anencephaly, mainly in Brazil, less in Argentina, and much less in Chile. Even though pregnancy terminations are illegal in all three countries, individual judge permissions can overrule the law, and they are becoming more common in Brazil, less in Argentina, while still nonexistent in Chile.

As in any hospital-based study, the investigated consecutive births were non-random, as well as biased, small, and non-representative samples of a universe of births, adding up to more than four million per year. Even though the crude observed/expected values were adjusted by hospital, this correction might have been incomplete.

Despite the 80% power to detect a 30–50% decrease in the rates of the selected anomalies, we may have lacked sufficient power to identify more subtle changes that could be expected for other birth defects.

We did not take into account possible differences in the use of supplements during the pre- and post-fortification periods. However, this was not seen as a limitation, because in these countries, supplements are usually prescribed after pregnancy has been detected, and, therefore, have no influence on the prevention of NTDs [Botto et al., 2006].

The strengths of the present work include availability of data from the years prior to fortification; large sample sizes for the baseline, as well as for the observation periods; detailed clinical descriptions of congenital anomalies (verbatim instead of codes), and the unbiased project design and data collection process, aimed at the study of causal risk factors for birth defects in general, instead of specifically evaluating a protective environmental factor, such as FA.

Since most congenital anomalies are heterogeneous from an etiological standpoint, we preferred to delineate them into presumably more homogeneous sub-phenotypes, and the detailed clinical descriptions available in the ECLAMC database allows for such precise delineations (e.g., "incomplete, two-thirds, left-sided, cleft of the lip, with ipsilateral gum notch, and normal hard and soft palate"). However, an intermediate degree of splitting was used, in order not to break down the material into diagnostic units too small to be evaluated.

## Folic Acid Fortification Effect on NTDs: Types and Sub-Types

In the present material, the occurrence of total spina bifida (cephalic, caudal, and unspecified levels) decreased significantly and consistently in all three countries, except for the total form (isolated plus associated) in Brazil, possibly related to the smaller sample size. Isolated anencephaly also decreased in the three countries, while the reduction of cephalocele was only significant in its isolated form in Chile.

The stronger effect of FA fortification on spina bifida than on anencephaly and cephalocele has already been reported in the Chilean sub-sample [López-Camelo et al., 2005], as well as in other parts of the world, such as the United States [Williams et al., 2002], Canada [De Wals et al., 2007], and South Africa [Sayed et al., 2008]. In our case series, open spina bifida is more accurately diagnosed than the other two NTDs. Some infants who were registered as having anencephaly or cephalocele actually have acrania or other disruptive defects of the cranial vault, not due to a failure of the neural tube closure process, and this is particularly expected to occur in the associated forms, such as those due to constriction bands or other exogenous factors. Our results clearly support the greater sensitivity of the rarer and more severe, higher level (cephalic) spina bifida aperta to FA prevention, described by De Wals et al. [2008] with Canadian data, while to our knowledge no other observations on NTD subtypes have been published. In conclusion, these observations suggest that isolated cephalic spina bifida is the most sensitive defect to FA, not only among NTDs, but among all congenital anomalies.

The direct correlation between baseline birth prevalence estimates and prevalence reduction rates after FA fortification, reported by De Wals et al. [2007] in different provinces in Canada, was also observed in the three South American sub-samples reported here. Unlike the Canadian material, our three regions correspond to three countries with different FA doses, length of fortified period, and strategies of fortification, and of which Chile has the largest post-fortification sample, and for the longest time period: 243,624 births during 7 years. Considering the isolated forms, the reduction rate of anencephaly attained in Chile was 50%, from 0.52 to 0.26 per 1,000, while in Argentina the reduction was 58%, from 0.69 to 0.29 per 1,000. The birth prevalence estimates, higher in Argentina than in Chile before fortification, leveled off after fortification (0.29 and 0.26, respectively). Assuming no interaction with other variables, this observation suggests that Chile has already reached the maximum reduction (Table IV).

As shown in Table I, the fortification policy in Brazil differs from those in Chile and Argentina, with half of the concentration of FA and a lower estimated consumption of wheat-flour bread than the other two countries. However, a careful literature review [Leoncini and Mastroiacovo, 2009] revealed no correlation between population blood folate levels and birth prevalence estimates of NTDs. Thus, the explanation for the observed differences among countries could be more complex than expected. For instance, different responses to FA fortification among different races/ethnicities cannot be discarded. Such ethnic differences have been reported for Blacks in the USA [Williams et al., 2005], as well as for the aboriginal groups of Australia [Bower et al., 2009], and despite their geographic vicinity, the three countries considered in our study have large ethnic admixture differences [Wang et al., 2008]. In Brazil, the significant reduction in the birth prevalence rate of anencephaly, but not of spina bifida, strongly suggests the coincidental effect of pregnancy terminations, as mentioned above.

## Folic Acid Fortification Effect on Other Defects

A number of reports on birth prevalence reductions of several congenital anomaly types, other than NTDs, after FA fortification, have been published [Simmons et al., 2004; Canfield et al., 2005; Robbins et al., 2006; Ionescu-Ittu et al., 2009]. In the present study, inconclusive results were obtained for three of the 47 non-NTD defects, namely, septal defects, transverse terminal limb defects, and subluxation of the hip.

Among the sub-types of congenital heart defects, isolated septal defects decreased, although without statistical significance, in the sub-samples of Argentina and Brazil, while the reduction of severe heart defects reported by Ionescu-Ittu et al. [2009] in Canada, was not observed in our material. Canfield et al. [2005] found a post-fortification reduction in transposition of the great arteries; however, this reduction was not observed by Robbins et al. [2006], in a study based on hospital records.

The observed prevalence reduction of transverse terminal limb defects was only significant for its total form and in the Brazilian sub-sample. Decreasing rates of limb defects after FA fortification were already published by other authors in the US [Simmons et al., 2004; Canfield et al., 2005; Robbins et al., 2006], without specification of the limb defect sub-types.

The prevalence reduction of subluxation of the hip, observed after fortification in Chile, Argentina, and with marginal significance in Brazil, has not been reported in the literature, nor has this defect been previously associated with FA.

Even though data are available in the ECLAMC database, the effects of FA fortification on other adverse pregnancy outcomes, such as preterm delivery, low birth weight, and twinning were considered out of the scope of this work, mainly because of the many intervening confounders. For instance, Nazer et al. [2006] reported increased twinning rates in an ECLAMC sample from Chile, in coincidence with the beginning of FA fortification. However, other putative factors were not considered, such as the increasing availability of assisted reproductive technologies, shown to be largely responsible for the raising multiple birth rates worldwide [Vollset et al., 2005].

## Reliability of These Observations

Many congenital anomalies within the ECLAMC sample have shown rising secular trends during the pre- and post-fortification periods, mainly due to better ascertainment (e.g., congenital heart defects), as well as for other unknown reasons (e.g., gastroschisis). The intra-hospital comparison approach, as well as the projection of the observed trend on the expected post-fortification prevalence, and the short interval between the two compared periods were expected to reduce this limitation, although not to eliminate it entirely. Thus, some spurious positive associations are expected. Nonetheless, other criteria could help understand the actual meaning of the crude results obtained, namely, inter-country consistency, coincidental published findings, marginal statistical significance, reduction effect greater in isolated than in associated forms, and biologic plausibility.

Under this scope, all of our findings on NTDs are consistent and reliable, while the relevance of those on the remaining three observed prevalence reductions (of septal defects, transverse terminal limb defects, and subluxation of the hip) is at least doubtful. The rate reduction of septal defects after fortification did not reach the pre-established critical level of significance, not even in Chile, with the largest fortified sample size and longest fortification period. However, the fact that this effect only occurred in the isolated form of the anomaly provides some biological support. No reasonable explanation could be found for the rate reduction of transverse limb deficiencies in just one country, only for the total and not for the isolated form; it is however consistent with previously published observations on limb defects in general. Although the rate reduction of subluxation of the hip was found in all three sub-samples, the low observational value of this diagnosis at birth, that is, the low concordance rate of the diagnosis by different observers, seriously affects its relevance.

## Congenital Anomalies Without a Significant Reduction in Prevalence in the Present Study

Previous studies have shown reductions in birth prevalence following FA fortification for oral clefts in general [Simmons et al., 2004], and for cleft palate only [Canfield et al., 2005], while negative results were reported by Robbins et al. [2006], and Sayed et al. [2008], as well as by us in a previous publication with preliminary data from Chile [Castilla et al., 2003].

For omphalocele, a significant reduction was reported by Canfield et al. [2005], and suggested by Simmons et al. [2004]. For Down syndrome, a non-significant reduction was observed by Simmons et al. [2004] in Arkansas, and a negative result by us, in our previous report with Chilean data [Castilla et al., 2003]. For renal agenesis and pyloric stenosis, reductions were only reported by Canfield et al. [2005]. For diaphragmatic hernia, a significant reduction was reported in a maternity hospital from Chile, whose data are included in the present work [Nazer et al., 2007].

The discussion of the present results on food fortification with FA excludes observations made after FA supplementation because of the large, and sometimes not well understood differences between these two different intervention types: supplementation and fortification [Botto et al., 1999].

## Recommendations for Developing Countries

Nationwide and mandatory FA food fortification strategies are recommended for the primary prevention of NTDs in transitional developing countries, such as most of the Latin American ones. This recommendation is mainly based on the high frequency of unintended pregnancies [Gadow et al., 1998], making periconceptional supplementation ineffective, as well as on the high cost-benefit ratio of a national fortification program, shown in Chile [Hertrampf and Cortés, 2008], and South Africa [Sayed et al., 2008].

As in any other large health intervention program, the larger the country, the more complex the FA fortification program organization. However, results from small or medium-sized countries, such as Costa Rica [Chen and Rivera, 2004] and Chile [Hertrampf and Cortés, 2008; present work], as well as from large and heterogeneous countries, such as Brazil or Argentina analyzed here, were conclusive about the effectiveness of the FA flour fortification program on the prevention of NTDs.

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**APPENDIX A. Number of cases by specific congenital anomaly types in their isolated (Iso) and total (isolated plus associated) forms, by year of birth, in Chile.**

| | Period (years) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1982–1987 (86,168[a]) | | 1988–1992 (113,095[a]) | | 1993–1997 (84,140[a]) | | 1998–2001 (69,677[a]) | | 2002–2004 (144,950[a]) | | 2005–2007 (98,674[a]) | |
| | Iso | Total | Iso | Total | Iso | Total | Iso | Total | Iso | Total | Iso | Total |
| Omphalocele | 10 | 19 | 13 | 35 | 10 | 22 | 8 | 19 | 11 | 38 | 7 | 28 |
| Gastroschisis | 2 | 3 | 3 | 6 | 9 | 9 | 12 | 14 | 19 | 21 | 27 | 31 |
| Anencephaly | 32 | 51 | 72 | 88 | 66 | 77 | 36 | 44 | 35 | 50 | 28 | 39 |
| Spina bifida-cephalic | 7 | 14 | 24 | 38 | 24 | 36 | 11 | 18 | 2 | 6 | 1 | 6 |
| Spina bifida-caudal | 34 | 47 | 49 | 73 | 24 | 34 | 38 | 50 | 31 | 56 | 19 | 36 |
| Spina bifida-total | 44 | 69 | 76 | 117 | 52 | 74 | 51 | 71 | 37 | 67 | 21 | 45 |
| Hydrocephaly | 28 | 53 | 34 | 68 | 29 | 53 | 30 | 56 | 76 | 130 | 35 | 70 |
| Cephalocele | 10 | 17 | 10 | 23 | 12 | 15 | 18 | 23 | 14 | 24 | 7 | 20 |
| Microcephaly | 11 | 39 | 6 | 16 | 13 | 29 | 15 | 30 | 54 | 98 | 25 | 55 |
| An/microtia | 24 | 43 | 29 | 53 | 24 | 41 | 33 | 42 | 72 | 92 | 33 | 68 |
| CHD-conotruncal | 14 | 20 | 26 | 37 | 28 | 48 | 38 | 53 | 99 | 142 | 40 | 57 |
| CHD-septal | 41 | 72 | 33 | 65 | 80 | 143 | 135 | 219 | 313 | 528 | 198 | 303 |
| CHD-valvular | 2 | 3 | 6 | 9 | 9 | 12 | 8 | 11 | 18 | 24 | 12 | 16 |
| CHD-other severe | 9 | 14 | 17 | 24 | 16 | 24 | 22 | 24 | 37 | 50 | 11 | 24 |
| CHD-unspecified | 87 | 113 | 64 | 97 | 30 | 42 | 13 | 18 | 14 | 36 | 16 | 33 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHD-severe-total (I-I) | 11 | 17 | 20 | 31 | 18 | 45 | 30 | 61 | 101 | 176 | 26 | 53 |
| CHD-severe-conotruncal (I-I) | 9 | 14 | 19 | 25 | 14 | 26 | 22 | 33 | 76 | 108 | 17 | 32 |
| CHD-severe-non-conotruncal (I-I) | 2 | 3 | 1 | 6 | 4 | 19 | 8 | 28 | 25 | 68 | 9 | 21 |
| Cleft palate only | 22 | 43 | 16 | 41 | 22 | 41 | 11 | 28 | 50 | 84 | 35 | 70 |
| Cleft lip ± cleft palate | 87 | 108 | 94 | 118 | 62 | 81 | 62 | 83 | 101 | 155 | 76 | 127 |
| Cleft lip only | 25 | 27 | 36 | 39 | 17 | 19 | 14 | 19 | 23 | 30 | 22 | 26 |
| Cleft lip and palate | 62 | 81 | 58 | 79 | 45 | 62 | 48 | 64 | 78 | 125 | 54 | 101 |
| Esophageal atresia | 12 | 21 | 20 | 45 | 11 | 22 | 9 | 15 | 12 | 24 | 7 | 19 |
| Duodenal atresia | 7 | 11 | 5 | 13 | 5 | 10 | 3 | 13 | 6 | 30 | 7 | 29 |
| Anal atresia | 18 | 39 | 23 | 54 | 21 | 45 | 13 | 37 | 32 | 90 | 24 | 62 |
| Ambiguous genitalia | 5 | 23 | 4 | 20 | 4 | 19 | 0 | 8 | 7 | 27 | 5 | 18 |
| Hypospadias-total | 61 | 72 | 51 | 66 | 52 | 70 | 54 | 57 | 107 | 126 | 68 | 93 |
| Hypospadias-distal | 53 | 62 | 43 | 55 | 43 | 53 | 52 | 55 | 97 | 109 | 62 | 80 |
| Hypospadias-proximal | 6 | 7 | 7 | 9 | 4 | 10 | 1 | 1 | 8 | 13 | 4 | 7 |
| Absent kidney/s | 4 | 18 | 1 | 19 | 6 | 28 | 4 | 12 | 9 | 30 | 10 | 27 |
| Polycystic kidneys | 13 | 30 | 15 | 40 | 16 | 31 | 21 | 35 | 38 | 64 | 19 | 41 |
| Hydronephrosis | 10 | 25 | 11 | 28 | 34 | 50 | 65 | 78 | 167 | 220 | 108 | 166 |
| Talipes equinovarus | 96 | 149 | 128 | 192 | 85 | 111 | 69 | 94 | 132 | 170 | 76 | 127 |
| Talipes calcaneovalgus | 60 | 81 | 62 | 90 | 53 | 63 | 62 | 78 | 73 | 100 | 35 | 59 |
| Polydactyly-post-axial | 61 | 84 | 80 | 100 | 78 | 96 | 65 | 86 | 149 | 190 | 90 | 121 |
| Polydactyly-pre-axial | 22 | 24 | 17 | 24 | 23 | 26 | 19 | 31 | 52 | 70 | 32 | 37 |
| Polydactyly-others | 3 | 5 | 3 | 6 | 4 | 8 | 1 | 3 | 7 | 12 | 5 | 8 |
| Syndactyly-toes 2–3 | 21 | 27 | 22 | 25 | 24 | 30 | 9 | 20 | 12 | 19 | 10 | 19 |
| Syndactyly-other types | 16 | 40 | 21 | 48 | 16 | 33 | 8 | 23 | 43 | 79 | 20 | 40 |
| LRD-TT: amputation | 17 | 21 | 15 | 26 | 13 | 20 | 9 | 17 | 22 | 43 | 5 | 13 |
| LRD-TTH: hypoplasia | 4 | 11 | 4 | 9 | 1 | 2 | 0 | 3 | 2 | 9 | 3 | 4 |
| LRD-pre-axial | 2 | 4 | 1 | 11 | 3 | 12 | 5 | 8 | 5 | 19 | 2 | 12 |
| Hip-subluxation | 95 | 121 | 65 | 79 | 45 | 49 | 53 | 58 | 69 | 84 | 35 | 49 |
| Hip-dislocation | 0 | 3 | 1 | 6 | 1 | 4 | 5 | 6 | 2 | 3 | 1 | 5 |
| Arthrogryposis | 3 | 9 | 4 | 19 | 4 | 17 | 6 | 15 | 12 | 29 | 2 | 17 |
| Diaphragmatic hernia | 17 | 30 | 23 | 48 | 16 | 35 | 27 | 36 | 40 | 55 | 29 | 42 |
| Abdominal muscle deficiency | 0 | 13 | 0 | 10 | 1 | 7 | 0 | 3 | 3 | 6 | 2 | 7 |
| Pectoralis hypoplasia | 2 | 4 | 2 | 2 | 1 | 5 | 2 | 6 | 3 | 6 | 2 | 4 |
| Skin ring constriction | 3 | 3 | 0 | 6 | 2 | 5 | 0 | 4 | 1 | 11 | 0 | 4 |
| Down total | 148 | 148 | 183 | 183 | 188 | 188 | 161 | 161 | 358 | 358 | 260 | 260 |
| Down ≤19 YMA | 13 | 13 | 13 | 13 | 14 | 14 | 17 | 17 | 18 | 18 | 20 | 20 |
| Down ≥35 YMA | 63 | 63 | 85 | 85 | 90 | 90 | 83 | 83 | 228 | 228 | 167 | 167 |

Spina bifida-cephalic, cervical, thoracic; spina bifida-caudal, lumbal, sacral; CHD, congenital heart disease; (I-I), CHD grouped as Ionescu-Ittu et al. [2009]; hypospadias-distal, balanic, balano-prepucial; hypospadias-proximal, penile, scrotal, perineal; LRD, limb reduction defect; TT, transverse terminal; TTH, transverse terminal hypoplasia (includes brachydactyly); YMA, years of maternal age; Down syndrome always considered as isolated by definition.

[a]Births.

APPENDIX B. Number of cases by specific congenital anomaly types in their isolated (Iso) and total (isolated plus associated) forms, by year of birth, in Argentina.

| | Period (years) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1982–1987 (349,895[a]) | | 1988–1992 (360,113[a]) | | 1993–1997 (350,069[a]) | | 1998–2001 (241,902[a]) | | 2002–2004 (193,509[a]) | | 2005–2007 (147,853[a]) | |
| | Iso | Total | Iso | Total | Iso | Total | Iso | Total | Iso | Total | Iso | Total |
| Omphalocele | 31 | 79 | 33 | 81 | 33 | 76 | 24 | 63 | 34 | 67 | 21 | 55 |
| Gastroschisis | 12 | 18 | 21 | 28 | 49 | 60 | 62 | 73 | 76 | 82 | 85 | 110 |
| Anencephaly | 180 | 234 | 223 | 288 | 224 | 271 | 160 | 197 | 134 | 167 | 43 | 54 |
| Spina bifida-cephalic | 22 | 52 | 34 | 63 | 62 | 98 | 52 | 84 | 46 | 72 | 3 | 7 |
| Spina bifida-caudal | 98 | 157 | 125 | 192 | 129 | 206 | 109 | 168 | 110 | 170 | 45 | 88 |
| Spina bifida-total | 132 | 242 | 160 | 263 | 194 | 310 | 163 | 255 | 158 | 246 | 49 | 98 |
| Hydrocephaly | 83 | 163 | 133 | 243 | 185 | 358 | 137 | 258 | 105 | 214 | 77 | 171 |
| Cephalocele | 37 | 63 | 42 | 67 | 42 | 71 | 46 | 74 | 41 | 61 | 15 | 29 |
| Microcephaly | 36 | 88 | 51 | 107 | 40 | 108 | 22 | 74 | 19 | 51 | 17 | 44 |
| An/microtia | 64 | 145 | 95 | 179 | 106 | 170 | 51 | 99 | 53 | 94 | 27 | 60 |
| CHD-conotruncal | 49 | 78 | 78 | 118 | 125 | 185 | 103 | 146 | 49 | 119 | 78 | 59 |
| CHD-septal | 145 | 214 | 144 | 241 | 361 | 558 | 250 | 437 | 145 | 424 | 144 | 223 |
| CHD-valvular | 13 | 16 | 18 | 22 | 25 | 33 | 15 | 27 | 13 | 30 | 18 | 18 |
| CHD-other severe | 17 | 24 | 34 | 50 | 68 | 86 | 59 | 81 | 17 | 63 | 34 | 36 |
| CHD-unspecified | 46 | 140 | 71 | 153 | 61 | 144 | 43 | 59 | 46 | 12 | 71 | 15 |
| CHD-severe-total (I-I) | 38 | 70 | 49 | 97 | 96 | 158 | 89 | 134 | 69 | 108 | 31 | 58 |
| CHD-severe-conotruncal (I-I) | 35 | 53 | 44 | 70 | 81 | 108 | 67 | 89 | 43 | 56 | 21 | 40 |
| CHD-severe-non-conotruncal (I-I) | 3 | 17 | 5 | 27 | 15 | 50 | 22 | 45 | 26 | 52 | 10 | 18 |
| Cleft palate only | 41 | 140 | 46 | 153 | 47 | 144 | 50 | 59 | 27 | 12 | 23 | 15 |
| Cleft lip ± cleft palate | 300 | 368 | 290 | 374 | 319 | 404 | 254 | 323 | 183 | 266 | 99 | 160 |
| Cleft lip only | 97 | 111 | 87 | 103 | 96 | 111 | 76 | 85 | 46 | 57 | 29 | 38 |
| Cleft lip and palate | 203 | 257 | 203 | 271 | 223 | 293 | 178 | 238 | 137 | 209 | 70 | 122 |
| Esophageal atresia | 50 | 91 | 48 | 95 | 40 | 82 | 33 | 78 | 39 | 65 | 21 | 49 |
| Duodenal atresia | 7 | 36 | 18 | 33 | 22 | 38 | 12 | 36 | 7 | 20 | 4 | 17 |
| Anal atresia | 47 | 133 | 67 | 177 | 82 | 192 | 40 | 135 | 45 | 121 | 26 | 83 |
| Ambiguous genitalia | 13 | 67 | 9 | 58 | 13 | 50 | 10 | 40 | 9 | 34 | 7 | 29 |
| Hypospadias-total | 156 | 204 | 183 | 225 | 145 | 186 | 122 | 149 | 99 | 124 | 55 | 70 |
| Hypospadias-distal | 133 | 170 | 165 | 198 | 121 | 148 | 108 | 121 | 86 | 102 | 51 | 61 |
| Hypospadias-proximal | 15 | 21 | 11 | 16 | 20 | 30 | 9 | 20 | 7 | 15 | 4 | 6 |
| Absent kidney/s | 1 | 15 | 2 | 26 | 6 | 61 | 14 | 62 | 16 | 56 | 17 | 49 |
| Polycystic kidneys | 9 | 31 | 13 | 39 | 41 | 79 | 53 | 87 | 49 | 83 | 27 | 47 |
| Hydronephrosis | 9 | 29 | 21 | 47 | 57 | 117 | 60 | 100 | 60 | 110 | 45 | 115 |
| Talipes equinovarus | 298 | 481 | 316 | 511 | 355 | 519 | 170 | 284 | 153 | 303 | 90 | 211 |
| Talipes calcaneovalgus | 106 | 176 | 88 | 147 | 90 | 138 | 52 | 86 | 42 | 71 | 23 | 42 |
| Polydactyly-post-axial | 268 | 341 | 309 | 384 | 278 | 367 | 215 | 283 | 188 | 237 | 97 | 143 |
| Polydactyly-pre-axial | 61 | 81 | 76 | 105 | 91 | 115 | 69 | 96 | 61 | 71 | 36 | 58 |
| Polydactyly-others | 8 | 19 | 8 | 26 | 11 | 26 | 3 | 7 | 10 | 14 | 3 | 6 |
| Syndactyly-toes 2–3 | 41 | 61 | 37 | 53 | 31 | 53 | 23 | 37 | 6 | 12 | 11 | 17 |
| Syndactyly-other types | 41 | 136 | 45 | 145 | 52 | 135 | 23 | 95 | 19 | 63 | 18 | 56 |
| LRD-TT: amputation | 53 | 92 | 43 | 93 | 34 | 80 | 31 | 73 | 40 | 81 | 16 | 30 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LRD-TTH: hypoplasia | 13 | 54 | 11 | 42 | 10 | 33 | 8 | 26 | 7 | 19 | 5 | 11 |
| LRD-pre-axial | 9 | 56 | 10 | 45 | 18 | 57 | 5 | 46 | 5 | 30 | 3 | 18 |
| Hip-subluxation | 797 | 904 | 458 | 530 | 319 | 371 | 94 | 125 | 56 | 69 | 13 | 29 |
| Hip-dislocation | 20 | 81 | 23 | 82 | 10 | 40 | 3 | 8 | 6 | 14 | 1 | 13 |
| Arthrogryposis | 10 | 33 | 21 | 53 | 27 | 109 | 19 | 82 | 14 | 64 | 0 | 13 |
| Diaphragmatic hernia | 33 | 59 | 38 | 66 | 71 | 103 | 78 | 105 | 50 | 81 | 47 | 73 |
| Abdominal muscle deficiency | 4 | 23 | 4 | 28 | 3 | 21 | 2 | 19 | 1 | 17 | 0 | 9 |
| Pectoralis hypoplasia | 9 | 32 | 20 | 40 | 18 | 48 | 5 | 19 | 4 | 16 | 3 | 13 |
| Skin ring constriction | 2 | 20 | 4 | 30 | 3 | 35 | 4 | 26 | 1 | 27 | 0 | 5 |
| Down total | 604 | 604 | 654 | 654 | 643 | 643 | 512 | 512 | 369 | 369 | 273 | 273 |
| Down ≤19 YMA | 51 | 51 | 48 | 48 | 55 | 55 | 45 | 45 | 20 | 20 | 32 | 32 |
| Down ≥35 YMA | 331 | 331 | 350 | 350 | 339 | 339 | 250 | 250 | 193 | 193 | 135 | 135 |

Spina bifida-cephalic, cervical, thoracic; spina bifida-caudal, lumbal, sacral; CHD, congenital heart disease; (I-I), CHD grouped as Ionescu-Ittu et al. [2009]; hypospadias-distal, balanic, balano-prepucial; hypospadias-proximal, penile, scrotal, perineal; LRD, limb reduction defect; TT, transverse terminal; TTH, transverse terminal hypoplasia (includes brachydactyly); YMA, years of maternal age; Down syndrome always considered as isolated by definition.

[a]Births.

APPENDIX C. Number of cases by specific congenital anomaly types in their isolated (Iso) and total (isolated plus associated) forms, by year of birth, in Brazil.

| | Period (month/years) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1982–1987 (291,825[a]) | | 1988–1992 (242,380[a]) | | 1993–1997 (189,106[a]) | | 1998–2002 (188,609[a]) | | 2003–2006/2005 (102,751[a]) | | 2007/2005–2007 (92,843[a]) | |
| | Iso | Total | Iso | Total | Iso | Total | Iso | Total | Iso | Total | Iso | Total |
| Omphalocele | 18 | 60 | 39 | 76 | 30 | 89 | 35 | 117 | 20 | 61 | 23 | 58 |
| Gastroschisis | 11 | 17 | 17 | 23 | 32 | 56 | 90 | 101 | 62 | 69 | 68 | 82 |
| Anencephaly | 126 | 169 | 98 | 129 | 110 | 157 | 156 | 208 | 92 | 115 | 42 | 64 |
| Spina bifida-cephalic | 11 | 28 | 13 | 31 | 15 | 34 | 29 | 61 | 19 | 34 | 6 | 13 |
| Spina bifida-caudal | 64 | 126 | 68 | 115 | 92 | 161 | 123 | 216 | 64 | 107 | 52 | 114 |
| Spina bifida-total | 80 | 175 | 86 | 157 | 111 | 203 | 160 | 288 | 88 | 149 | 64 | 132 |
| Hydrocephaly | 76 | 128 | 106 | 202 | 197 | 358 | 220 | 394 | 128 | 226 | 78 | 181 |
| Cephalocele | 24 | 44 | 31 | 64 | 38 | 65 | 41 | 72 | 32 | 59 | 11 | 30 |
| Microcephaly | 33 | 91 | 27 | 61 | 28 | 72 | 27 | 86 | 31 | 55 | 19 | 40 |
| An/microtia | 17 | 58 | 28 | 62 | 24 | 75 | 14 | 51 | 17 | 41 | 16 | 38 |
| CHD-conotruncal | 30 | 48 | 58 | 88 | 76 | 126 | 84 | 131 | 48 | 66 | 36 | 63 |
| CHD-septal | 127 | 190 | 183 | 279 | 136 | 263 | 219 | 431 | 125 | 234 | 93 | 175 |
| CHD-valvular | 7 | 10 | 22 | 33 | 21 | 31 | 23 | 34 | 10 | 13 | 9 | 16 |
| CHD-other severe | 15 | 25 | 22 | 37 | 30 | 53 | 34 | 54 | 12 | 21 | 17 | 30 |
| CHD-unspecified | 53 | 122 | 49 | 99 | 23 | 60 | 64 | 95 | 30 | 54 | 16 | 30 |
| CHD-severe-total (I-I) | 28 | 46 | 53 | 91 | 55 | 105 | 58 | 138 | 29 | 57 | 27 | 43 |
| CHD-severe-conotruncal (I-I) | 22 | 35 | 44 | 66 | 47 | 71 | 46 | 79 | 20 | 37 | 19 | 37 |
| CHD-severe-non-conotruncal (I-I) | 6 | 11 | 9 | 25 | 8 | 34 | 12 | 59 | 9 | 20 | 8 | 6 |
| Cleft palate only | 35 | 93 | 32 | 91 | 19 | 70 | 33 | 102 | 21 | 61 | 7 | 39 |
| Cleft lip ± cleft palate | 178 | 233 | 157 | 222 | 148 | 207 | 147 | 236 | 72 | 114 | 61 | 121 |
| Cleft lip only | 63 | 77 | 55 | 69 | 59 | 73 | 57 | 70 | 27 | 36 | 24 | 35 |
| Cleft lip and palate | 115 | 156 | 102 | 153 | 89 | 134 | 90 | 166 | 45 | 78 | 37 | 86 |
| Esophageal atresia | 39 | 79 | 33 | 63 | 22 | 60 | 28 | 84 | 19 | 46 | 6 | 21 |
| Duodenal atresia | 7 | 13 | 14 | 28 | 13 | 32 | 9 | 27 | 9 | 20 | 5 | 19 |
| Anal atresia | 30 | 91 | 28 | 103 | 16 | 89 | 15 | 85 | 9 | 50 | 4 | 29 |
| Ambiguous genitalia | 21 | 67 | 12 | 54 | 11 | 58 | 18 | 76 | 16 | 38 | 4 | 47 |
| Hypospadias-total | 349 | 414 | 297 | 354 | 250 | 308 | 323 | 385 | 193 | 224 | 143 | 188 |
| Hypospadias-distal | 294 | 343 | 251 | 292 | 219 | 263 | 286 | 337 | 178 | 198 | 127 | 155 |
| Hypospadias-proximal | 43 | 56 | 34 | 44 | 18 | 27 | 31 | 38 | 13 | 20 | 9 | 20 |
| Absent kidney/s | 3 | 29 | 18 | 56 | 19 | 72 | 33 | 83 | 18 | 42 | 9 | 36 |
| Polycystic kidneys | 13 | 43 | 30 | 71 | 46 | 113 | 96 | 155 | 32 | 58 | 24 | 52 |
| Hydronephrosis | 26 | 55 | 58 | 105 | 133 | 213 | 287 | 388 | 98 | 138 | 128 | 190 |
| Talipes equinovarus | 395 | 556 | 390 | 569 | 279 | 451 | 320 | 500 | 226 | 344 | 148 | 295 |
| Talipes calcaneovalgus | 113 | 177 | 415 | 482 | 129 | 170 | 146 | 200 | 111 | 141 | 72 | 111 |
| Polydactyly-post-axial | 685 | 791 | 626 | 699 | 482 | 565 | 467 | 574 | 311 | 382 | 282 | 356 |
| Polydactyly-pre-axial | 64 | 79 | 40 | 63 | 39 | 55 | 36 | 56 | 26 | 36 | 25 | 44 |
| Polydactyly-others | 13 | 24 | 9 | 20 | 9 | 19 | 9 | 23 | 2 | 10 | 4 | 8 |
| Syndactyly-toes 2–3 | 73 | 103 | 51 | 70 | 40 | 53 | 44 | 65 | 16 | 23 | 15 | 27 |
| Syndactyly-other types | 59 | 134 | 38 | 107 | 24 | 77 | 21 | 72 | 16 | 43 | 7 | 42 |
| LRD-TT: amputation | 59 | 85 | 41 | 76 | 38 | 77 | 34 | 74 | 34 | 61 | 6 | 18 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LRD-TTH: hypoplasia | 10 | 32 | 14 | 31 | 8 | 17 | 6 | 13 | 5 | 11 | 2 | 5 |
| LRD-pre-axial | 3 | 22 | 6 | 18 | 7 | 28 | 2 | 30 | 6 | 19 | 2 | 10 |
| Hip-sub-dislocation | 572 | 661 | 523 | 583 | 309 | 356 | 376 | 432 | 168 | 193 | 119 | 150 |
| Hip-dislocation | 42 | 65 | 14 | 36 | 12 | 34 | 3 | 11 | 0 | 4 | 1 | 6 |
| Arthrogryposis | 4 | 14 | 9 | 27 | 13 | 73 | 29 | 118 | 8 | 40 | 2 | 14 |
| Diaphragmatic hernia | 12 | 33 | 19 | 59 | 34 | 72 | 53 | 94 | 34 | 51 | 19 | 34 |
| Abdominal muscle deficiency | 2 | 20 | 0 | 21 | 1 | 27 | 1 | 37 | 0 | 17 | 0 | 8 |
| Pectoralis hypoplasia | 3 | 7 | 6 | 8 | 1 | 2 | 2 | 3 | 0 | 2 | 0 | 3 |
| Skin ring constriction | 0 | 6 | 0 | 17 | 2 | 26 | 1 | 22 | 2 | 15 | 2 | 13 |
| Down total | 342 | 342 | 333 | 333 | 277 | 277 | 312 | 312 | 204 | 204 | 147 | 147 |
| Down $\leq$19 YMA | 20 | 20 | 23 | 23 | 29 | 29 | 19 | 19 | 19 | 19 | 12 | 12 |
| Down $\geq$35 YMA | 158 | 158 | 145 | 145 | 125 | 125 | 157 | 157 | 102 | 102 | 76 | 76 |

Spina bifida-cephalic, cervical, thoracic; spina bifida-caudal, lumbal, sacral; CHD, congenital heart disease; (I-I), CHD grouped as Ionescu-Ittu et al. [2009]; hypospadias-distal, balanic, balano-prepucial; hypospadias-proximal: penile, scrotal, perineal; LRD, limb reduction defect; TT, transverse terminal; TTH, transverse terminal hypoplasia (includes brachydactyly); YMA, years of maternal age; Down syndrome always considered as isolated by definition.

[a]Births.