MATERNAL HISTORY OF MISCARRIAGES AND SPECIFIC BIRTH DEFECTS. PROPOSED MECHANISMS FOR THEIR ASSOCIATION

Journal:	*Birth Defects Research Part A: Clinical and Molecular Teratology*
Manuscript ID	Draft
Wiley - Manuscript type:	Original Research Article
Date Submitted by the Author:	n/a

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Key Words:	miscarriage, ECLAMC, spina bifida, hypospadias, omphalocele, gastroschisis, talipes, club foot

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Running title: Miscarriages and specific birth defects

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ABSTRACT

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Some studies, mainly of the older literature, have observed a significant association between miscarriages and infants with certain birth defects (BDs) in the same sibship. However, few have deepened into the BD/miscarriage relationship, and lately nothing has been added to the knowledge of mechanisms possibly responsible for both events.

The purpose of this work was to identify specific BDs associated with maternal miscarriages, to establish if the risk depended on the number of losses, and to suggest specific factors for each BD/miscarriage association.

Methods: The study included the ECLAMC data base registries of 26,906 live and stillborn infants with one of 19 selected isolated BDs and of 93,853 normal controls. Infants born to primigravid mothers were excluded. Demographic and reproductive variables were compared between control mothers with and without previous miscarriages. Number of miscarriages, their frequencies, and distribution were shown for each BD and for controls. A conditional logistic regression was performed to evaluate the miscarriage risk for each BD.

Results: Control mothers with vs. without previous miscarriages were older, had had more pregnancies, and were less educated. Three risk models for miscarriages were observed: highest risk for gastroschisis, omphalocele, and talipes; only one miscarriage for spina bifida, and only two or more for hypospadias.

Conclusion: Based on these three models, the following common factors for each BD/miscarriage association are proposed: infertility therapies for hypospadias, vascular disruption for gastroschisis and talipes, while for spina bifida, the trophoblastic cell residue theory could not be discarded.

Key words: miscarriage; ECLAMC; spina bifida; hypospadias; omphalocele; gastroschisis; talipes; club foot

INTRODUCTION

INTRODUCTION

Approximately 10-15% of all clinically recognized pregnancies end in miscarriage (Schaeffer et al., 2004). Most of them occur during the first trimester and are mainly due to chromosome anomalies (Hassold et al., 1980). Other genetic, environmental, or combined causes have also been shown to be involved, while for many the cause remains unknown.

Recurrence of pregnancy losses occurs in 1-2% of fertile women, often again without an identifiable cause (Brigham et al., 1999). Eventually, a history of abortions includes the birth of a viable although malformed fetus, due to inherited chromosome anomalies (De Krom et al., 2015). Some authors have analyzed the association between previous miscarriages and the occurrence of certain birth defects of unknown etiology (Khoury and Erickson, 1993; Paz et al., 1992; Martínez-Frías and Frías, 1997), and a number of hypotheses have been brought forward. For instance, it has been suggested that the same defect shown by the liveborn infant was also present, although undetected, in the aborted conceptus, being the cause of the pregnancy loss (Khoury and Erickson, 1993). Martínez-Frías and Frías (1997), who found more abortions in the sibship of infants with more than with less severe defects, reached similar conclusions.

Clarke et al. (1975) hypothesized that residual trophoblastic cells from a previous miscarriage interfered with the embryo development, causing a neural tube defect. This hypothesis was supported by some authors (Gardiner et al., 1978) and rejected by others (Martínez-Frías and Frías, 1997).

Vascular disruption has been suggested as responsible for certain defects, such as club foot and gastroschisis (Van Allen, 1981; Lubinsky, 2014), while Rittler et al. (2015)

have demonstrated an association between miscarriages and gastroschisis, proposing a disruptive mechanism for both. The purpose of this work was to identify among a number of selected birth defects those specifically associated with a history of miscarriages, if the magnitude of the risk for those birth defects depended on the number of miscarriages, if the risk of an immediately previous miscarriage differed from one not immediately previous, and to propose involved mechanisms for each birth defects/miscarriage association.

MATERIAL AND METHODS

ECLAMC (Latin American Collaborative Study of Congenital Malformations) is a program dedicated to the research of birth defects (BDs), through a network of maternity hospitals where health professionals, mainly pediatricians, identify birth defects in live and stillborn infants. Data on socioeconomic and demographic characteristics, previous birth outcomes, and prenatal factors are obtained from medical records and by interviewing the mothers of malformed infants and of healthy controls (non-malformed infant born immediately after each affected newborn and paired by sex), before their discharge. Detailed descriptions of the registry and methodology have been previously published (Castilla and Orioli, 2004).

The present study included the ECLAMC data base registries of live and stillborn infants with isolated, major defects born between 1967 and 2013, and those of healthy controls. Infants with more than one major defect were excluded, as were cases and controls born to primigravid mothers.

A total of 19 BDs were selected, with at least 160 cases, in order to detect a 1.5 risk, with a 15% exposure, an 80% power of the test, and an alpha error of 0.05 (Appendix).

The definition of miscarriage was based on the mother´s description of a spontaneous first trimester pregnancy loss, without any identifiable fetus.

The following variables were compared between control mothers with and without previous miscarriages:
Demographic: maternal and paternal age, gravidity, low maternal and paternal education (< 7 years schooling), low paternal occupation (unemployed or unqualified worker), and ethnic ancestry (Native American, African American, and European).
Reproductive and of current pregnancy: short inter-birth interval (< 1 year), medication and chronic maternal illness in first trimester of pregnancy.
Anova and t-test were applied for continuous, chi-square for discrete variables. A Bonferroni correction was applied to adjust for the large number of comparisons. Significance level was set at 0.05.
The number of maternal miscarriages, their frequencies over total pregnancies and their distribution by number were obtained for each BD and for controls.
Cases and healthy controls (4 per case) were matched by hospital, year of birth and maternal gravidity. To establish the miscarriage risk for each BD, a conditional logistic regression was performed, adjusting by maternal and paternal ages, Native ancestry, and maternal education and chronic illnesses.
The same analysis was performed, taking the infants with the remaining BDs as malformed controls.
The risk between immediately vs. not immediately previous miscarriages was compared, only using case and control mothers with a history of miscarriages.
Data were analyzed with the statistical software StataCorp LP, version 12.0, College Station, TX.

RESULTS

The study included the ECLAMC data base registries of 26,906 infants with the 19 selected BDs and a total of 93,853 healthy controls.

The comparison of epidemiologic characteristics of control mothers with and without previous miscarriages is shown in Table 1. Mothers with previous miscarriages showed a higher frequency of certain risk factors, such as older age, more pregnancies, and less education. The magnitude of these variables increased as the number of miscarriages increased.

In Table 2, the number of miscarriages, their frequencies over total pregnancies, and their distribution by number are shown for each BD and for controls.

A conditional logistic regression, using healthy controls, showed that mothers with previous miscarriages were at a statistically significant risk for five of the 19 selected BDs, namely gastroschisis, omphalocele, talipes equinovarus, spina bifida, and hypospadias (Table 3). For the former two, the risk increased with increasing number of miscarriages.

When comparing with malformed controls, the risk remained significant for omphalocele (OR: 1.83(1.33-2.53), $p<0.001$), gastroschisis (OR: 2.00(1.51-2.65), $p<0.001$), and talipes (OR: 1.18(1.04-1.35), $p<0.01$).

For hypospadias, only mothers with more than one miscarriage were at risk, while for spina bifida, only one previous miscarriage, but not more than one, represented a risk factor.

When comparing immediately vs. not immediately previous miscarriages, no risk difference was found for any of the selected defects.

DISCUSSION

Here we have shown that among a number of selected birth defects, five were associated with a maternal history of miscarriages.

In coincidence with one of our findings, Paz et al. (1992) observed a significant association between previous

miscarriages and talipes equinovarus. They also found that anencephaly and spina bifida were significantly associated, but with stillbirth, not miscarriage, having joined both as a single variable (pregnancy loss).

Other methodological differences were, for instance, having only considered the immediately previous abortion, while mothers with miscarriages at any other time were included into the control group; and that gastroschisis and omphalocele were included into a global category of "other single defects", precluding comparisons with our results.

Khoury and Erickson (1993) evaluated the risk for 57 selected categories of birth defects in infants of women with a history of pregnancy losses (miscarriages and stillbirths). In coincidence with our work, the authors found a significant risk for positional limb defects and hypospadias, while in opposition, they found no risk for spina bifida, omphalocele, or gastroschisis.

Types of birth defect/miscarriage associations

The present work revealed three models of birth defect-miscarriage associations: one, with only one miscarriage (spina bifida); two, with only two or more miscarriages (hypospadias); and three, where the risk was highest (gastroschisis, omphalocele, talipes), and increased for the former two as the number of miscarriages increased.

This finding was not unexpected, given that different mechanisms are assumed to be involved in birth defects, as well as in their associated miscarriages.

Therefore, it seemed reasonable to search for specific factors common to each defect/miscarriage association.

Spina bifida

One possible hypothesis explaining its association with miscarriages is the polygenic-multifactorial model of inheritance. Within the spectrum of neural tube defects

(NTDs), a miscarriage within the sibship of an infant with spina bifida could represent an anencephalic embryo, lost because of its greater severity (Laurence and Roberts, 1977; Carmi et al, 1994).

On the other hand, Clarke et al. (1975) have shown that mothers of infants with NTDs had more miscarriages immmediately previous to the current birth than after. They hypothesized that residual trophoblastic cells from the preceding abortion interfered with the subsequent developing embryo, leading to anencephaly or spina bifida. This cell rest hypothesis was supported by some authors (Gardiner et al., 1978), some have even related it to other defects (Sheiner et al., 1998), while others (Martínez-Frías and Frías, 1997) found that the rate of miscarriages immediately preceding the birth of an affected infant did not differ from that of miscarriages at any other time. Lu et al. (2011) showed that an immediately previous abortion was a risk factor for a subsequent fetus with anencephaly only if the interpregnancy interval between both was short. Todoroff and Shaw (2000) reached similar conclusions.

We analyzed this hypothesis by evaluating the interaction between interbirth interval and miscarriage on the occurrence of each birth defect and found that for spina bifida such an interaction actually existed. The 1.01 (0.77-1.29) risk for a long interval increased to 1.70 (1.11-2.60) if it was short.

Based on these results, the cell rest theory could not be entirely descarded. However, other factors related to a short interpregnancy interval, such as nutritional or vitamin deficiencies (Czeizel, 2009), should also be taken into consideration.

Hypospadias

The present work showed that only women with a history of two or more miscarriages were at risk for hypospadias in a male infant.

Many studies have demonstrated an association between infertility treatments and hypospadias. Some authors have found such an association with sex hormone medication (Carmichael et al., 2005), suggesting interference with the development of male genitalia, while others have not (Källén et al., 1991). However, an excess of hypospadias was also observed after treatment with assisted reproductive therapies, other than medication (Heisey et al., 2015), and this finding would require further research. Nevertheless, and whatever the factor interfering with male genitalia development, it seems reasonable to expect that any such therapy would only be applied after a history of at least two miscarriages.

Talipes equinovarus

Club foot has repeatedly been related to vascular disruptions through factors such as early amniocentesis (Evans and Wapner, 2005), the loss of a twin fetus (Pharoah, 2005), vasoconstrictive medication (Werler, 2006), or maternal smoking during pregnancy (Skelly et al., 2002).

Merrill et al. (2011) have identified vascular anomalies in the lower limbs of patients with club foot, in support of a vascular role in the pathogenesis of this defect.

In an epidemiologic study on club foot, Werler et al. (2013) have observed, among other findings, that case mothers were more often primiparous (a recognized risk factor for club foot, through fetal constraint) than control mothers. However, they also found that gravidity did not differ between cases and controls, which suggests that case mothers could have had more previous abortions (which do not add to parity) than controls. Unfortunately,

previous miscarriages were not included among their analyzed variables.

In further support of vascular disruption, the authors observed associated anomalies, possibly due to the same mechanism, in four of their cases: one with septooptic dysplasia, two with hydrocephaly, and one with a brain infarct.

Talipes equinovarus is one of the most frequent and conspicuous defects of the Moebius sequence, while other findings, such as an expressionless face or neurologic dysfunction, can be missed or not reported. The use of misoprostol as abortifacient, due to its uterotonic property, has often been associated with the Moebius sequence, as well as with other defects also related to vascular disruption, such as terminal transverse limb defects (da Silva et al., 2006), mainly in regions where terminations of pregnancy are illegal (Pastuszak et al., 1998; Vargas et al., 2000).

It could be hypothesized that abortions preceding the birth of an infant with talipes were in fact terminations of pregnancy (not admitted as such due to their illegal condition), being the current outcome an attempted although missed abortion. The observed association between miscarriages and hip dislocation (Paz et al., 1992), also often present in the Moebius sequence, adds further support to this hypothesis.

Gastroschisis

Lubinsky (2014) proposed two disruptive pathogenetic models for gastroschisis. The first was not age-specific and acted through vasoconstrictive factors, such as certain medication (Werler, 2006), or smoking (Skarsgard et al., 2015), among others.

The second was age-specific, and acted through the thrombotic effect of estrogens, typical in young women who show higher levels of estrogens at early stages of

pregnancy (Lubinsky, 2012). In support of this hypothesis, the association between gastroschisis and certain endocrine disruptors with estrogenic effect have been demonstrated (Agopian et al., 2013).

In a previous work (Rittler et al., 2015), we have shown that a maternal history of miscarriages was the main risk factor for gastroschisis. To our knowledge, an association between gastroschisis and pregnancy losses has only been mentioned by Getz et al. (2012), whose study however was limited to mothers with short interpregnancy intervals. They found that the risk factor was the short interval, with the risk increasing if the previous pregnancy had ended in a miscarriage. Contrarily, in our study the identified risk factor was the miscarriage, regardless of the interpregnancy interval.

Both hypertension and thrombophilia are recognized risk factors for pregnancy losses (Kutteh and Triplett, 2006), and although to our knowledge the association between thrombophilia and gastroschisis has not been mentioned, other, less frequent hyperthrombotic conditions could by involved.

Omphalocele

In coincidence with the here observed association between omphalocele and a history of miscarriages, Agopian et al. (2009) found significantly more omphalocele cases among mothers without than with previous livebirths.

However, the relationship between omphalocele and miscarriages does not seem to suggest any straightforward hypothesis. The chances of misdiagnosing omphalocele for gastroschisis were low in our sample, since for a previous study on gastroschisis (Castilla et al., 2008), all abdominal wall defect cases were reviewed, enhancing diagnostic reliability.

One possible explanation could be the fact that omphalocele is often present in syndromes, mainly due to chromosome

anomalies (Stoll et al., 2001), and couples carrying a chromosome rearrangement may be at risk for malformed fetuses as well as for recurrent miscarriages (De Krom et al., 2015). Although in this work only infants with isolated defects were included, chromosome studies, which are not requested as part of the ECLAMC procedure, were not always done, and consequently the inclusion of some cases with chromosome anomalies could not be entirely ruled out. However, this explanation did not seem able to account for the observed number of cases.

On the other hand, some authors (Reefhuis and Honein, 2004; Marshall et al., 2015) have observed that both old and young maternal age (< 20 years) were associated with omphalocele, and given the recognized association between gastroschisis and young maternal age, perhaps in a subgroup, factors similar to those responsible of gastroschisis could also be involved in the occurrence of omphalocele.

Strengths and weaknesses

The main strengths of this study resided in the magnitude of ECLAMC series of infants with birth defects, having adjusted by number of pregnancies, and the use of malformed controls, thereby reducing the memory bias effect in mothers of sick vs. healthy newborns.

A further strength was that ascertainment and reporting was performed by pediatricians specially trained in diagnosis and description of birth defects, thereby assuring homogeneous data by following clearly defined rules.

Limitations were those related to retrospective case control studies, such as the recall bias for data obtained by interviewing the mothers. Information on abortions, if spontaneous or induced, might be unreliable, especially in countries, such as most South American, where terminations of pregnancy are illegal.

CONCLUSIONS

The present work revealed three models of birth defect-miscarriage associations: one, where the risk increased as the number of miscarriages increased; two, with only one miscarriage, and three, with only two or more.
The recognition of different types of associations, as well as the obtained heterogeneity reduction among some defects, might increase the chances of recognizing specific links between birth defects and miscarriages, as a way to approach the identification of underlying common causes.

ACKNOWLEDGMENTS

The authors wish to thank all the people working in collaboration in ECLAMC, a network that has been active for more than 40 years.

LITERATURE CITED

Agopian A, Marengo L, Mitchell LE. 2009. Descriptive epidemiology of nonsyndromic omphalocele in Texas, 1999-2004. Am J Med Genet A 149A(10):2129-2133.

Agopian AJ, Langlois PH, Cai Y, Canfield MA, Lupo PJ. 2013. Maternal residential atrazine exposure and gastroschisis by maternal age. Matern Child Health J 17:1768-1775.

Brigham SA, Conlon C, Farquharson RG. 1999. A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. Hum Reprod 14(11):2868-2871.

Carmi R, Gohar J, Meizner I, Katz M. 1994. Spontaneous abortion high risk factor for neural tube defects in subsequent pregnancy. Am J Med Genet 51(2):93-97.

Carmichael SL, Shaw GM, Laurent C, Croughan MS, Olney RS, Lammer EJ. 2005. Maternal progestin intake and risk of hypospadias. Arch Pediatr Adolesc Med 159(10):957-962.

Castilla EE, Orioli IM. 2004. ECLAMC: The Latin American Collaborative Study of Congenital Malformations. Community Genet 7:76-94.

Castilla EE, Mastroiacovo P, Orioli IM. 2008. Gastroschisis: international epidemiology and public health perspectives. Am J Med Genet C Semin Med Genet 148C:162-179.

Clarke C, Hobson D, McKcndrick OM, Rogers SC, Sheppard PM. 1975. Spina bifida and anencephaly: Miscarriage as possible cause. Br Med J 4:743-746.

Czeizel AE. 2009. Periconceptional folic acid and multivitamin supplementation for the prevention of neural tube defects and other congenital abnormalities. Birth Defects Res A Clin Mol Teratol 85(4):260-268.

da Silva Dal Pizzol T, Knop FP, Mengue SS. 2006. Prenatal exposure to misoprostol and congenital anomalies: systematic review and meta-analysis. Reprod Toxicol 22(4):666-671.

De Krom G, Arens YH, Coonen E, Van Ravenswaaij-Arts CM, Meijer-Hoogeveen M, Evers JL, Van Golde RJ, De Die-Smulders CE. 2015. Recurrent miscarriage in translocation carriers: no differences in clinical characteristics between couples who accept and couples who decline PGD. Hum Reprod 30(2):484-489.

Evans MI, Wapner RJ. 2005. Invasive prenatal diagnostic procedures 2005. Semin Perinatol 29(4):215-218.

Gardiner A, Clarke S, Cowen J, Finn R, McKendrick OM. 1978. Spontaneous abortion and fetal abnormalities in subsequent pregnancy. Br Med J 1:1016-1018.

Getz KD, Anderka MT, Werler MM, Case AP. 2012. Short interpregnancy interval and gastroschisis risk in the National Birth Defects Prevention Study. Birth Defects Res A Clin Mol Teratol 94:714-720.

Hassold T, Chen N, Funkhouser J, Jooss T, Manuel B, Matsuura J, Matsuyama A, Wilson C, Yamane JA, Jacobs PA. 1980. A cytogenetic study of 1000 spontaneous abortions. Ann Hum Genet 44(Pt 2):151-178.

Heisey AS, Bell EM, Herdt-Losavio ML, Druschel C. 2015. Surveillance of congenital malformations in infants conceived through assisted reproductive technology or other fertility treatments. Birth Defects Res A Clin Mol Teratol 103(2):119-126.

Källén B, Mastroiacovo P, Lancaster PA, Mutchinick O, Kringelbach M, Martínez-Frías ML, Robert E, Castilla EE. 1991. Oral contraceptives in the etiology of isolated hypospadias. Contraception 44(2):173-182.

Khoury MJ, Erickson JD. 1993. Recurrent pregnancy loss as an indicator for increased risk of birth defects: a population-based case-control study. Paediatr Perinat Epidemiol 7(4):404-416.

Kutteh WH, Triplett DA. 2006. Thrombophilias and recurrent pregnancy loss. Semin Reprod Med 24(1):54-66.

Laurence KM, Roberts CJ. 1977. Spina bifida and anencephaly: are miscarriages a possible cause? Br Med J 2(6083):361-362.

Lu QB, Wang ZP, Gao LJ, Gong R, Sun XH, Zhao ZT. 2011. Previous abortion and the risk of neural tube defects. A case-control study. J Reprod Med 56(9):431-436.

Lubinsky M. 2012. Hypothesis: Estrogen related thrombosis explains the pathogenesis and epidemiology of gastroschisis. Am J Med Genet A 158A(4):808-811.

Lubinsky M. 2014. A vascular and thrombotic model of gastroschisis. Am J Med Genet A 164A(4):915-917.

Marshall J, Salemi JL, Tanner JP, Ramakrishnan R, Feldkamp ML, Marengo LK, Meyer RE, Druschel CM, Rickard R, Kirby RS; National Birth Defects Prevention Network. 2015. Prevalence, correlates, and outcomes of omphalocele in the United States, 1995-2005. Obstet Gynecol 126(2):284-293.

Martínez-Frías ML, Frías JL. 1997. Are blastogenetic anomalies sporadic? Am J Med Genet 68(4):381-385.

Merrill LJ, Gurnett CA, Siegel M, Sonavane S, Dobbs MB. 2011. Vascular abnormalities correlate with decreased soft tissue volumes in idiopathic clubfoot. Clin Orthop Relat Res 469(5):1442-1449.

Pastuszak AL, Schüler L, Speck-Martins CE, Coelho KE, Cordello SM, Vargas F, Brunoni D, Schwarz IV, Larrandaburu M, Safattle H, Meloni VF, Koren G. 1998. Use of misoprostol during pregnancy and Möbius' syndrome in infants. N Engl J Med 338(26):1881-1885.

Paz JE, Otaño L, Gadow EC, Castilla EE. 1992. Previous miscarriage and stillbirth as risk factors for other unfavourable outcomes in the next pregnancy. Br J Obstet Gynaecol 99(10):808-812.

Pharoah PO. 2005. Causal hypothesis for some congenital anomalies. Twin Res Hum Genet 8(6):543-550.

Reefhuis J, Honein MA. 2004. Maternal age and non-chromosomal birth defects, Atlanta--1968-2000: teenager or thirty-something, who is at risk? Birth Defects Res A Clin Mol Teratol 70(9):572-579.

Rittler M, Campaña H, Ermini ML, Gili JA, Poletta FA, Pawluk MS, Giménez LG, Cosentino VR, Castilla EE, López-Camelo JS. 2015. Gastroschisis and young mothers: What makes them different from other mothers of the same age? Birth Defects Res A Clin Mol Teratol 103(6):536-543.

Schaeffer AJ, Chung J, Heretis K, Wong A, Ledbetter DH, Lese Martin C. 2004. Comparative genomic hybridization-array analysis enhances the detection of aneuploidies and submicroscopic imbalances in spontaneous miscarriages. Am J Hum Genet 74(6):1168-1174.

Sheiner E, Katz M, Fraser D, Gohar J, Carmi R. 1998. The relationship between congenital cardiovascular malformations and spontaneous abortion in preceding pregnancy. Paediatr Perinat Epidemiol 12(2):128-135.

Skelly AC, Holt VL, Mosca VS, Alderman BW. 2002. Talipes equinovarus and maternal smoking: a population-based case-control study in Washington state. Teratology 66(2):91-100.

Skarsgard ED, Meaney C, Bassil K, Brindle M, Arbour L, Moineddin R; Canadian Pediatric Surgery Network (CAPSNet).

2015. Maternal risk factors for gastroschisis in Canada. Birth Defects Res A Clin Mol Teratol 103(2):111-118.

Stoll C, Alembik Y, Dott B, Roth MP. 2001. Risk factors in congenital abdominal wall defects (omphalocele and gastroschisi): a study in a series of 265,858 consecutive births. Ann Genet 44(4):201-208.

Todoroff K, Shaw GM. 2000. Prior spontaneous abortion, prior elective termination, interpregnancy interval, and risk of neural tube defects. Am J Epidemiol 151(5):505-511.

Van Allen MI. 1981. Fetal vascular disruptions: mechanisms and some resulting birth defects. Pediatr Ann 10:219-233.

Vargas FR, Schuler-Faccini L, Brunoni D, Kim C, Meloni VF, Sugayama SM, Albano L, Llerena JC Jr, Almeida JC, Duarte A, Cavalcanti DP, Goloni-Bertollo E, Conte A, Koren G, Addis A. 2000. Prenatal exposure to misoprostol and vascular disruption defects: a case-control study. Am J Med Genet 95(4):302-306.

Werler MM. 2006. Teratogen update: pseudoephedrine. Birth Defects Res A Clin Mol Teratol 76(6):445-452.

Werler MM, Yazdy MM, Mitchell AA, Meyer RE, Druschel CM, Anderka M, Kasser JR, Mahan ST. 2013. Descriptive epidemiology of idiopathic clubfoot. Am J Med Genet A 161A(7):1569-1578.

LEGENDS

LEGENDS

Appendix: ECLAMC data base. 209 participating hospitals in 10 South American countries, 1967 - 2013.

Table 1: Epidemiologic characteristics of control mothers with and without previous miscarriages.

Table 2: Total number and distribution of miscarriages for each birth defect.

Table 3: Previous miscarriage/s and risk for congenital anomalies.

Table 1: Epidemiologic characteristics of control mothers with and without previous miscarriages.

Characteristics	Miscarriage						Number of miscarriages							
	No N=76244		Yes N=17609		t	p	1 N= 13534		2 N= 3026		3+ N= 1049		F	p
	N	X±sd	N	X±sd			N	X±sd	N	X±sd	N	X±sd		
Maternal age	74442	26.7±6.3	17564	28.7±6.4	-37.80	<0.001	13497	28.2±6.3	3018	30.3±6.2	1049	31.4±5.9	240.35	<0.001
Gravidity	75872	3.3±1.8	17605	4.5±2.3	-79.40	<0.001	13530	4.1±2.0	3026	5.7±2.3	1049	7.3±2.5	1650.3	<0.001
Paternal age	72213	30.4±7.6	17145	32.1±7.7	-26.34	<0.001	13185	31.6±7.6	2939	33.4±7.9	1023	34.6±7.6	123.85	<0.001
	N	%	N	%	χ^2_1	p	N	%	N	%	N	%	χ^2_2	p
Low maternal education	20810	27.3	5062	28.8	15.12	<0.001	3782	27.9	921	30.4	359	34.2	23.829	<0.001
Low paternal education	17995	23.6	4348	24.7	9.37	0.002	3236	23.9	800	26.4	312	29.7	23.798	<0.001
Low paternal occupation	29126	38.2	6161	35.0	62.95	<0.001	4703	34.8	1075	35.5	383	36.5	1.792	0.408
Native	29996	42.1	6636	38.8	60.95	<0.001	5129	39.1	1138	38.7	369	36.6	2.406	0.300
African	15218	21.3	3700	21.6	0.68	0.409	2768	21.1	683	23.2	249	24.7	12.287	0.002
European	26273	36.6	6748	39.6	50.72	<0.001	5236	39.8	1122	38.1	390	38.7	3.355	0.187
Short inter-birth interval	22351	29.3	5526	31.4	29.26	<0.001	4289	31.7	931	30.8	306	29.2	3.513	0.173
Medication	41533	54.5	10483	59.5	148.15	<0.001	7966	58.9	1873	61.9	644	61.4	11.074	0.004
Chronic maternal illness	7930	10.4	2524	14.3	223.53	<0.001	1845	13.6	461	15.2	218	20.8	42.941	<0.001

X±sd: mean ± standard deviation

Table 2: Total number and distribution of miscarriages for each birth defect

			Miscarriages		Mothers by number of miscarriages			
	Mothers	Gravidities total	N	%	0	1	2	3+
Omphalocele	438	1667	166	10.0	329	70	29	10
Hypoplastic left heart	208	756	62	8.2	168	27	7	6
Gastroschisis	864	2525	202	8.0	706	123	30	5
Truncus arteriosus	821	3045	222	7.3	664	114	31	12
Transverse limb reduction	442	1653	120	7.3	368	49	13	12
Diaphragmatic hernia	594	2168	153	7.1	488	75	25	6
Spina bifida	1,739	6718	464	6.9	1,383	267	73	16
Microtia	1,081	4229	281	6.6	880	145	40	16
Cephalocele	461	1826	118	6.5	369	71	18	3
Septal heart defect	2,247	8313	540	6.5	1,858	285	70	34
Esophageal atresia	502	1879	121	6.4	418	60	16	8
Hypospadias	1,179	4211	270	6.4	980	143	45	11
Talipes equinovarus	3,459	12590	808	6.4	2,869	441	109	40
Cleft palate	654	2548	156	6.1	536	97	13	8
Cleft lip +/- cleft palate	3,541	14384	874	6.1	2,896	482	121	42
Preaxial polydactyly	868	3233	198	6.1	716	116	29	7
Postaxial polydactyly	5,397	20610	1258	6.1	4,489	667	175	66
Anencephaly	1,800	7177	417	5.8	1,496	224	58	22
Anorectal atresia	602	2408	122	5.1	511	67	20	4
Controls	93,853	329921	23258	7.0	76,244	13,534	3,026	1,049

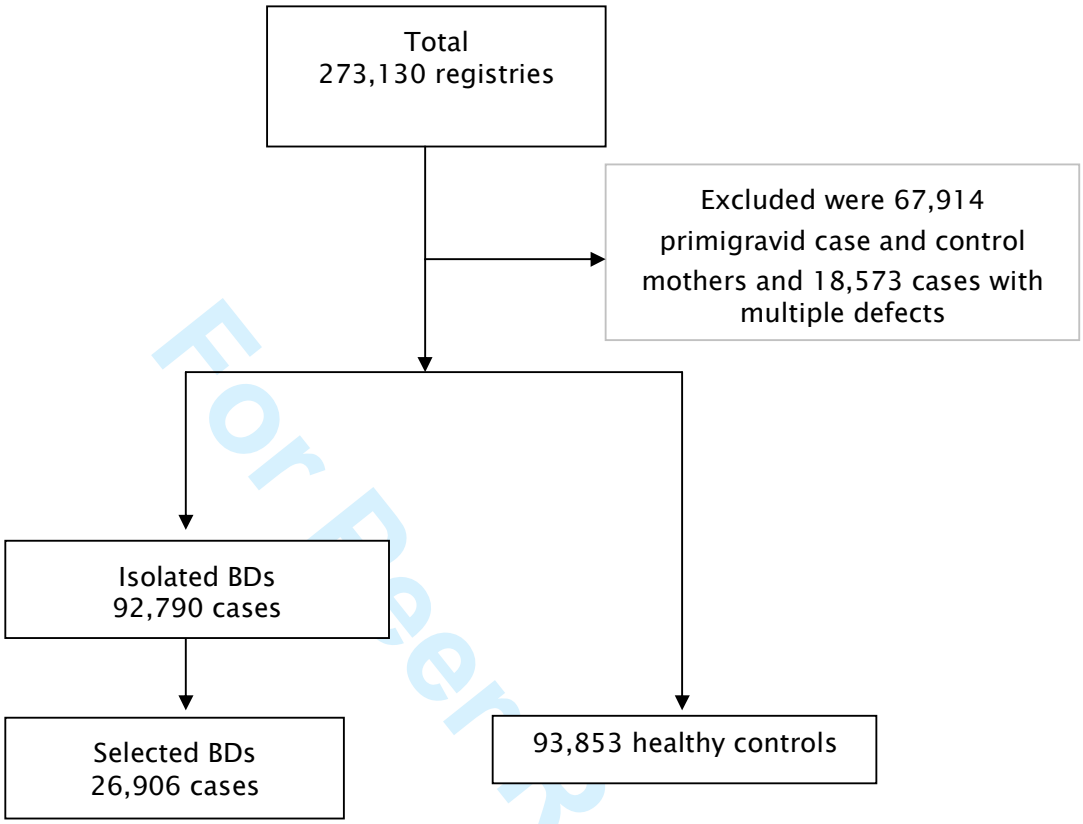
%: N / total gravidities
+/-: with or without

Table 3: Previous miscarriage/s and risk for birth defects.

Birth defect	Previous miscarriage/s		Number of previous miscarriages			
			1		2 +	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Gastroschisis	1.91 (1.46-2.99)	<0.001	1.76 (1.33-2.33	<0.001	3.04 (1.35-1.72)	<0.001
Omphalocele	1.72 (1.26-2.36)	<0.001	1.49 (1.06-2.10)	<0.001	3.13 (1.79-5.49)	<0.001
Talipes equinovarus	1.48 (1.31-1.68)	<0.001	1.49 (1.30-1.70)	<0.001	1.45 (1.14-1.84)	0.002
Hypoplastic left heart	1.31 (0.78-2.21)	0.306	1.25 (0.72-2.18)	0.434	1.70 (0.60-4.80)	0.313
Spina bifida	1.27 (1.08-1.48)	0.001	1.29 (1.08-1.43)	0.003	1.18 (0.88-1.58)	0.262
Cephalocele	1.23 (0.91-1.28)	0.174	1.17 (0.85-1.62)	0.334	1.65 (0.87-3.10)	0.334
Hypospadias	1.18 (0.97-1.44)	0.089	1.06 (0.86-1.32)	0.578	1.84 (1.27-2.65)	0.001
Esophageal atresia	1.18 (0.85-1.63)	0.326	1.13 (0.79-1.60)	0.498	1.40 (0.75-2.60)	0.293
Preaxial polydactyly	1.14 (0.91-1.44)	0.255	1.14 (0.89-1.46)	0.299	1.17 (0.73-1.89)	0.517
Cleft lip +/- cleft palate	1.13 (1.00-1.27)	0.031	1.10 (0.97-1.25)	0.118	1.26 (0.99-1.59)	0.052
Microtia	1.10 (0.90-1.36)	0.345	1.08 (0.36-1.86)	0.498	1.19 (0.81-1.75)	0.379
Truncus arteriosus	1.09 (0.86-1.39)	0.460	1.07 (0.83-1.38)	0.609	1.22 (0.76-1.94)	0.411
Transverse limb reduction	1.08 (0.73-1.51)	0.677	1.04 (0.71-1.43)	0.838	1.18 (0.66-2.08)	0.574
Anorectal atresia	1.04 (0.77-1.42)	0.788	1.01 (0.73-1.41)	0.940	1.18 (0.65-2.13)	0.583
Diaphragmatic hernia	1.03 (0.77-1.38)	0.839	1.01 (0.74-1.39)	0.928	1.10 (0.64-1.87)	0.736
Cleft palate	1.00 (0.76-1.31)	0.962	1.09 (0.82-1.44)	0.557	0.69 (0.39-1.21)	0.194
Anencephaly	0.98 (0.83-1.16)	0.825	0.99 (0.82-1.19)	0.915	0.96 (0.67-1.30)	0.736
Septal heart defect	0.97 (0.83-1.13)	0.684	0.96 (0.81-1.12)	0.593	1.02 (0.76-1.37)	0.870
Postaxial polydactyly	0.96 (0.89-1.05)	0.365	0.94 (0.85-1.05)	0.273	1.01 (0.84-1.22)	0.903

OR= odds ratio; CI= confidence interval
+/-: with or without

Appendix: ECLAMC data base. 209 participating hospitals in 10 South American countries, 1967 – 2013.



Birth defect	N
Omphalocele	438
Gastroschisis	864
Anencephaly	1,800
Spina bifida	1,739
Cephalocele	461
Microtia	1,081
Cleft palate	654
Cleft lip +/- cleft palate	3,541
Esophageal atresia	502
Ano-rectal atresia	602
Truncus arteriosus	821
Septal defects	2,247
Hypoplastic left heart	208
Hypospadias	1,179
Transverse limb reduction	451
Talipes equinovarus	3,459
Preaxial polydactyly	868
Postaxial polydactyly	5,397
Diaphragmatic hernia	594