**CEOG** Clinical and Experimental Obstetrics & Gynecology

# Uterine contractility as a cause of amniotic band syndrome

**A. Galvan Montaño[1], H. Rios[1], L. Armijo[1], M.L. Suárez Roa[1], E. Ureta[1], A. Perez Gonzalez[2]**

*[1] General Hospital "Manuel Gea González", Mexico City*
*[2] Health Sciences and Plastic Surgeon of National Pediatric Institute, Mexico City (Mexico)*

**Summary**

*Objective:* The objective of this study was to determine whether puncturing the uterine wall and the amnion causes uterine contractions that result in fetal abnormalities. *Materials and Methods:* An experimental study was performed using four groups of three female rabbits. Group A received a puncture of the amniotic membranes of one of the uteri on day 15 of gestation followed by group B on day 16, group C on day 17, and group D on day 18. The duration and force of contractions and fetal abnormalities were determined. *Results:* There were immediate contractions after the puncture, which lasted 20 to 132 seconds with forces that ranged from 309 to 4,411 mg. All of the experimental fetuses exhibited anomalies of the head and extremities, exencephaly, cleft palates, and an absence of eyelids. *Conclusion:* Injury to the uterine wall and the amnion can immediately cause uterine contractions, which are associated with different types of fetal abnormalities.

*Key words:* Amniotic band syndrome; Fetal abnormalities; Therapy.

## Introduction

Amniotic band syndrome (ABS) has an international incidence that ranges from 1:1,200 to 15,000 births without a preference for sex or race. In Latin America, the reported prevalence is 1:11,200 births [1-3]. ABS is a set of fetal malformations that are caused by fibrous adhesions (amniotic bands), which trap and strangle fetal parts, such as the extremities and can cause ischemic damage to the tissue distal to the bands. This phenomenon can result in hypoplasia, amputation, syndactyly, and polydactyly. When the amniotic fluid is ingested and swallowed by the fetus, the fetal development of the face can be disrupted, leading to craniofacial malformations and a cleft palate. Other malformations that have been described include anencephaly, encephalocele, ectopia cordis, and a ventral diaphragmatic defect [1-3].

There are two theories on the mechanism of fetal malformations in this disease. The endogenous theory states that the defects that are associated with ABS occur in response to fetal development complications in the formation of connective tissue [4]. The exogenous theory was proposed by Torpin in 1968 and states that the rupture of the amnion, specifically when the chorion remains undamaged, causes a transitory oligohydramnios secondary to the loss of amniotic fluid through the chorion. The fetus passes from the amniotic cavity to the chorionic cavity via this defect. The contact between the fetus and the "adherent" mesoderm of the chorionic surface of the amnion leads to tangling of fetal parts and cutaneous lesions, which have been observed in cases of ABS [5].

Animal research has provided evidence that a puncture or rupture of the amnion disrupts the embryological vessels during development, which causes ischemia and injuries, such as hypoplasia and the amputation of the extremities [6, 7]. Following the Kino model, the rabbit can be used as an animal model for the prenatal development of amniotic bands due to the number and diversity of anomalies that ABS presents [8].

The objective of this study was to determine whether puncturing the uterine wall and amnion causes uterine contractions that cause anomalies in fetal rabbits.

## Materials and Methods

In accordance with the Official Mexican Standard NOM-062-ZOO-19998 [9], all procedures were approved by the Research and Ethics Committee of the Dr. Manuel Gea Gonzalez General Hospital and the Institutional Committee for the Care of Laboratory Animals (ICCLA). The design of this study was comparative, open, experimental, prospective, and transverse.

A total of 12 female New Zealand rabbits that weighed three to 3.5 kg were housed in individual cages. A female was brought to the male cages for mating, which was verified by the veterinarian and zootechnician, and this day was considered day 0. Four groups of three rabbits were formed. The uterine wall and amniotic membranes of one of the uteri was punctured in group A on day 15 of gestation followed by group B on day 16, group C on day 17, and group D on day 18. The female rabbits were sacrificed on day 29 of gestation.

Preoperatively, the female rabbits were administered 35 mg/kg ketamine and five mg/kg xylazine intramuscularly on the day when the uterine wall and amnion were punctured. An IV was started with a 22-G catheter, and the IV tubing was placed with a microdrip that was connected to warm saline solution (50 ml for two

Revised manuscript accepted for publication March 16, 2015

hours). Enrofloxacin was administered at ten mg/kg (IV), and a 2.5-mm (internal diameter) endotracheal tube was inserted via the nose with a previous instillation of 2% lidocaine. Once fixed, the tube was connected to the anesthesia circuit for the passage of isoflurane at 5% over three minutes for induction and at 3% for maintenance during the surgery.

During the laparotomy, a trichotomy was performed, and the abdominal wall was cleaned with 0.7% iodine and 74% isopropyl alcohol. An abdominal midline incision was made, and the uteri that were closest to the ovaries (right and left) were identified. The uterus of the doe is formed by two horns right and left and each horn has a cervix that opens into the vagina. You can have on average four uteri and each uterus behaves as an independent unit. With its monochorial placenta, amniotic sac, and the uterine wall placenta.

The normal period of gestation in the doe is on average 31 days in 98% of cases, but can vary from 29 to 35 days, depending on the number of fetuses in gestation (varies from four to 12), to minor number of fetuses the greater the period of gestation and vice versa. One of the wombs (experimental) was randomly chosen for the physiographical study, in which an "S" hook was placed on the wall of the uterus and a baseline recording of contractile activity was obtained as a control. The wall of the uterus and the amniotic membranes were punctured using an insulin syringe with a 0.2-mm needle (outer diameter) that avoided blood vessels and prevented injury to the fetus. A single puncture was performed regardless of whether amniotic fluid was extracted; uterine muscle activity (pitch and duration) was immediately recorded over 240 seconds (the time when contractions appeared, which had previously been standardized with two female rabbits per group). A digital data acquisition system and the Biopac Student Lab PRO (v. 3.7) were used to obtain the recordings, which were later analyzed offline. The contralateral uterus that was closest to the ovary was used as a control, those who took the baseline uterine tone control and no puncture was performed

After the procedure, the abdominal wall was closed at two levels: the muscle was closed with Vicryl 2-0, and the skin was closed with Prolene 3-0. During the postoperative period, two mg/kg of flunixin meglumine was administered intramuscularly every 12 hours for three days, and ten mg/kg of enrofloxacin was administered intramuscularly every 12 hours for six days.

On day 29 of gestation, the female rabbits and the fetuses were sacrificed with a sodium pentobarbital overdose. The fetuses in the uterus, control, experimental, and remaining uteri were examined macroscopically. The uteri were identified (experimental and control) and extracted with the products of the uterus. The uteri were infiltrated with formalin, and a macroscopic examination of the fetuses was conducted after eight days.

The following variables were analyzed: the day of gestation, the quantity of fluid amniotic extracted, fetal deformities, the force of the contractions as measured in mg, the number of contractions in 240 seconds, and the duration of the contractions as measured in seconds. The variables were analyzed using the following descriptive statistics: the mean, the standard deviation (SD), and the range.

## Results

The punctures were performed on 12 uteri, three per group. In the punctured uteri of group A (punctured at 15 days of gestation), there was an average of three contractions in 240 seconds. The average duration of the contractions was 56.4 seconds with a range of 36-80 seconds and a SD of 14.62 seconds. The average basal uterine tone measured before the puncture was 125.8 mg, and the average force of the contractions was 497.3 mg with a range of 309.3 to 909.2 mg and a SD of 216.52 mg (Table 1). During the macroscopic examination of the three experimental fetuses, one abortion, one death, and one fetus with exencephaly, an absence of eyelids, a cleft palate, and limb compression were observed (Figure 1). Among the three control fetuses, one abortion occurred. In the remaining fetuses, one death, one abortion, one forelimb compression, and one compression of the facial area occurred.

In group B (punctured at 16 days of gestation), an average of three contractions was observed in 240 seconds. The average duration of the contraction wave was 63.2 seconds, with a range of 38-120 seconds and a SD of 26.57 seconds. The average basal uterine tone was 178.9 mg, and the average force of the contractions was 997.1 mg with a range of 434.4 to 2,059.4 mg and a SD of 532.83 mg (Table 2). The anomalies that were found in the three experimental fetuses included cephalic compression and two deaths (Figure 2). In the three control fetuses, a limb deformity was observed. In the remaining fetuses, there was one fetus with exencephaly, eight fetuses with cephalic compression, two fetuses with clubfoot, eight limb deformities, and four deaths.

In group C (punctured at 17 days of gestation), there was an average of three contractions in 240 seconds. The average duration of the contraction wave was 51.6 seconds with a range of 20-84 seconds and a SD of 23.5 seconds. The average basal uterine tone was 192.4 mg, and the average force of the contractions was 1,936.5 mg with a range of 661.2 to 3,189.1 mg and a SD of 742.4 mg (Table 3). The anomalies that were found in the three experimental fetuses included limb and tail deformities and two deaths (Figure 3). In the three control fetuses, a deformity of the right hind limb was found. In the remaining fetuses, there were six fetuses with cephalic compression, six limb deformities, four fetuses with clubfoot, and one death.

In group D (punctured at 18 days of gestation), there was an average of three contractions in 240 seconds. The average duration of the contractions was 56 seconds, with a range of 24-132 seconds and a SD of 36.15 seconds. The average basal uterine tone was 261.1 mg, and the average force of the contractions was 2,035.4 mg with a range of 957.6 to 4,411.5 mg and a SD of 1,195.74 mg (Table 4). In the three experimental fetuses, the following abnormalities were found: one case of cephalic compression, one case of exencephaly, two forelimb deformities, three cases of clubfoot, and two tail deformities (Figure 4). In the three control fetuses, one case of cephalic compression was observed. In the remaining fetuses, there were seven fetuses with cephalic compression, four forelimb deformities, nine hind limb deformities, and eight tail deformities. An average of 0.3 ml of amniotic fluid was extracted from ten of the 12 punctured uteri.

Table 1. — *Time and force measurements of uterine contractions and the abnormalities in fetuses from uteri that were punctured at 15 days of gestation.*

*Duration of the contractions in seconds*

| Case | 1st contraction | 2nd contraction | 3rd contraction |
|---|---|---|---|
| 1 | 44 | 80 | 68 |
| 2 | 60 | 52 | 44 |
| 3 | 52 | 72 | 36 |
| Range | 36-80 | | |
| Mean | 56.4 | | |
| *SD | 14.62 | | |

*Force measurements of contractions (mg)*

| Case | Basal | 1st contraction | 2nd contraction | 3rd contraction |
|---|---|---|---|---|
| 1 | 271.13 | 351.07 | 427.13 | 357.97 |
| 2 | 255.15 | 547.30 | 387.61 | 378.90 |
| 3 | 257.69 | 309.32 | 806.86 | 909.28 |
| Basal mean | 261.32 | | | |
| Mean | 497.3 | | | |
| Range | 309.32–909.28 | | | |
| *SD | 216.52 | | | |

*Anomalies found*

| Case | Amount of amniotic liquid extracted (ml) | Experimental fetus | Control fetus | Other fetus |
|---|---|---|---|---|
| 1 | 0.7 | Abortion | Abortion | Abortion |
| 2 | 0.3 | Death | None | 1 death and 1 fetus with hind limb compression |
| 3 | 0.5 | Exencephaly, cleft palate, hind and forelimb compression | None | 1 fetus with facial area compression |

*SD = standard deviation.

Table 2. — *Time and force measurements of uterine contractions and the abnormalities in fetuses from uteri that were punctured at 16 days of gestation.*

*Duration of the contractions in seconds*

| Case | 1st contraction | 2nd contraction | 3rd contraction |
|---|---|---|---|
| 1 | 80 | 120 | – |
| 2 | 52 | 44 | 56 |
| 3 | 68 | 38 | 48 |
| Range | 38-120 | | |
| Mean | 63.2 | | |
| *SD | 26.57 | | |

*Force measurements of contractions (mg)*

| Case | Basal | 1st contraction | 2nd contraction | 3rd contraction |
|---|---|---|---|---|
| 1 | 141.74 | 990.76 | 1445.37 | – |
| 2 | 222.38 | 841.22 | 807.80 | 2059.43 |
| 3 | 172.75 | 471.54 | 434.44 | 926.76 |
| Basal mean | 178.95 | | | |
| Mean | 997.16 | | | |
| Range | 434.44–2059.43 | | | |
| *SD | 532.83 | | | |

*Anomalies found*

| Case | Amount of amniotic liquid extracted (ml) | Experimental fetus | Control fetus | Other fetus |
|---|---|---|---|---|
| 1 | 0.4 | Cephalic compression | None | 4 fetuses with cephalic compression, 1 fetus with exencephaly, 2 fetuses with forelimb compression, 2 fetuses with a clubfoot, and 2 fetuses with a hind limb deformity |
| 2 | 0.2 | Death | None | 1 death, 2 fetuses with cephalic compression, and 2 fetuses with a hind limb deformity |
| 3 | 0.4 | Death | Deformity of the left hind limb | 3 deaths, 2 fetuses with cephalic compression, and 2 fetuses with forelimb deformity |

*SD = standard deviation.

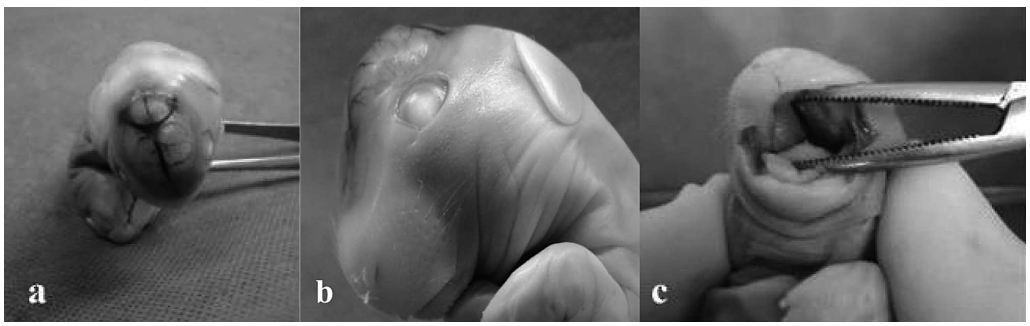


Figure 1. — An experimental fetus from group A (at 15 days of gestation): (a) exencephaly, (b) an absence of eyelids, and (c) a cleft palate.

Table 3. — *Time and force measurements of uterine contractions and the abnormalities in fetuses from uteri that were punctured at 17 days of gestation.*

*Duration of the contractions in seconds*

| Case | 1st contraction | 2nd contraction | 3rd contraction | 4th contraction |
|---|---|---|---|---|
| 1 | 84 | 64 | 52 | – |
| 2 | 32 | 32 | 20 | 80 |
| 3 | 56 | 72 | 24 | – |
| Range | 20–84 | | | |
| Mean | 51.6 | | | |
| *SD | 23.5 | | | |

*Force measurements of contractions (mg)*

| Case | Basal | 1st contraction | 2nd contraction | 3rd contraction | 4th contraction |
|---|---|---|---|---|---|
| 1 | 176.94 | 3189.18 | 2470.25 | 2146.85 | – |
| 2 | 185.73 | 895.29 | 1719.25 | 1870.27 | 2394.81 |
| 3 | 214.69 | 180712 | 2208.30 | 661.21 | – |
| Basal mean | 192.45 | | | | |
| Mean | 1936.25 | | | | |
| Range | 661.21–3189.18 | | | | |
| *SD | 742.44 | | | | |

*Anomalies found*

| Case | Amount of amniotic liquid extracted (ml) | Experimental fetus | Control fetus | Other fetus |
|---|---|---|---|---|
| 1 | 0 | Deformity of the forelimb, hind limb, and tail | None | 3 fetuses with cephalic compression, 3 fetuses with a forelimb deformity, and 2 fetuses with a bilateral clubfoot |
| 2 | 0.5 | Death | Deformity of a hind limb | 1 fetus with cephalic compression, 1 fetus with compression of the left forelimb, and 1 fetus with a clubfoot |
| 3 | 0.3 | Death | None | 1 death, 2 fetuses with cephalic compression, 2 fetuses with a deformity of the right forelimb, and 1 fetus with a bilateral clubfoot |

*SD = standard deviation.

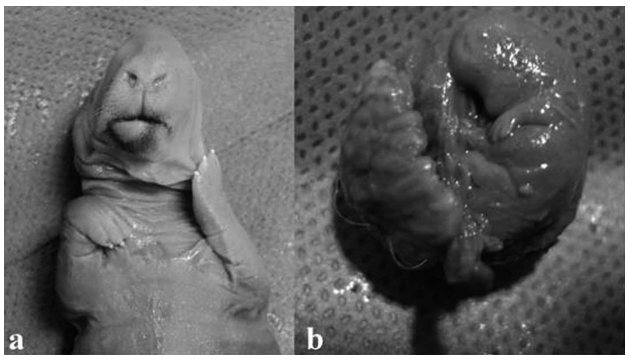


Figure 2. — Experimental fetuses from group B: (a) cephalic compression and (b) fetal death.

Table 4. — *Time and force measurements of uterine contractions and the abnormalities in fetuses from uteri that were punctured at 18 days of gestation.*

*Duration of the contractions in seconds*

| Case | 1st contraction | 2nd contraction | 3rd contraction |
|---|---|---|---|
| 1 | 132 | 36 | 24 |
| 2 | 76 | 72 | – |
| 3 | 40 | 40 | 28 |
| Range | 24–132 | | |
| Mean | 56 | | |
| *SD | 36.15 | | |

*Force measurements of contractions (mg)*

| Case | Basal | 1st contraction | 2nd contraction | 3rd contraction |
|---|---|---|---|---|
| 1 | 271.13 | 4411.51 | 1192.92 | 1232.21 |
| 2 | 255.15 | 1795.97 | 3251.50 | – |
| 3 | 257.69 | 1484.06 | 1957.88 | 957.64 |
| Basal mean | 261.32 | | | |
| Mean | 2035.46 | | | |
| Range | 957.64–4411.51 | | | |
| *SD | 1195.74 | | | |

*Anomalies found*

| Case | Amount of amniotic liquid extracted (ml) | Experimental fetus | Control fetus | Other fetus |
|---|---|---|---|---|
| 1 | 0.1 | Cephalic compression, bilateral clubfoot, and tail deformity | None | 5 fetuses with cephalic compression, 2 fetuses with a forelimb deformity, 4 fetuses with a bilateral clubfoot, and 3 fetuses with a tail deformity |
| 2 | 0.3 | | None | 1 death, 1 fetus with cephalic compression, 2 fetuses with a deformity of the right forelimb, 2 fetuses with a bilateral clubfoot, and 3 fetuses with a tail deformity |
| 3 | 0.0 | | Cephalic compression | 1 death, 1 fetus with cephalic compression, 3 fetuses with a clubfoot, and 2 fetuses with a tail deformity |

*SD = standard deviation.

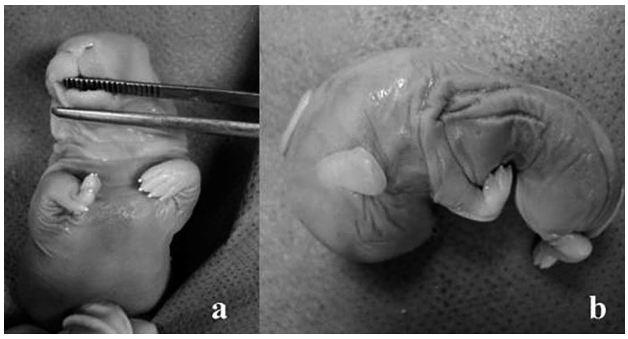


Figure 3. — Fetuses from group C: (a) front view of a forelimb deformity and (b) side view of a forelimb deformity.

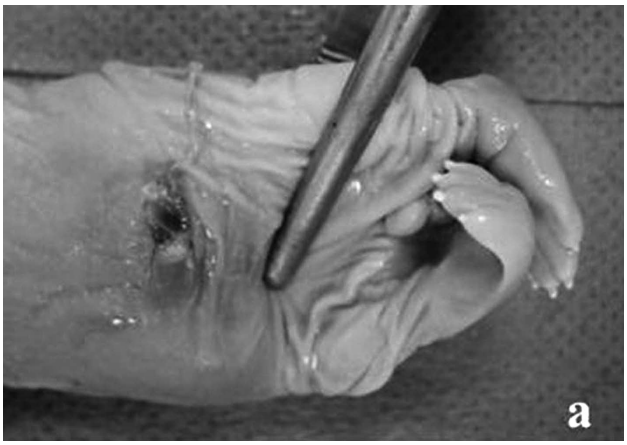


Figure 4. — Experimental fetuses from group D: (a) a hind limb deformity.

## Discussion

To date, the pathogenesis of ABS is unclear. Several studies have associated ABS with a history of maternal trauma, oophorectomy during pregnancy, the use of intrauterine devices, and invasive procedures, such as amniocentesis. Amniotic bands do not increase maternal risk during pregnancy; however, these bands generally cause premature labor [10] and can result in numerous irreversible and severely disfiguring fetal injuries that are incompatible with life [11, 12]. However, no study has demonstrated that uterine contractions can cause ABS anomalies.

In the present study, the authors found that after puncturing the wall and amniotic membranes of rabbit uteri using a fine needle and without injuring the fetus, uterine contractions immediately occurred and lasted between 20 and 132 seconds. These contractions generated changes in the fetal environment, which resulted in a variety of abnormalities, such as exencephaly and limb deformities. The contraction force increased from a baseline average of 189.6 mg to at least 309.32 mg (1.6-fold increase) and up to a maximum of 4,411.51 mg (22-fold increase).

There was an association between the increase in the force of the contractions and the day of gestation, which began with an average force of 497.3 mg at 15 days of gestation and increased up to 2,035.46 mg of force at 18 days of gestation. This finding indicates that the strength of the uterine contractions increased with the day of gestation.

All the experimental fetuses had an anomaly, such as exencephaly, a cleft palate, an absence of eyelids, cephalic compression, a clubfoot, a limb deformity, death, and abortion. Previous data indicated that there is a direct relationship between the contractions and the anomalies that are found.

As discussed by Kino [7], uterine contractions can cause decreased blood flow from the placenta to the embryo, which results in a type of injury that depends on the gestational age. The present authors hypothesize that the different anomalies that we found were associated with the following three factors: the force and the duration of the contractions, the day of gestation, and the loss of amniotic fluid. On days 15 and 16 of gestation, the injuries were more evident, such as exencephaly, an absence of eyelids, a cleft palate, death, and abortion. On days 17 and 18, the authors found skull, limb, and tail compressions and cases of clubfoot.

Nakayama *et al.* observed that uterine and fetal surgery procedures on rhesus monkeys between 123-152 days of gestation, such as amniocentesis, maternal laparotomy without uterine manipulation, and hysterectomy with and without fetal surgery, induced patterns of electromyographic activity (EMG) and that the uterine contractions were similar to the contractions that are observed during preterm delivery. A characteristic of this response is bursts in EMG activity, called type I [13].

The study was based on the study of Kino [7] that mentions that uterine contractions, decrease blood flow from the placenta to the embryo, which causes lesions in the extremities, but he only made histopathological studies, and the present study measured electro-graphically uterine contractions. If it intends that uterine contractions may be an important factor in the presence of SBA, it is independent of the theories already described for its origin as the endogenous and exogenous.

In the ABS: these bands occur when uterine contractions break the amnion forming amniotic bands that strangle extremity or in case of being swallowed by the fetus caused cleft palate. Amniotic bands do not necessarily have to exist to cause anomalies; uterine contractions can not break the amnion and compression of tissues of the fetus can lead to another series of severe anomalies.

According to the above described, there can or cannot be the loss of the amniotic liquid, and when there are contractions lesions may be more severe. Rather, these have been described in the context of the amnion ruptura sequence (ARS) in previable fetuses, as reported by Kalousek and Bamforth [14].

Other questions should be answered in future studies, such as whether uterine contractions cause a decrease in blood flow from the placenta to the fetus and whether substances or mediators are released before the stimulus that causes uterine contractions.

The results of this study may explain anomalies that occur in children conditioned by some external stimulus caused to the mother during the first and second trimester of pregnancy in this study caused by uterine contractions. Which would lead us to consider preventive measures.

## Conclusions

The present authors identified mechanisms that are involved in the anomalies present in ABS and differ from the mechanisms of the previously proposed theories (endogenous and exogenous). Uterine contractions were the main factor in these mechanisms.

The results of this study could explain the anomalies caused by external agents during second trimester of gestation; those agents would begin the uterine contractions.

Other questions should be answered in future studies, such as whether uterine contractions cause a decrease in blood flow from the placenta to the fetus and whether substances or mediators are released before the stimulus that causes uterine contractions.

## References

[1] Gilbert-Barnes E.: "Potter's pathology of the fetus, infant and child". 2nd ed. Philadelphia: Mosby-Elsevier, 2007.

[2] Pons G.A, Saez A.R, Sepulveda L.W.: "Amniotic band, intrauterine synechiae, and mullerian septum: etiopathogenic, differential diagnosis and prognosis". *Rev. Chil. Ultrasonog*., 2005, *8*, 51.

[3] Pardini A.G. Jr,, Dos Santos M.A, Freitas A.D.: "Congenital constriction bands". *Acta Ortop. Bras.,* 2001, *19*, 3.

[4] Streeter G.L.: "Focal deficiency in fetal tissues and their relation to intrauterine amputation". *Contrib. Embryol.,* 1930, *33*, 41.

[5] Rivas L.R, Juarez A.A, Islas D.L, Duran M.A, Oviedo R.I.: "Amniotic bands syndrome associated with a Potter sequence. A case of autopsy". *Rev. Mex. Pediatr.,* 2005, *72*, 78.

[6] Poswillo D.: "Observations of fetal posture and caudal mechanisms of congenital deformity of palate, mandible, and limbs". *J. Dent. Res.,* 1966, *45*, 584.

[7] Kino Y.: "Clinical and experimental studies of the congenital constriction band syndrome, with an emphasis on its etiology". *J. Bone Joint Surg. Am.,* 1975, *57*, 636.

[8] Galvan A., Alvarez E., Parraguirre S., Suarez M.L., Perez A.: "Development of a rabbit fetal model for the study of the Amniotic Band Syndrome". *Fetal. Pediatr. Pathol.,* 2012, *31*, 300.

[9] Mexican Official Norm: "NOM-062-ZOO-1999". Available at: http://www.fmvz.unam.mx/fmvz/principal/archivos/062ZOO.PDF

[10] De La Cabada C., Naranjo H., Duque L.: "Prenatal constriction bands, a case and literature review (Amniotic band syndrome)". *Dermatol. Venez.,* 1992, *30*, 121.

[11] Schloo R., Miny P., Holzgreve W., Hors J., Lenz W.: "Distal limb deficiency following chorionic villus sampling?" *Am. J. Med. Genet.*, 1992, *42*, 404.

[12] Capdevila R.L., Nualart H.L.: "Circumferential amniotic bands. Liberation in surgical time". *Rev. Mex. Ortop. Traum.,* 1999, *13*, 60.

[13] Nakayama D., Harrison M., Seron-Ferre M., Villa R.: "Fetal Surgery in the primate II. Uterine electromyographic response to operative procedures and pharmacologic agents". *J. Pediatr. Surg.,* 1984, *19*, 333.

[14] Kalousek D.K., Bamforth S.: "Amnion rupture sequence in previable fetuses". *Am. J. Med. Genet.*, 1988, *31*, 63.

Address reprint requests to:
G. MONTAÑO ALFONSO, M.D.
General Hospital "Manuel Gea González"
Calzada de Tlalpan 4800, Colonia Sección XVI.
Del. Tlalpan, Distrito Federal (Mexico)
e-mail: gamagq3@hotmail.com