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Full Length Research Paper

Incidental finding of *Mycoplasmas* in developmental dysplasia of the hip and hip dislocation

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A non-controlled prospective trial was carried out to investigate ultrastructure of acetabular roof and joint capsule by electron microscopy in dislocated developmental dysplasia of the hip (DDC). Nineteen randomly selected babies aged 18 months to 8 years and ten months, were operated on by a medial approach for open hip reduction between January and July 2006, obtaining biopsies at surgery. No healthy hips were opened just for harvesting biopsies. Surprisingly *Mycoplasma* corpuscles were found in 15 out of the 19 patients (78%, p = 0.019 binominal distribution test). Corpuscles appeared in 3 cases in roof and capsule, in 5 only in cartilage and in 7 only in capsule. Four out of 5 children aged 18 - 22 months (80%) had *Mycoplasma* in cartilage, as well as in 4 out of those 14 (28.6%) older than 22 months. Three out of 5 under 22 months (60%) had *Mycoplasma* in joint capsule: 40% in cartilage and capsule and 20% only in capsule. In those greater than 22 months only 7.2% had *Mycoplasma* at both levels and 42.8% only in the joint capsule. *Mycoplasma* had a tendency to decrease as patients grew-up in age. It is proposed that *Mycoplasma* in acetabulum could contribute to impair its full ossification in DDC since it would compete for cartilage nutrients, thus producing deterioration of chondral cells and matrix, and a difficult acetabular physis advancement inside the chondral acetabulum. Also a decreased capsule tensile strength could result in slacking capsule and joint dislocation.

Word keys: Congenital, developmental, hip dysplasia, mycoplasma.

INTRODUCTION

There are many predisposing factors traditionally related to developmental dysplasia of the hip (DDH) and its dislocation, such as the female gender, the caucasic population, a family trend, oligohidramnios, the left hip and the breech presentation at delivery, but the true etiology for the dysplasia still seems to deserve more investigation. Frequency of DDH in Mexico has been reported in 1 to 2/1000 newborns (Beltrán, 1968; Chávez-Rojas, 1969; Fox, 1972).

The embrionary development of the hip takes place at four to six weeks of pregnancy as a mesenchimal condensation, which evolves to a chondral mold that will gap at the interzone in the future articular space. Hip dysplasia consists of an insufficient ossification of the

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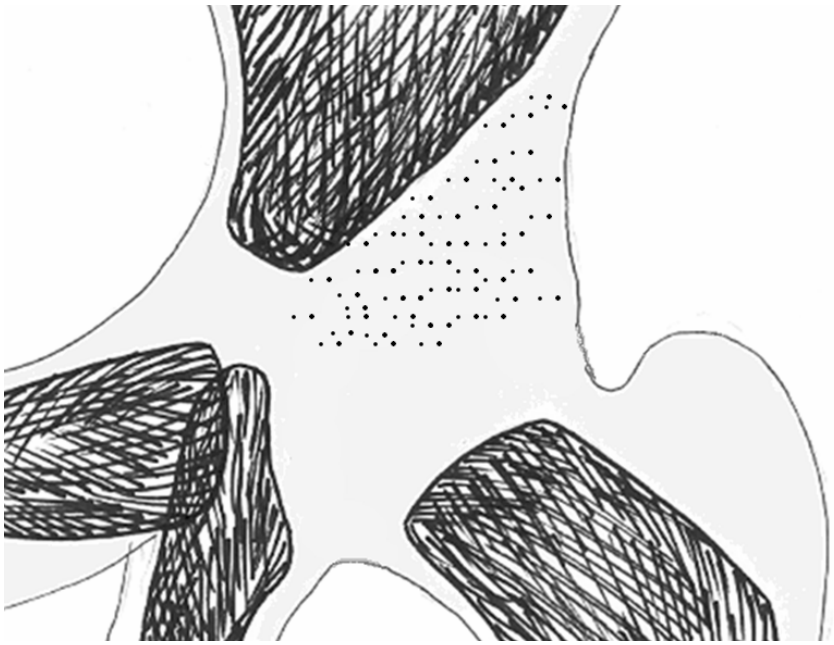

Figure 1. Drawing that pretends to explain the conception of hip dysplasia before the interzone. Points are trying to illustrate the presence of Mycoplasmas in the chondral acetabular mold. Microorganisms could be competing for cartilage nutrients and preventing the appropriate advancement downwards of the ossification nucleus of the iliac bone.

acetabular roof. However, the roof is always well conformed as a chondral mold of the acetabular cavity once the interzone appears. Acetabular dysplasia is not obligated to coexist with hip dislocation. When dislocation occurs, it could be due to a slackening of the joint capsule, which must happen far later after the joint space has taken place. (Figure 1)

Such a point of view, should allow us to enhance a rationale that roof dysplasia and hip dislocation could formally be different anatomical lesions. In our records, some 35% of dysplastic hips (160 out of 458 cases) are dysplastic but non-dislocated.

This paper illustrates the incidental findings that have been obtained in an attempt to know something more about the ultrastructure of the dysplastic acetabular roof and about the nature of the joint capsule. We reviewed a short series of patients by transmission electron microscopy (EM) of the embryonic acetabular cartilage and of the redundant capsule of the dislocated hip, through biopsies taken at the time of the reduction surgery.

PATIENTS AND METHODS

Our protocol for treatment of children who have DDH and dislocation involves conservative procedures such as the Pavlik harness or diverse abduction devices in younger babies, reserving the reduction surgery for those greater than 18 months of age, in whom the risk of postoperative necrosis seems to be lower (Mankey et al., 1993; Weiner et al., 1977).

The present paper involved 19 children with 22 dislocated hips, one male and 18 female. Age at the time of surgery ranged from one year and 6 months through 8 years and 10 months. Patients were randomly selected and operated on between February and July 2006. There were 12 left hips and 10 right (Table 1).

Three cases out of the 19 (Nos. 1, 6 and 9) had already been operated on elsewhere and they were admitted to our institution for revision surgery because of postoperative re-dislocated hips. Operative procedure in all patients was through medial approach (Ferguson 1973; Ludloff, 1913). Since obtaining tissue for examination means an invasive procedure, samples were taken at the time of the hip reduction surgery (Figure 2). No other case, either sick or healthy, was operated on just for taking tissue samples.

The operative technique included, in 2 cases, a femoral varus-derotational osteotomy in the same operating time in order to improve centering of the femoral head into the acetabulum as well as to improve postoperative hip stability.

A square piece of 4 × 4 and 2 mm deep of cartilage was obtained from the anterior aspect of the iliac bone, above the anterior rim of the acetabular roof in an area where the dysplastic acetabulum is still not ossified. A similar size sample from the joint capsule was taken from its lateral flap after it had been incised for opening and cleansing the joint cavity prior to the hip reduction. Biopsies were examined at the Electron Microscope Philips Tecnai-10 of our institution.

Preoperative radiological imaging for assessing acetabular dysplasia and hip dislocation as well as for evaluating the postoperative hip reduction was performed by the method of the acetabular index and by the concentrical centering of the hip (Fernández, 1978; Muñoz, 1999).

After surgery, patients were immobilized in the so-called human position for 3 months and afterwards in the so-called second

Table 1. Cases of DDH and CDH studied by biopsies, electron microscopy and culture For Mycoplasma

Number*	Gender	Age at surgery years * months	Bone acetabular index in degrees (right/left)	Cartilage acetabular index in degrees ** (arthrogram)	Preoperative centering of the hip *** in millimeters	Result of biopsy for Mycoplasma		Tissue available for late culture	Late culture
						Cartilage	Capsule		
1	Female	1 + 6	35 / 30	10 / 12	+8 / +22	+	-----	Yes	
2	Male	1 + 6	14 / 32	----- / 14	+2 / +13	+	+	Yes	
3	Female	1 + 7	45 / 15	12 / -----	+24 / -3	-	+		
4	Female	1 + 9	14 / 30	----- / 10	+2 / +30	+	-	Yes	
5	Female	1 + 10	32 / 45	----- / 10	+3 / +18	+	+	Yes	
6	Female	2 + 0	42 / 18	18 / -----	+22 / 0	-	-		
7	Female	2 + 1	18 / 36	----- / 0	0 / +26	-	+		
8	Female	2 + 6	38 / 18	15 / -----	+28 / +2	-	+		
9	Female	3 + 6	38 / 29	5 / 12	+18 / +9	-	+	Yes	
10	Female	3 + 8	20 / 35	----- / 20	0 / +17	-	-	Yes	Positive
11	Female	4 + 0	14 / 34	----- / 0	+1 / +20	+	-		
12	Female	4 + 2	44 / 40	14 / 0	+24 / +28	-	+	Yes	
13	Female	4 + 6	40 / 20	6 / -----	+26 / 0	-	-	Yes	
14	Female	5 + 4	48 / 48	30 / 15	+25 / +25	+	-	Yes	
15	Female	5 + 4	20 / 36	----- / 0	+3 / +25	-	+	Yes	
16	Female	5 + 6	46 / 22	0 / -----	+45 / -2	-	-	Yes	
17	Female	6 + 8	40 / 40	26 / 25	+46 / +46	+	+	Yes	Positive
18	Female	7 + 0	38 / 12	22 / -----	+41 / +4	+	-		
19	Female	8 + 10	8 / 37	----- / 18	+1 / +33	-----	+		

* Progressive number was fixed according to age at the time of surgery
** Cartilage acetabular index was transoperatively measured by arthrogram, only in hips to be operated on.
*** Centering of the hip was measured in millimeters as by the Fernández method in which $\pm$ 3 mm above or below to the 45 degrees line from the center of the acetabulum is normal, +4 to +10 degrees means subluxated hip and +11 or higher means dislocated hip. [4, 11]
----- means that no measure was taken as no arthrogram was performed in normal or non-operated on hips, or no tissue was taken for biopsy.

position for 3 more months with hips in extension and mild abduction. The 2 patients in whom a femoral osteotomy was carried-out were only immobilized in the second position for 4 months. A statistical binominal distribution test was applied, with a minimal expectancy of $p = 0.5$ for good probability, but ideally $p \leq 0.05$ for more reliable results.

RESULTS

A decrease in density of the cartilaginous stroma was found inside the dysplastic acetabular roof. Chondrocytes appeared with degenerative changes such as big vacuoles in the cytoplasm. However, nucleus seemed to be viable. The joint capsule demonstrated also a decrease in the amount of collagen fibers.

More surprising was the finding of corpuscles of Mycoplasma, which was present in 15 out of the 19 patients (78%, $p = 0.019$ by a binominal distribution test). For global results, corpuscles were present in cartilage and joint capsule in 3 cases, cartilage in 5 and capsule in 7 cases.

Corpuscles found in our cases were round-shaped and from 50 - 240 nm in diameter as described in literature for most Mycoplasmas (Waites et al., 2005), although, some times they can be pleomorphic thus addressing their great capacity of adaptation to a diversity of tissue environments. Pleomorphic mollicutes were found only in case 17, a girl aged 6 years and 8 months.

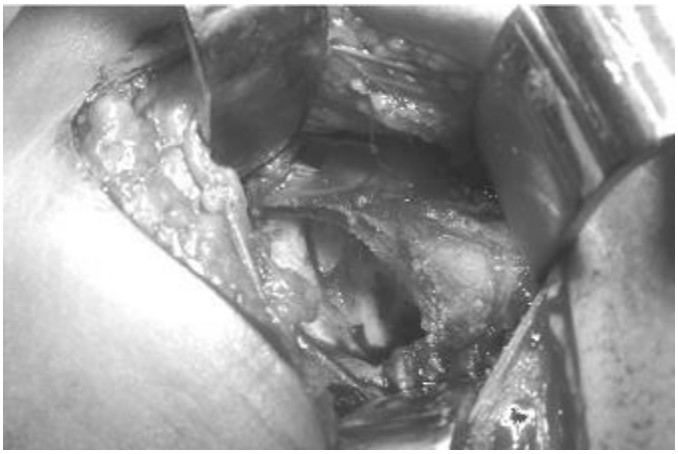

Figure 2. Trans-operative print of a hip (Case No. 6) in which the acetabular cavity has been debrided and cleansed. A small square above the acetabular rim show the area where sample of cartilage from the anterior wall of the acetabulum has been taken.

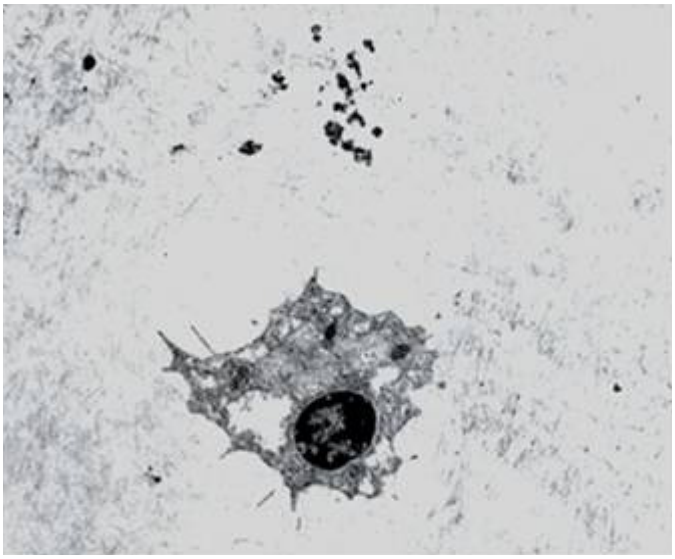

Figure 3. EM images of the roof cartilage in a girl of one year and 6 months (case 1). A. At 3,700×, they appear in the upper section corpuscles of *Mycoplasma* with a tendency to agglomerate. The cartilage tissue is scarce. In the lower section a degenerative chondrocyte can be observed with vacuolated cytoplasm.

According to age groups, there were some differences. In 4 out of the 5 children from 18 - 22 months of age for (80 %) *Mycoplasma* was present in cartilage (Figures 3 and 4), compared to those 15 girls greater than 22 months in whom it was present in the cartilage in 4 cases (28.6 %). *Mycoplasma* was present in the joint capsule of 3 out of the 5 children under 22 months (60%), in cartilage and capsule in 2 (40%) and one case only in capsule. In those 14 cases above 22 months, only one had *Mycoplasma* at both levels and 6 (42.8%) only in capsule.

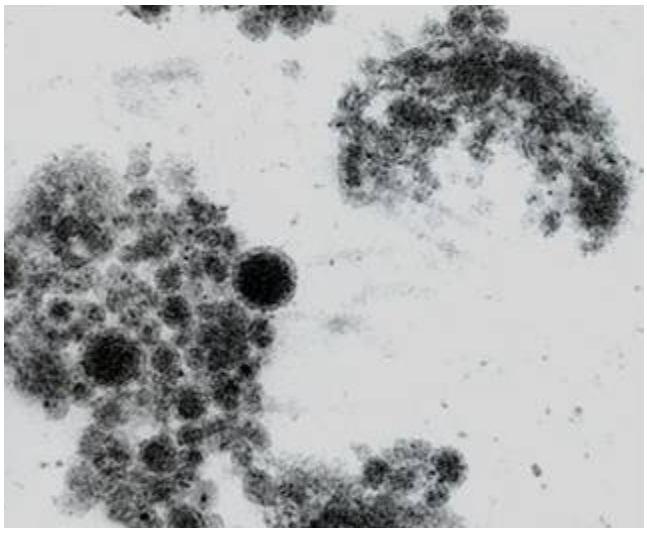

Figure 4. This is the case of figure 3 at 65,000×. The upper section of the previous image has been enlarged, where the corpuscles of *Mycoplasma* can be better seen.

When *Mycoplasma* was identified in the joint capsule, it was observed that, there was a decrease in the amount of collagen fibers, which was more notorious particularly in those areas surrounding the packs of *Mycoplasma* corpuscles (Figures 5 and 6).

In relationship to transoperative arthrograms for determining the true level of chondral acetabular index (not roof dysplasia) was significantly higher in those cases with *Mycoplasma* in cartilage with a chondral acetabular index of 22.0° ± 8.6° against 10.0° ± 6.4° in negative cases ($p = 0.02$). In cases with *Mycoplasma* in capsule arthrogram for chondral roof was the same with 14.3° for positive and negative cases.

Concentrical centering of the hip is considered to be normal if the center of the femoral head is ± 3 mm above or below the 45° axis line from the center of the acetabulum. Figures for dislocated hips in this series were found at an average of +26 mm of lateral displacement of the femoral heed in relationship to the central 45° axis of the acetabulum in positive cases with *Mycoplasma* in capsule and +27.5 mm in negative cases to *Mycoplasma* in capsule which means no significant difference. Otherwise, in cases under 22 months of age centering figures were at an average of +21.4 mm of lateral displacement of the femoral head and +28.5 mm in those cases above 22 months, $p = 0.5$ as a result of a student-t test, which means a slight difference related to age but not statistically significant. All those findings related to the presence of *Mycoplasma* in acetabulum as well as in the hip joint capsule, which were obtained incidentally.

After 3 years, tissue samples that had been stored in laboratory (only in 12 patients) were late cultured in search for *Mycoplasma*. Results were weak positive only

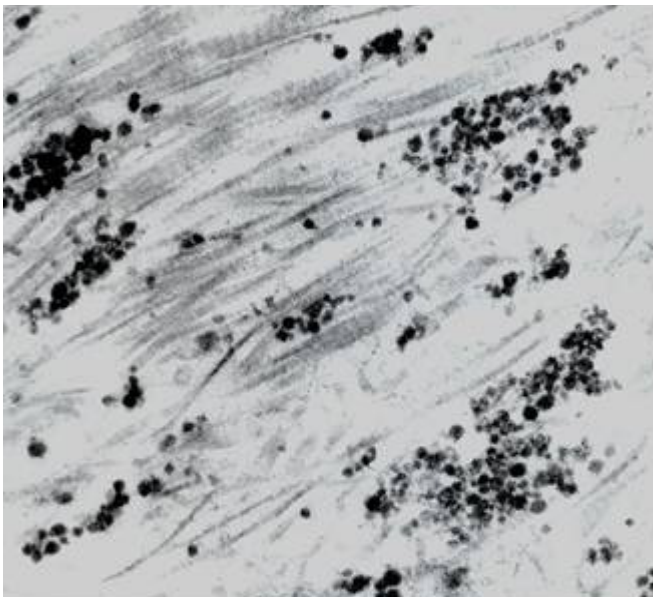

Figure 5. Transmission EM from the capsule of a girl aged 8 years and 10 months (Case No. 19). At 24,000×, there is an abundant amount of corpuscles compatible with *Mycoplasma* in the middle of the capsular tissue, where a certain decreased amount of collagen fibers can be seen.

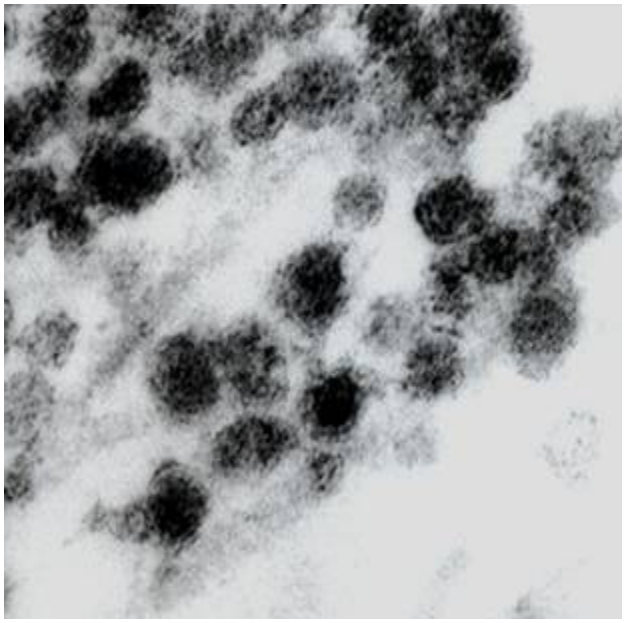

Figure 6. Enlarged area from Figure 4. At 93,000 x, several conglomerates of *Mycoplasma* are seen in the same patient of the previous image. Collagen fibers of the joint capsule are rather scarce.

in 2 cases, one in whom EM had been negative and one in whom EM had been intensely positive for both, cartilage and capsule. Cultures were intended to obtain a significant amount of biological material for polymerase

Figure 7. This is the culture in Agar-Eaton from the cartilage of Patient No. 10, a girl of 3 years and 8 months of age. Arrows are pointing out the growth of *Mycoplasma* colonies at 40×.

Figure 8. The culture of cartilage in Agar-Eaton of Patient no 17, a girl of 6 years and 8 months of age. Arrows as well are demonstrating colonies of *Mycoplasma* at 40×.

chain reaction (PCR), however, it was not possible to identify microorganisms in view of the weakness of cultures. Any case, cultures were macroscopically characteristic for *Mycoplasma*, with their typical aspect of the so-called fried egg. (Figures 7 and 8)

DISCUSSION

Mycoplasmas, also called mollicutes, are the smallest living organisms, they lack cell membrane and it has been described that they can be saprophytic (Hayflick et al., 1965). There are sometimes more than 10 species of

Mycoplasma and *Ureaplasma* of human interest as a result of diverse findings in human beings (Rivera-Tapia, et al., 2006). Mollicutes do not necessarily cause typical purulent infections, but otherwise can produce atypical infections such as pneumonia in newborns (Waites, 2005).

Mycoplasmas can be isolated from female genital tract (Waites et al., 2005) and can be transmitted to the fetus in 3 main ways, first is by contiguity thus infecting the amnios. In those cases fetus can aspirate infected amniotic fluid and may undergo atypical pneumonia of the newborn. The second way of transmission can be vascular. In such cases, mollicutes should come into the fetal blood stream. The final way is by contact with body of the newborn at the moment of delivery, with possible cases of dermatitis.

The vascular pattern of transmission could be the one interesting in humans for the particular case of the hip dysplasia and dislocation. In those cases, mollicutes in the blood stream could be seeded into the iliac cartilaginous acetabular mass in the embryo, as well as in the joint capsule.

Blood vessels for each structure are different. Iliac bone is irrigated by collateral branches of the iliac intern or hypogastric artery (iliolumbar and gluteal branches), one inside and one outside at the same level of the iliac bone wing. Those arteries will arise the ossification center of the iliac wing, which involves a physis in its lower part that will normally ossify the acetabular roof through the first half of pregnancy (Redon, 2006).

The emergencies of blood vessels for the iliac bone and the umbilical artery is very close to each other. So a true blood pumping mechanism is occurring to the iliac bone as in the embryo, that is, an almost terminal blood circulation. It could be an easy way to seed *Mycoplasmas* from the blood stream into the iliac bone.

Competing for nutrients inside the cartilage has been described in animals (Thorp, 2008) as Turkey 65 syndrome which consists of chondrodystrophy produced by either *Mycoplasma gallisepticum or Mycoplasma iowae*. As well, destructive polyarthropathy possibly related to *Ureaplasma diversum* has been reported in aborted bovine fetuses (Himsworth et al., 2010).

On the other hand, circumflex arteries for the joint capsule are given from the deep femoral artery. So if a seeding of mollicutes is to happen at the hip joint capsule, this is going to be through a different vascular way and necessarily at a latter moment after the acetabular roof has been involved, since the joint capsule will appear after the interzone has formed in the future place of the articular space. The interzone appears as a result of the action of temporary molecules (Pacifici, 2008) such as the component Gdf5 of the bone morphogenetic protein (BMP), other polypeptides, the β-factor transforming of growth, the family of Wnt proteins, one more protein related to the parathyroid hormone and 2 transcription factors.

Hip evolves on its formation as a mesenchimal condensation, later on as a condral mold of a ball and socket and finally the joint. There is no possibility of evolving on separate mesenchimal condensations in the embryo. So the hip dislocation will necessarily occur after its formation, realizing any abnormality inside the capsular tissue that slacken the capsule and allows dislocation.

This rationale should allow us to elucubrate about the existence of acetabular dysplasia and hip dislocation as formally separate anatomical lesions. An argument in favor of this rationale should be the number of cases who have acetabular roof dysplasia but no hip dislocation as it happens in about 35% of our series, as outlined in the third paragraph.

After birth, why the acetabulum re-shapes once the hip has been reduced in spite of the potential damage produced by Mycoplasma? It may be because of the traditional Darwinian conception of remodelation as a result of function. And what could be the role of *Mycoplasma* in the dysplastic acetabular roof in spite or remodeling? There are only speculating responses to such a question, that could be the non-purulent atypical infection produced by *Mycoplasma* and only mild silent tissue impairment. The smaller the child, the better ossification and remodeling of acetabulum as a result of the minimum damage produced in the chondral stroma by mollicutes at a younger age.

On the other hand, in something more than a 30% of cases, acetabular dysplasia is persistent through the children's growth in spite of a concentrical centering of the hip (Mankey et al., 1993). That could be the rate of severity of acetabular involvement by *Mycoplasma*.

Why the hip? By the same reason, the hip is the most frequently affected joint by septic arthritis in the newborn. The iliac intern artery which will irrigate the iliac bone, has its origin at a very near point to the emergency of the umbilical artery, so that some hydrodynamic turbulence seems to be produced at such a point, giving a true pumping mechanism into the iliac bone. This is an area of high blood consumption and an almost terminal circulation in embryo, whose lower limbs are at the starting buds.

Multiple publications have reported the presence of mollicutes in joints of patients who have rheumatoid arthritis (Ramírez et al., 2005) as well as after prosthetic joint replacements (Lee et al., 2009) but no publications have been found for DDH and Mycoplasma.

Findings as reported in the present paper, which have been obtained without being expected are called 'serendipia'. That is the ending point of this first series.

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