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Maturity of the Myenteric Plexus Is Decreased in the Gastroschisis Rat Model

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Gastroschisis · Myenteric plexus · Gut development · Neurofilaments

Abstract
Background: Amniotic fluid (AF) and its components, such as fetal urine and meconium, may lead to intestinal alterations in gastroschisis, which cause immaturity of the myenteric plexus and consequent intestinal hypomotility and malabsorption. In this study we identified morphological and histological alterations of the intestine and the myenteric plexus with two different times of exposure to AF. ***Methods:*** The experimental gastroschisis was achieved at two different gestational ages, on day 18.5 (E18.5) and day 19.5 (E19.5) of gestation, in fetal rats which were divided into 3 subgroups: control, sham and gastroschisis. We measured fetal body weight (BW), intestinal weight (IW) and intestinal length (IL). The layers of intestinal wall and myenteric plexus were evaluated by hematoxylin and eosin staining (HE staining) and immunofluorescence (α-internexin), respectively. ***Results:*** BW was not significantly different among the control, sham and gastroschisis groups at both ages. IW and IL were larger and shorter, respectively, in the gastroschisis fetuses ($p < 0.001$) at both ages. Intestinal diameters and wall layers presented significant differences among control, sham and gastroschisis fetuses at both ages ($p < 0.001$), but the time of exposure to AF compromised the serous membrane, D-II (diameter II, $p < 0.001$) and IL ($p = 0.001$). α-Internexin presented more intensive immunoreactivity in gastroschisis fetuses at E18.5. ***Conclusions:*** In gastroschisis, the longer the time of exposure to AF, the more severe bowel impairment will be, especially with regard to IL and the serous layer, and the more immature the myenteric plexus will be.

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Introduction

Gastroschisis is a congenital defect of the abdominal wall, characterized by a small abdominal wall defect (AWD) usually located to the right of the navel that allows herniation and permanent exposure of intestinal loops to amniotic fluid (AF) until the end of gestation [1].

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Experimental studies in which constriction of the vascular pedicle of the eviscerated bowel by a narrow AWD was analyzed to try to explain bowel thickening, edema and ischemic lesions have been made in chick embryo and lamb models, but their conclusions showed no relationship of intestinal changes with moderate or mild constriction at the site of the AWD [2]. These authors affirmed that there were no differences between constriction by an AWD 1.5 or 3 times the normal intestinal diameter. Despite chronic constriction of the bowel at the site of the AWD being an important contributing factor to the intestinal damage seen in gastroschisis, the effect of AF on the myenteric neurons (intrinsic nervous system) is present and could respond to transitory intestinal dysmotility in the postnatal period [2].

In gastroschisis, the degree of damage to the extruded bowel loops is directly related to the time of exposure to the AF [3] and to the meconium, causing morphological and histological alterations to the intestine such as shortening, increased weight, diameter and thickness of the intestinal wall [4, 5], in addition to the development of a fine fibrous coating over the serous layer that favors the development of adherences between the bowel loops [6, 7]. In gastroschisis such lesions will eventually lead to decreased intestinal peristalsis and deficiency in absorption of nutrients [8], contributing to increased morbidity and medical-hospital costs [9].

Intestinal hypoperistalsis in gastroschisis is attributed to the disorganization and immaturity of the myenteric plexus [10]. These characteristics may function as markers of the degree of bowel lesion expression and may be measured by the presence of neurofilaments along the intrinsic intestinal nervous plexus [11, 12]. Recently other authors have reported that the interstitial cells of Cajal serve as electrical pacemakers, providing pathways for the active propagation of slow waves, acting as mediators of enteric motor neurotransmission, and playing a role in afferent neural signaling; they also discuss the complexity of the physiology and histological identification of the interstitial cells of Cajal [13]. Despite the cell threshold potential for action generation being typically constant, the baseline membrane potential can be controlled by neurotransmitters, hormones, and pharmacologic agents, but slow-wave depolarization does not cause muscular contraction [14].

Based on that information we decided to use α-internexin to identify the maturity of the myenteric nervous system in order to obtain a direct measurement of the degree of damage to the extruded bowel loops, since the interstitial cells of Cajal are not the single factors responsible for intestinal motility [15]. In the present study, we tested the hypothesis that the time of exposure of bowel loops to AF may result in different degrees of intestinal wall lesion, which will likewise produce different degrees of neuronal immaturity. Therefore, the morphometric and histological alterations in rat bowels were studied and compared, emphasizing the maturity of the myenteric plexus in experimental gastroschisis of rat fetuses with two different times of exposure to AF.

Materials and Methods

This study was submitted to the Animal Experimentation Ethics Committee of the State University of Campinas (CEEA-UNICAMP) and approved as research project No. 281-1.

Sprague-Dawley female rats weighing between 250 and 300 g were placed in cages with male rats and the presence of a vaginal smear in the female was a sign that copulation had occurred; this was considered as day 0 of gestation (total gestation 22 days). The females received abundant food and were kept in a 12-hour day-night cycle. Ten pregnant rats were used in the study and divided equally into 2 groups, with 5 rats in group E18.5, composed of fetuses operated on day 18.5 of gestation, and the other 5 rats in group E19.5, composed of fetuses operated on day 19.5 of gestation.

The gastroschisis model used was that of Correia-Pinto et al. [5]. Pregnant rats were anesthetized on days 18.5 and 19.5 of gestation, respectively, with ketamine 50 mg/ml (175 mg/kg; Ketamina®, Pfizer do Brasil Ltda.) in association with xylazine 10 mg/ml (2.5 mg/kg; Rompum®, Bayer do Brasil Ltda.). Surgery was carried out using a microscope with ×2.5 magnification. The womb was exposed and a pouch wall suture with 6–0 Prolene® thread was always made on the 2nd, 3rd and 4th fetuses in each cornu, excluding the fetuses in the extremities due to the great discrepancy in size. After that the fetuses were partially externalized up to the navel level and experimental gastroschisis was established by means of a small opening to the right of the navel, allowing externalization of bowel loops. Before closure of the womb AF was reconstituted with 0.5 ml of 0.9% warmed saline solution in both groups, E18.5 and E19.5. Nothing was done with the control fetuses, and the sham fetuses had only the right lower limb externalized.

On day 21.5 of gestation, the rats were once again submitted to cesarean section to remove the control, sham and gastroschisis fetuses, which were weighed on high-precision scales (body weight, BW) with measures provided in grams. Their intestines were removed and also weighed (intestinal weight, IW), isolated from the mesentery and straightened to measure their length (intestinal length, IL) from the duodenal pylorus up to the rectum at the peritoneal reflection. In addition, the IW/BW and IW/IL ratios were also analyzed in the 3 subgroups of the 2 operation days, to verify the influence of the time of exposure to AF in the E18.5 and E19.5 groups.

The fetal intestine was divided into 4 segments from the duodenum to distal colon, each one having approximately 4 cm in length, for the histological study. The first 4-cm segment was tak-

en between the Treitz angle and proximal jejunum; the second came from the distal jejunum and proximal ileum; the third came from the ileum and proximal colon, and the fourth was taken from the colon. All segments had the proximal extremity marked with a 4–0 cotton suture and were embedded in sequence in the same paraffin block. To avoid possibilities of taking a far proximal or distal extremity of the intestines and analyzing portions that were not affected, we sliced the transverse median portion of the 4 segments in the paraffin block. Thereby we excluded the possibility of assaying a segment that was not exposed to AF. After embedding, 5-μm transverse sections of the intestine were stained using hematoxylin and eosin (HE). HE-stained cross-sections were photographed using a Nikon Eclipse E800 photomicroscope (Nikon, Tokyo, Japan; final magnification ×100 for intestinal diameter, ID, and ×200 for intestinal layers). The slides were digitalized and the ID (ID-I = mesenteric to anti-mesenteric border, and ID-II = perpendicular axis to ID-I), total intestinal wall, mucosa, circular muscular layer, longitudinal muscular layer and serous thicknesses were measured in 5 transversal sections using Image Pro Plus software (Epix, Buffalo Grove, Ill., USA). To avoid the possibility of taking very proximal or distal extremities of the intestines and analyzing portions that were not affected, we sliced the transverse median portion of the 4 segments in the paraffin block. Thereby we excluded the possibility of assaying a segment that was not exposed to AF. Measurements were taken in micrometers from the serosa to the villous sinus, at each of the 4 quadrants of each section, in radial orientation, totaling 20 measurements for each variable per animal from the 3 subgroups at gestational ages E18.5 and E19.5.

According to Faussoni-Pellegrini et al. [11], α-internexin is a neurofilament protein and one of those responsible for the cytoskeleton formation and is considered a marker of neuronal maturity. The immunoreactivity (IR) of the α-internexin is intense early in gestation but decreases with aging, and maintenance of a high IR of this protein in the postnatal period or adult life is a sign of neuronal immaturity [9]. The degree of maturity of the myenteric plexus (intrinsic neuronal system, localized between the mucosa and each layer of muscle) [16] was analyzed by the intensity of IR on immunomarking to α-internexin and was observed through a fluorescence microscope according to Vannucchi et al. [10].

The slices were deparaffinized and then incubated for 1 h in solution containing BSA (3%) and Tween-20 (0.1%) in a PBS (0.1 M) buffer to block unspecific sites. After that, the samples were incubated at 4°C for 12 h, with goat polyclonal primary antibody anti-internexin C-18 (sc-7570, Santa Cruz, Calif., USA, 1:100), diluted in PBS (0.1 M) containing 1% BSA and 0.3% Triton X-100 and incubated for 12 h. On the following day, the slides were rinsed in PBS 0.1 M (three 5-min washes) and incubated for 1 h at room temperature, with anti-goat secondary antibody produced in a donkey and conjugated with TRITC (1:100, diluted in PBS 0.1 M and 0.3% Triton X-100). The samples were then washed 3 times in PBS for 5 min and covered with a solution of 4′,6′-diamidine-2-phenylindol (0.25 μg/ml; Sigma) for 5 min in order to show cell nuclei. After washing in PBS for 5 min, the slides were then mounted in DABCO (protector of immunofluorescence) and glycerol. The immunomarking was observed through a fluorescence microscope (Nikon 50i, USA) connected to a digital camera (Nikon DMX 1200F, USA), with images acquired by means of the ATC-1 software.

Descriptive statistics of continuous variables were used to describe the sample profile according to the study variables, with mean, standard deviation, minimum, maximum and median values of bowel segment diameters and thickness of the 4 main layers: mucosa, circular muscular layer, longitudinal muscular layer and serosa. The variance analysis was used for repeated measures (Repeated Measures ANOVA) to compare the variables among the subgroups and other features, considering two main factors: the control, sham and gastroschisis subgroups and the 2 gestational days, E18.5 and E19.5. The comparison of the control, sham and gastroschisis subgroups as well as days E18.5 and E19.5 was made with the Tukey's post hoc test. In view of the absence of normal distribution of measures, the analyses were carried out after logarithmic transformation of data ($\log_{10}$). The level of significance adopted for the statistical tests was 5%, that is $p < 0.05$.

Results

Ten Sprague-Dawley female rats, with a total of 49 fetuses, were used in this study. After 5 fetal deaths (10%) 44 live fetuses (90%) remained. Of these 44 live fetuses, 23 were studied on the E18.5 and distributed into the 3 subgroups: 8 control, 7 sham, and 8 gastroschisis fetuses. Another 21 fetuses were studied on E19.5 and also distributed into 3 subgroups: 7 control, 7 sham and 7 gastroschisis fetuses.

Morphological Measures of the Fetuses

IW, IL, IW/BW and IW/IL presented significant differences when comparing the three subgroups control, sham and gastroschisis ($p < 0.001$) for the 2 days studied. The IL presented significant differences when the measures for both days were compared ($p < 0.001$), that is IL on E18.5 was shorter than IL on E19.5. There were no significant differences among the BW measures when the fetuses of the 3 subgroups were compared for the 2 days studied ($p = 0.793$). In the comparative analysis of IW/IL measures in the subgroups and on the days studied, there was no significant effect of interaction among subgroups and days ($p = 0.347$), except for the ratio IW/BW ($p = 0.035$). The results of morphological measures are shown in table 1.

Intestinal Histomorphometric Measures

The total thickness of the intestinal wall presented significant differences only when the 3 subgroups in each group were compared ($p = 0.013$ for E18.5 and $p < 0.001$ for E19.5; table 2). There were no significant differences when the measures of the intestinal wall were compared between the 2 days ($p = 0.476$; fig. 1). In the comparative analysis of the intestinal wall measures

Table 1. Results of measurements in fetuses submitted to gastroschisis on days 18.5 and 19.5, as well as their control and sham litter mates (mean ± SD)

	E18.5			E19.5			Group C/S/G	Day 18.5 × 19.5
	C (n = 8)	S (n = 7)	G (n = 8)	C (n = 7)	S (n = 7)	G (n = 7)	p	p
Body weight, g	5.65 ± 0.59	5.91 ± 0.26	5.54 ± 0.68	6.02 ± 0.30	5.81 ± 0.96	5.89 ± 0.44	NS	NS
Intestine weight, g	0.22 ± 0.03	0.21 ± 0.03	0.31 ± 0.07	0.24 ± 0.01	0.24 ± 0.04	0.29 ± 0.06	<0.001	NS
Intestine length, mm	211.00 ± 17.24	200.29 ± 23.04	139.88 ± 30.17	236.00 ± 17.77	235.00 ± 24.39	162.43 ± 16.54	<0.001	<0.001
IW/BW, mg/mg	3.96 ± 0.23	3.59 ± 0.51	5.64 ± 0.87	4.00 ± 0.24	4.09 ± 0.32	5.02 ± 1.18	<0.001	0.035
IW/IL, mg/mm	1.06 ± 0.12	1.07 ± 0.17	2.33 ± 0.67	1.02 ± 0.06	1.01 ± 0.10	1.86 ± 0.58	<0.001	NS

G = Gastroschisis; C = control; S = sham; IW/BW = intestine weight/body weight; IW/IL = intestine weight/intestine length.

Table 2. Results of histological measurements in fetal rats submitted to gastroschisis on days 18.5 and 19.5, as well as their control and sham litter mates (mean ± SD)

	E18.5			E19.5			Group C/S/G	Day 18.5 × 19.5
	C (n = 32)	S (n = 28)	G (n = 32)	C (n = 28)	S (n = 28)	G (n = 28)	p	p
Measure D-I and D-II	850.25 ± 158.46	852.48 ± 73.77	1,124.25 ± 283.03	934.21 ± 84.03	971.38 ± 114.81	1,154.60 ± 193.49	<0.001 (D-I) 0.015 (D-II)	0.800 (D-I) <0.001 (D-II)
Mucosa	26.76 ± 5.40	26.10 ± 3.07	33.00 ± 10.14	22.35 ± 5.65	23.38 ± 4.54	39.07 ± 10.33	<0.001	NS
Circular layer	10.81 ± 1.50	9.95 ± 1.11	16.38 ± 4.31	13.23 ± 2.16	9.94 ± 1.58	17.58 ± 4.25	<0.001	NS
Longitudinal layer	8.14 ± 0.75	7.55 ± 0.87	10.69 ± 0.69	7.09 ± 1.21	5.66 ± 1.54	12.85 ± 4.67	<0.001	NS
Serous layer	2.48 ± 0.36	2.53 ± 0.26	3.38 ± 0.62	0.19 ± 0.21	1.10 ± 0.24	2.09 ± 1.01	<0.001	<0.001
Total wall	48.19 ± 7.13	46.13 ± 7.13	63.46 ± 16.54	43.85 ± 7.36	40.08 ± 5.75	71.60 ± 16.17	<0.001	NS

G = Gastroschisis; C = control; S = sham; D-I = diameter I; D-II = diameter II; NS = no statistical significance.

among the subgroups and days, there was no significant effect of the interaction among these subgroups and the days of exposure ($p = 0.104$). All the layers of the ileal wall analyzed presented significant differences in the comparison of measures of fetuses in the 3 control, sham and gastroschisis subgroups on E18.5 and E19.5 ($p < 0.001$). The longitudinal muscular layer also tended to be compromised as a consequence of the longer exposure to AF, but did not show statistical differences between the days ($p = 0.083$); the serosa presented significant differences in the comparison of the 3 subgroups ($p < 0.001$ for E18.5 and $p = 0.004$ for E19.5) and 2 days when comparing the measures between E18.5 and E19.5 ($p < 0.001$). The results of the histological measures are shown in table 2.

Evaluation of the Myenteric Plexus by α-Internexin

The myenteric plexus of gastroschisis fetuses presented more intensive α-internexin immunoreactivity both in the E18.5 and E19.5 groups, with higher expression in the myenteric plexus of gastroschisis fetuses of group E18.5. Immunofluorescence presented low or no immunoreactivity for α-internexin in the intestinal walls of control and sham fetuses in both groups (fig. 2).

Discussion

Gastroschisis is a rare congenital disease of unknown etiology in which the bowel loops exposed to AF lead to functional alterations in the postnatal period, such as intestinal hypomotility and disorders in the ability to absorb nutrients [8, 17]. The anatomic and functional damage to the intestine occurs not only by direct contact with AF, but mainly through the effect of meconium released by the fetus during gestation [18, 19] and the time of exposure to AF that exacerbates the intestinal wall lesion [3, 4].

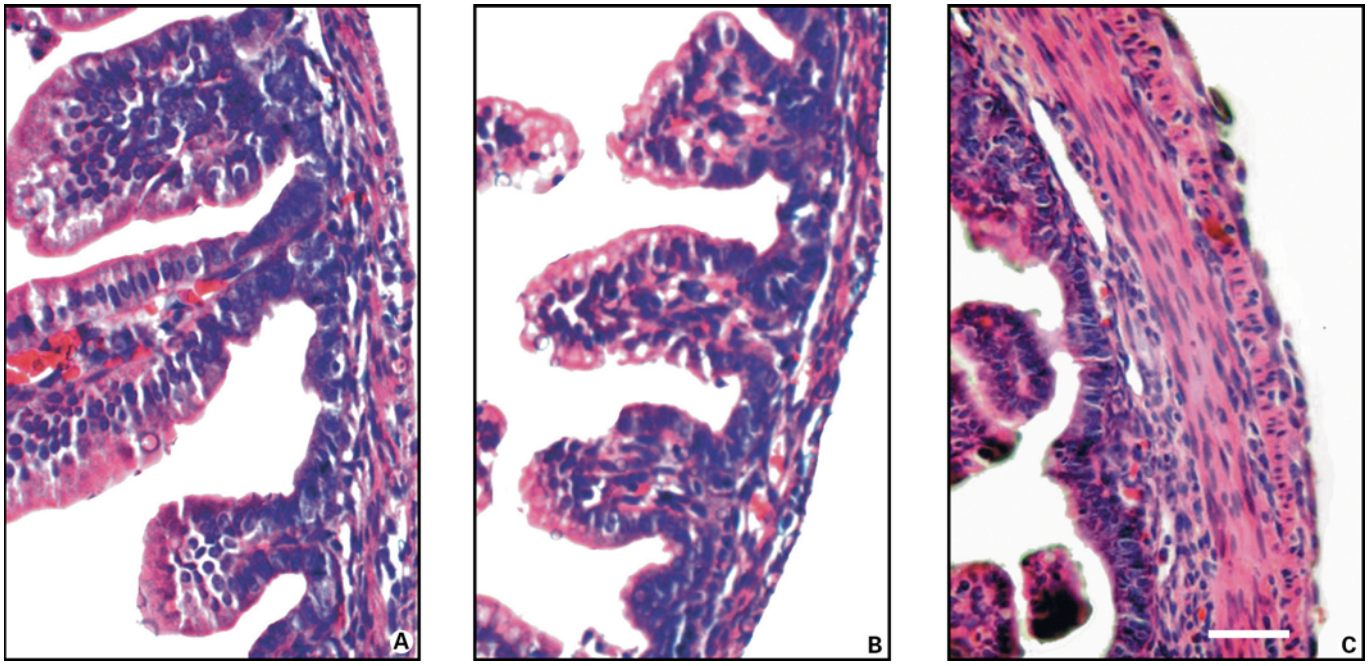

Fig. 1. Thickness of the intestinal wall of the 3 subgroups, control (**A**), sham (**B**) and gastroschisis (**C**), of fetuses of the E18.5 group. Note that the intestinal wall in the gastroschisis subgroup (**C**) is thicker than the control and sham subgroups at both ages ($p < 0.001$), and although no statistical difference was observed, the intestinal wall in gastroschisis at E19.5 is thicker than at E18.5. ×40. Bar = 30 μm.

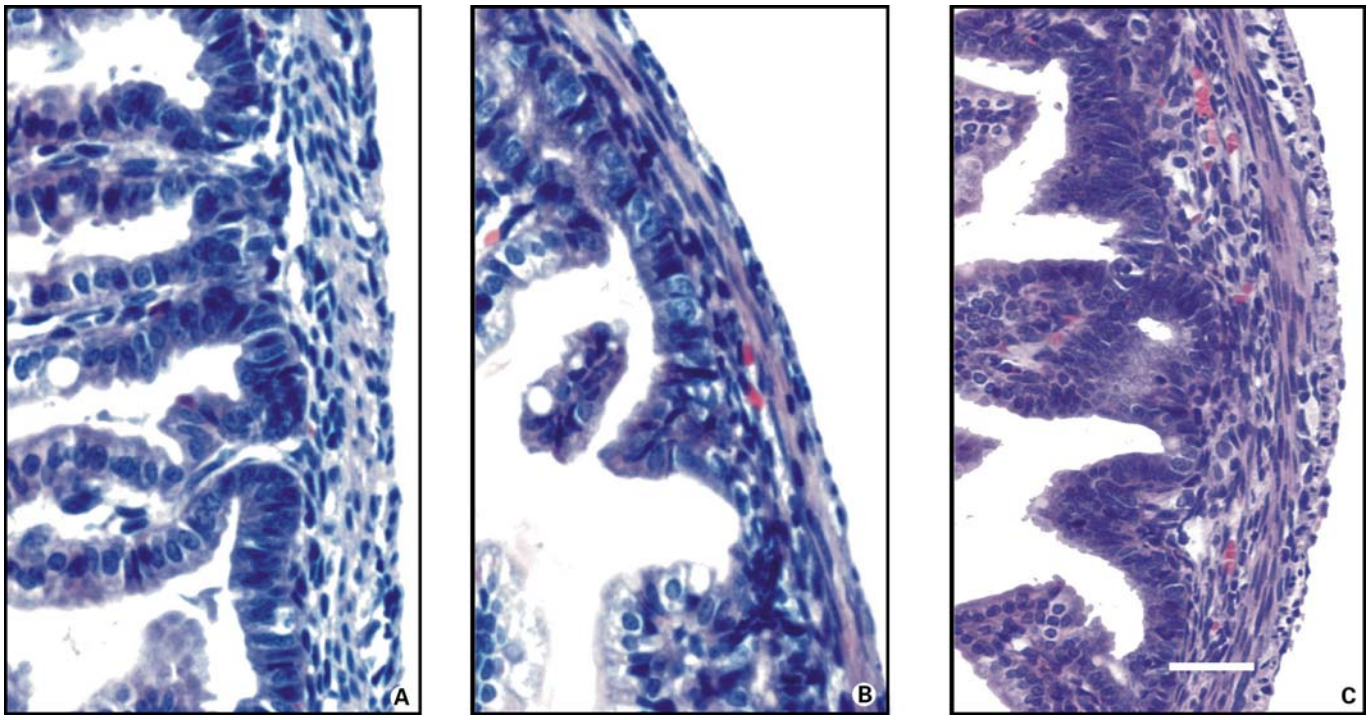

Fig. 2. Thickness of the intestinal wall of the 3 subgroups, control (**A**), and sham (**B**) and gastroschisis (**C**), of fetuses of the E19.5 group. Note that the intestinal wall is in the gastroschisis subgroup (**C**) is thicker than the control and sham subgroups at both ages ($p < 0.001$), and although no statistical difference was observed, the intestinal wall in gastroschisis at E19.5 is thicker than at E18.5. 40×. Bar = 30 μm.

The impairment of bowel loops in gastroschisis occurs more frequently at the end of gestation; in human fetuses the lesions are more noticeable after the 30th week of gestation, when a fine fibrous coating is formed which promotes progressive alterations in the intestinal serosa [20, 21].

The most frequently found structural alterations in the gastroschisis intestine are: thickened walls, increased weight, shortened length and the appearance of a fine fibrous coating that covers the serosa [22, 23]. In experimental gastroschisis models in rabbits, which evaluated a single period of intestinal exposure to AF, a larger intestinal diameter, smaller body weight, intestinal weight and intestinal length were found [24, 25]. Similar results were obtained in experimental gastroschisis in sheep [3].

Although disorganization and immaturity of the myenteric plexus have not been properly demonstrated in the lamb or ewe models, Langer et al. [3], in an in vitro bowel motility assay, showed that the maximal contractile response to acetylcholine was impaired in gastroschisis lamb fetuses and stated that this was a possible reproducible model of gastroschisis, with features similar to the human condition.

In this gastroschisis study in rats, we found a shorter IL ($p < 0.001$) while the IW was higher ($p < 0.001$) in gastroschisis fetuses when compared to control and sham fetuses in both E18.5 and E19.5 groups, results that were similar to those found by Correia-Pinto et al. [5]. However, we found that the IL was shorter in gastroschisis fetuses of the E18.5 than in those of the E19.5 groups ($p = 0.001$), that is, the longer time of exposure to AF caused more severe intestinal shortening.

The diameters of the intestines of gastroschisis fetuses (D-I and D-II) were larger that those in control and sham fetuses ($p < 0.001$ and $p = 0.015$, respectively) in both groups, results that were comparable to those of Albert et al. [26]. With a similar behavior to IL, D-II also presented significant differences when compared to measures of both groups, that is D-II in gastroschisis fetuses of E18.5 was smaller than in gastroschisis fetuses of E19.5 ($p < 0.001$). BW was not as altered in the subgroups ($p = 0.793$) as in the E18.5 and E19.5 groups ($p = 0.271$); these results are different from those of Albert et al. [2] and Franchi-Teixeira et al. [27] who found a smaller BW in gastroschisis fetuses.

In a comparative analysis of the serous measures, we verified that it was enlarged in the gastroschisis fetuses ($p < 0.001$), in both the E18.5 and E19.5 groups ($p = 0.035$), as was also found in the rabbit [26] and chicken [28] models. Once again, these results show the deleterious effects in the course of gestation on bowel loops, mainly in the serous layer.

In the histomorphometric evaluation of the intestinal wall in gastroschisis in lambs, the circular and longitudinal muscular layers were both enlarged, but the circular muscular layer presented greater growth and began to thicken (hyperplasia) at 100 days of gestation, earlier than the longitudinal layer when compared with controls, but both layers were enlarged at 135 days of gestation [4]. Opposite results were obtained by Langer et al. [20] who stated that only the longitudinal muscle layer was enlarged at 120 days of gestation ($p < 0.05$), with a much larger increase at term (145 days, $p < 0.001$). These results [4, 20] suggested that the longer the time of exposure to AF, the more the lesion in this intestinal wall layer is affected. These results demonstrate that when the muscular layers are compromised this might contribute to explain intestinal hypomotility and malabsorption. We also found in gastroschisis in rats a thickening of the circular and longitudinal layers in the 3 subgroups ($p < 0.001$) in both gestational days E18.5 and E19.5; although we have not found significant differences when comparing the longitudinal muscular layer between the days E18.5 and E19.5 ($p = 0.083$), we believe that the longer the time of exposure to AF is, the trend is for this layer to become thicker.

The mucous layer was distended in gastroschisis for both subgroups ($p < 0.001$), but not between E18.5 and E19.5; mucosal thickening was also verified in lamb gastroschisis, with corresponding enlargement of the submucosa [4]. The same occurred in rabbit gastroschisis [27] and it seems that this thickening of the intestinal wall is related with a decrease in disaccharidase activity, contributing to neonatal intestinal malabsorption [17].

The α-internexin is a neurofilament protein present in the central and peripheral nervous system. It is involved in the embryonic differentiation of cells of the myenteric neuronal lineage (intrinsic neuronal system, localized between the mucosa and each layer of muscle) [11]. It is also involved in cytoskeleton formation and may be considered as a marker of neuronal maturity, since it appears earlier in embryonic life and diminishes its immunoreactivity when going from fetal to neonatal life; if present in the adult phase, it is indicative of neuronal immaturity [10].

Santos et al. [8] found decreased activity of acetylcholinesterase and more immaturity of the myenteric plexus caused by lactate dehydrogenase. By means of α-internexin immunoreactivity in gastroschisis bowel loops in E18.5

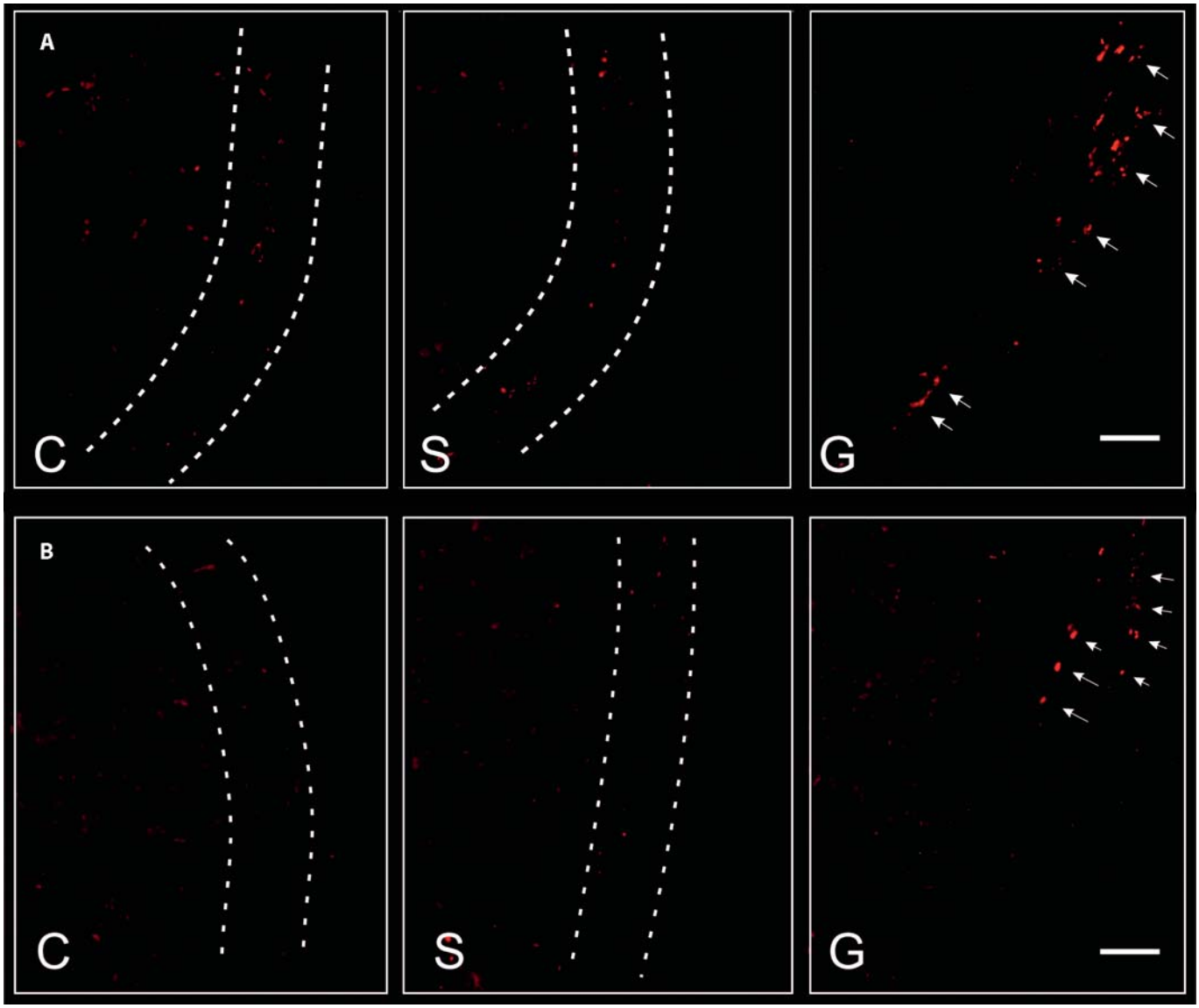

Fig. 3. Immunofluorescence labeling for α-internexin in gastroschisis fetuses (G; arrows) in the E18.5 (**A**) and E19.5 (**B**) age groups, where the most damaged plexus with immature neural cells is more intensely marked in gastroschisis at E18.5 (**A**, G) while gastroschisis at E19.5 (**B**, G) is weakly marked probably due to a less immature plexus. Control (C) and sham (S) fetuses at both ages (**A**, **B**) show no labeling for α-internexin probably due to adequate maturity of the plexus. The interrupted lines show the whole extent of the intestine wall. ×40. Bar = 30 μm.

fetuses, Vannucchi et al. [12] found that there was diminished reaction and consequent delay in neuronal maturation in addition to disorganization of the myenteric plexus. Both findings could explain the alterations in synaptic activity of the myenteric neurons that affect the control of intestinal motility in gastroschisis. The present study found greater immunoreactivity of α-internexin in gastroschisis fetuses of the E18.5 and E19.5 groups than in control and sham fetuses of both groups; however, the immunoreactivity of gastroschisis fetuses of group E18.5 was higher that in the gastroschisis fetuses of group E19.5, that is, the longer the period of exposure to AF (fig. 3), the longer the myenteric plexus will remain immature. These are additional results to those found by Vannucchi et al. [10, 12] which were presented for only 1 gestational day, E18.5.

Preterm delivery could help to avoid some complications after birth and could improve the postoperative period, be less costly, and shorten the requirements for total parenteral nutritional [29]. All this damage in the intestinal wall is due to prolonged exposure of the intestinal loops to AF containing inflammatory substances: interleukin-8 [7], fetal urine [30], and fetal meconium [19].

Exchange of the AF during gestation has been studied in order to prevent or decrease damage in the intestinal wall in fetuses with gastroschisis [24, 31]. More recently experimental studies based on the information that the intestinal wall is damaged by components of AF, in particularly meconium, have shown that changing the concentration of AF could in fact decrease this damage, even by amnio-infusion or by amnio-exchange [32].

This procedure in human gestation may bring some difficulties due to the high risk of uterus contractions and an increased cycle of AF production, where flow measurements following drainage of the amniotic cavity indicate a production rate of 16–42 ml/h, which means a complete change in AF every 2–3 h. Besides amnio exchange would have to be a continued procedure to maintain a constant low concentration of AF and would not be totally safe for most pregnancies [33].

Despite the not completely clarified problems regarding the adequate volume of AF to be exchanged, the time during pregnancy, and how often the procedure should be done, amnio exchange could prevent functional and morphological adverse effects on the enteric nervous system and perhaps improve the clinical results postnatally [34].

We conclude that, in the gastroschisis model in rats, intestinal wall alterations, mainly in the serosa and intestinal length, as well as the immaturity of the myenteric plexus were more intensive in fetuses whose exposure to AF was longer (gastroschisis/E18.5). Further experimental studies should be developed and directed toward comprehending intestinal motility in gastroschisis, aiming at minimizing neuromuscular damage to bowel loops exposed to AF, and allowing the evaluation of the supposed advantages of early delivery for human gastroschisis fetuses.

References

1 Bealer JF, Graf J, Brush SW, Adzick NS, Harrison MR: Gastroschisis increases small bowel nitric oxide synthase activity. J Pediatr Surg 1996;31:1043–1048.

2 Albert A, Sancho MA, Julia V, Diaz F, Bombi JA, Morales L: Intestinal damage in gastroschisis is independent of the size of the abdominal defect. Pediatr Surg Int 2001;17:116–119.

3 Langer JC, Longaker MT, Crombleholme TM, et al: Etiology of intestinal damage in gastroschisis. I: Effects of amniotic fluid exposure and bowel constriction in a fetal lamb model. J Pediatr Surg 1989;24:992–997.

4 Srinathan SK, Langer JC, Blennerhassett MG, Harrison MR, Pelletier GJ, Lagunoff D: Etiology of intestinal damage in gastroschisis. III: Morphometric analysis of the smooth muscle and submucosa. J Pediatr Surg 1995;30:379–383.

5 Correia-Pinto J, Tavares ML, Baptista MJ, Estevao-Costa J, Flake AW, Leite-Moreira AF: A new fetal rat model of gastroschisis: development and early characterization. J Pediatr Surg 2001;36:213–216.

6 Nichol PF, Hayman A, Pryde PG, Go LL, Lund DP: Meconium staining of amniotic fluid correlates with intestinal peel formation in gastroschisis. Pediatr Surg Int 2004;20:211–214.

7 Fasching G, Haeusler M, Mayr J, Schimpl G, Haas J, Puerstner P: Can levels of interleukins and matrix petalloproteinases in the amniotic fluid predict postnatal bowel function in the fetuses with gastroschisis? J Pediatr Surg 2005;40:1887–1891.

8 Santos MM, Tannuri U, Maksoud JG: Alterations of enteric nerve plexus in experimental gastroschisis: is there a delay in the maturation? J Pediatr Surg 2003;38:1506–1511.

9 Sydorak RM, Sbragia L, Nijagal A, Phibbs RH, Harrison MR, Albanese CT: A big hole in the system: gastroschisis. J Pediatr Surg 2002;37:1669–1672.

10 Vannucchi MG, Midrio P, Flake AW, Faussone-Pellegrini MS: Neuronal differentiation and myenteric plexus organization are delayed in grastroschisis: an immunohistochemical study in a rat model. Neurosci Lett 2003;339:77–81.

11 Faussone-Pellegrini MS, Matini P, DeFelici M: The cytoskeleton of the neurons during murine embryonic life. Anat Embryol (Berl) 1999;199:459–469.

12 Vannucchi MG, Midrio P, Zardo C, Faussone-Pellegrini MS: Neurofilament formation and synaptic activity are delayed in the myenteric neurons of the rat fetus with gastroschisis. Neurosci Lett 2004;364:81–85.

13 Ward SM, Sanders KM: Involvement of intramuscular ICC in neuroeffector transmission in the gastrointestinal tract. J Physiol 2006;14:1–31.

14 Zenilman ME: Origin and Control of gastrointestinal motility. Surg Clin North Am 1993;73:1081–1099.

15 Hirst GDS, Suzuki H: Involvement of interstitial cells of Cajal in the control of smooth muscle excitability. DOI: 10.1113/jphysiol.2006.121178. http://jp.physoc.org/cgi/content/abstract/jphysiol.2006.121178v.

16 Omari TI, Rudolph CD: Physiology of the gastrointestinal tract in the fetus and neonate; in Polin RA, Fox WW, Abman SH (eds): Fetal and Neonatal Physiology, ed 3. Philadelphia, Saunders, 2004, vol 2, pp 1125–1138.

17 Guo W, Swaniker F, Fonkalsrud EW, Vo K, Karamanoukian R: Effect of intraamniotic dexamethasone administration on intestinal absorption in a rabbit gastroschisis model. J Pediatr Surg 1995;30:983–987.

18 Api A, Olguner M, Hakgüder G, Ates O, Özer E, Akgür FM: Intestinal damage in gastroschisis correlates with the concentration of intraamniotic meconium. J Pediatr Surg 2001;36:1811–1815.

19 Ramon y Cajal CL, Martinez RO: Defecation in utero: a physiologic fetal function. Am J Obstet Gynecol 2003;188:153–156.

20 Langer JC, Bell JG, Castillo RO, et al: Etiology of intestinal damage in gastroschisis. II: Timing and reversibility of histological changes, mucosal function, and contractility. J Pediatr Surg 1990;25:1122–1126.

21 Tibboel D, Vermey-Keers C, Klünck P, Gaillard JLJ, Koppenberg J, Molenaar JC: The natural history of gastroschisis during fetal life: development of the fibrous coating on the bowel loops. Teratology 1986;33:267–272.
22 Deans KJ, Mooney DP, Meyer MM, Shorter NA: Prolonged intestinal exposure to amniotic fluid does not result in peel formation in gastroschisis. J Pediatr Surg 1999;34:975–976.
23 Correia-Pinto J, Tavares ML, Baptista MJ, Henrique-Coelho T, Estevao-Costa J, Flake AW, Leite-Moreira AF: Meconium dependence of bowel damage in gastroschisis. J Pediatr Surg 2002;37:31–35.
24 Albert A, Julia V, Morales L, Rovira J, Sancho A, Bombi JA: Role of the amniotic fluid in gastroschisis. An experimental study (in Spanish). Cir Pediatr 1992;5:12–16.
25 Albert A, Julia MV, Morales L, Parri FJ: Gastroschisis in the partially extraamniotic fetus: experimental study. J Pediatr Surg 1993;28:656–659.
26 Albert A, Margarit J, Julia V, Sancho MA, Galan X, Lopez D, Morales L: Morphology and mucosal biochemistry of gastroschisis intestine in urine-free amniotic fluid. J Pediatr Surg 2003;38:1217–1220.
27 Franchi-Teixeira AR, Weber Guimarães Barreto M, Nogueira B, Bittencourt D, Violin L, Sbragia L: Amniotic fluid and intrauterine growth restriction in a gastroschisis fetal rat model. Fetal Diagn Ther 2005;20:494–497.
28 Yu J, Gonzalez-Reyes S, Diez-Pardo JA, Tovar JA: Local dexamethasone improves the intestinal lesions of gastroschisis in chick embryos. Pediatr Surg Int 2004;19:780–784.
29 Dunn JCY, Fonkalsrud EW, Atkinson JB: The influence of gestational age and mode of delivery on infants with gastroschisis. J Pediatr Surg 1999;34:1393–1395.
30 Olguner M, Akgür FM, Api A, Özer E, Aktung T: The effects of Intraamniotic human neonatal urine and meconium on the intestines of the chick embryo with gastroschisis J Pediatr Surg 2000;35:458–461.
31 Aktug T, Erdag G, Kargi A, Akgur FM, Tibboel D: Amnio-allantoic fluid exchange for the prevention of intestinal damage in gastroschisis: an experimental study on chick embryos. J Pediatr Surg 1995;30:384–387.
32 Aktug T, Uçan B, Olguner M, Akgür FM, Özer E: Amnio-allantoic fluid exchange for prevention of intestinal damage in gastroschisis. II: Effects of exchange performed by using two different solutions. Eur J Pediatr Surg 1995;8:308–311.
33 Biggs JS, Duncan RO: Production rate and sources of amniotic fluid at term. J Obstet Gynaecol Br Commonw 1970;77:326–332.
34 Sencan A, Gümüstekin M, Gelal A, Arslan O, Özer E, Manisa EM: Effects of amnio-allantoic fluid exchange on bowel contractility in chick embryos with gastroschisis. J Pediatr Surg 2002;37:1589–1593.