



Comparative study of intracisternal kaolin injection techniques to induce congenital hydrocephalus in fetal lamb

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Abstract

Purpose Kaolin (aluminum silicate) has been used to generate hydrocephalus by direct cisterna magna injection in animal models. The aim of the present study is to compare which method of Kaolin injection into fetal cisterna magna is feasible, safer, and more effective to induce hydrocephalus in fetal lambs.

Methods Twenty-five well-dated pregnant ewes at gestational 85–90 days (E85-90) were used to compare three different kaolin injection puncture techniques into the fetal cisterna magna. Group 1, ultrasound guidance in a maternal percutaneous transabdominal (TA); group 2, without opening the uterus in a transuterine (TU) technique; group 3, by occipital direct access after exteriorizing fetal head (EFH); and group 4, control group, was normal fetal lambs without injection. The fetal lambs were assessed using lateral ventricle diameter ultrasonographic measurements prior the kaolin injection and on the subsequent days. We analyzed the effectivity, mortality, and fetal losses to determine the best technique to create hydrocephalus in fetal lamb.

Results After fetal intracisternal kaolin (2%, 1mL) injection, lateral ventricle diameters increased progressively in the three different interventional groups compared with the normal values of the control group ($p \leq 0.05$). We observed that the transabdominal method had a 60% of fetal losses, considering failure of injection and mortality, compared with the 12.5% in the open group (EFH), and 0% for the transuterine group.

Conclusions Based on our study, we believe that both, open uterine (EFH) and transuterine approaches are more effective and safer than the transabdominal ultrasound-guided method to induce hydrocephalus.

Keywords Fetal · Hydrocephalus · Kaolin · Sheep · Ventriculomegaly

Introduction

The first known attempt to produce hydrocephalus experimentally in animals was made at the beginning of the past

century [1]. Since then, various types of experimental models have been reported. During the first 3 decades of the twentieth century, the major focus and interest in the experimental studies was the analysis of the cerebrospinal fluid (CSF) circulation, both in normal and in pathological conditions. In this period, various methods inducing mechanical and/or chemical blocking of the CSF circulation were employed. The first experimental production of hydrocephalus was incidental, accomplished in 1907, by means of the production of meningitis in monkeys [1]. With the development of teratology, other models of intrauterine hydrocephalus were generated, such the first murine hydrocephalus in a mutant, reported by Clark in 1932 [2]. Blockage of the CSF circulation by a chemical agent was also performed with the introduction of the intracisternal injection of colloidal kaolin (aluminum silicate) by Lindauer [3] in 1938. Gradually, the major interest of the experiments shifted to focus on hydrocephalic brain damage. Cambria et al. [4], from Italy, reported their first studies with experimental creation of hydrocephalus in the fetus. In the

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subsequent years, until 1990, the laboratory investigations in hydrocephalus have focused on the analysis of neuronal development in the immature brain and on the functional recovery. During this time, congenital teratologic models have become the preference for experimental studies [5–10].

The most widely used method has been the injection of aluminum silicate into the cisterna magna [5, 6, 9–12]. Some authors reported that fetal percutaneous puncture of the cisterna magna was accomplished after opening the uterus and expose the fetal head [9]. The research group of Dr. Harrison also described a transuterine method without opening the uterus, where the needle can be placed within the cisterna magna by palpation of the posterior atlanto-occipital membrane through the thin and floppy sheep uterine wall [6, 10].

This method is inexpensive, simple, reliable, minimally invasive leaving no visible wound, and to some extent titratable. Kaolin causes an inflammatory response of the leptomeninges and the subarachnoid space around the brainstem and cerebellum, thus causing obstruction at the fourth ventricle outlets [9].

The aim of the present study is to compare which method of kaolin injection into fetal cisterna magna is feasible, safer, and more effective to induce hydrocephalus in fetal lambs.

Materials and methods

A total of 25 well-dated pregnant ewes (JUMISC, Caceres, Spain) were studied at gestational 85–90 days (E85–90) to compare three different techniques to inject kaolin into the fetal cisterna magna to create prenatal hydrocephalus.

The injection of 1 mL of kaolin (2% in saline) suspension into the fetal cisterna magna was performed by three different techniques (Table 1).

We distributed the animals into 4 groups:

- Group 1, we placed the injection needle within the cisterna magna with ultrasound guidance by a maternal percutaneous transabdominal (TA) approach (Fig. 1A).
- Group 2, we placed the needle within the cisterna magna exposing but without opening the uterus (Fig. 1B) in a transuterine (TU) technique.

- Group 3, we inserted the needle into the fetal cisterna magna by occipital direct access after opening the uterus (Fig. 1C) and exteriorization of fetal head (EFH).
- Group 4 was a control without injection in time-dated matched normal fetal lambs.

In the first three groups, puncture of the cisterna magna was performed using a 22 Gauge needle. Correct positioning and location of the tip was verified by the flow and dropping of clear CSF. Prior to the kaolin injection, the same 1 mL volume of CSF was withdrawn (Fig. 1). Fetal lateral ventricle diameters were calculated using ultrasonography prior to injection and periodically on the subsequent 20 days (Table 1).

Animal preparation

The pregnant ewes were admitted to the CCMIJU animal facility 1 week prior to the study for acclimatization and health checks, and they were evaluated by ultrasound to determine the age of the gestation and not pre-existing lesions. The pregnant mothers were fed ad libitum on hay and concentrates with unrestricted access to clean water 12 h previous surgery.

All animals received an intramuscular injection of Borgal® (trimethoprim (2.5 mg/kg) and sulfadoxine (12.5 mg/kg)) 1 day prior to surgery, and daily for 4 days post-surgery. Also, 30 min before the surgery, the sheep were infused with 1 g of IV cefazolin in 100 mL of saline over a 30-min time period and gentamicin (5 mg/kg) into the amniotic fluid before closing the uterine cavity. Each animal also received an intramuscular injection of buprenorphine (10 µg/kg/12 h) on the day of the surgery and daily for 4 days post-surgery and meloxicam (1 mg/kg/24 h) for 4 days post-surgery.

Anesthesia was induced intravenously with propofol 1% (6 mg/kg). The animals were then intubated with a 9.0- to 10.0-mm endotracheal tube connected to a closed circuit from anesthetic system. Anesthesia was maintained with sevoflurane (EtSEVO = 2.9–3.3%) in 50% oxygen (FiO₂ = 0.5), and the ewes were mechanically ventilated to maintain normocapnia (EtCO₂ 35 to 40 mmHg). For intraoperative analgesia, ketorolac was administered (1 mg/kg/IM) and constant monitoring of ECG, blood pressure, and maternal pulse was measured.

Table 1 Different cisterna magna injection methods and after injection lateral ventricle diameter (mm)

Kaolin injection approach into cisterna magna	n	Lateral ventricular diameter (mm)							Failed	Abortion	Death
		Injection	2 days	4 days	8 days	12 days	16 days	20 days			
Transabdominal (TA)	10	3.98 (±0.33)	4.00 (±0.61)	4.14 (±0.34)	4.44 (±0.30)	5.2 (±0.65)	6 (±0.71)	6.65 (±0.96)	3	2	1
Transuterine (TU)	4	3.33 (±0.23)	4.50 (±0.42)	4.60 (±0.41)	5.05 (±0.21)	5.60 (±0.95)	4.75 (±0.63)	6.43 (±0.65)	–	–	–
Open (EFH)	8	4.28 (±0.45)	3.87 (±0.71)	3.71 (±0.68)	4.15 (±0.49)	4.91 (±0.92)	5.45 (±0.86)	6.0 (±0.97)	–	1	1
Control	3	3.75 (±0.35)	3.40 (±0.14)	2.06 (±0.41)	2.56 (±0.15)	2.93 (±0.20)	3.33 (±0.23)	3.43 (±0.40)	N/A	N/A	N/A

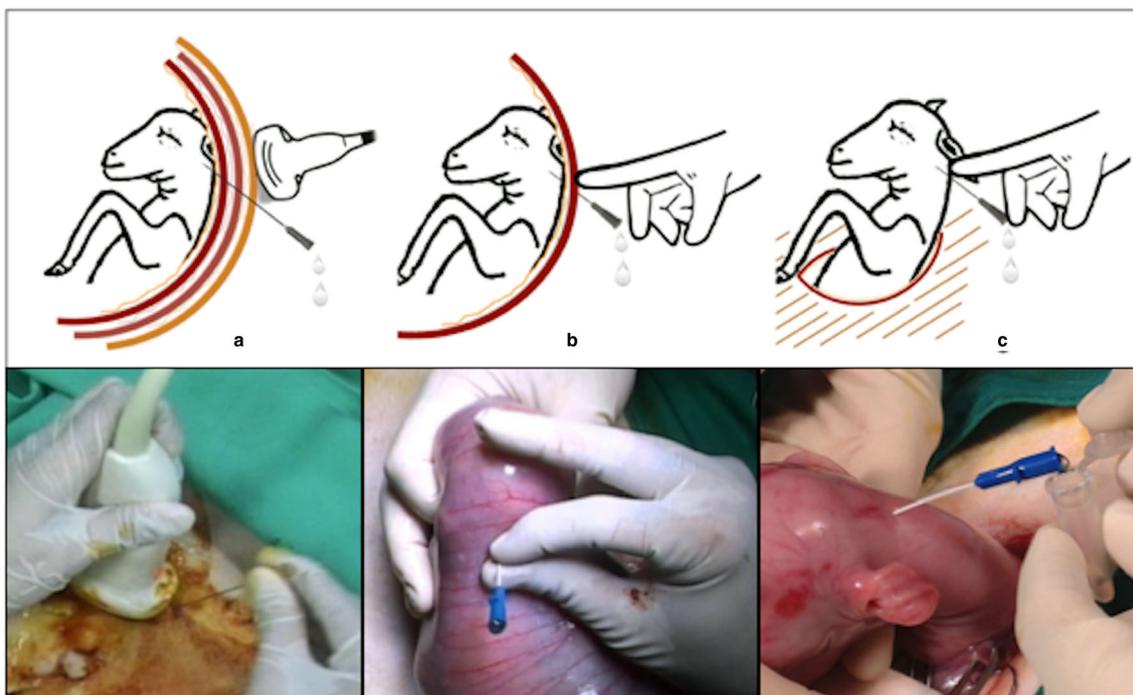


Fig. 1 Schematic illustration (upper) and image (below) of (a) maternal percutaneous transabdominal (TA) approach with needle within the cisterna magna under ultrasound guidance. (b) transuterine (TU) technique, and (c) injection with exteriorization of fetal head (EFH)

In group 1, TA percutaneous needle injection through the maternal abdominal wall into the fetal cisterna magna was performed under ultrasound guidance in 10 fetal lambs. Identification and access of fetal cisterna magna was achieved in all cases (Fig. 2A), even in some of them with some difficulties depending on fetal position. In groups 2 and 3, TU and EFH respectively, the laparotomy site has been shaved, cleaned with 70% alcohol and prepped with povidone-iodine

scrub. After laparotomy, in group 2, we used the transuterine injection method that Glick et al. have described previously [10]. This access was performed in four fetuses. We proceeded to exteriorize the uterus and, after manual head fixation and under transuterine palpation without any uterine opening, we have introduced the 22 G needle in the fetal posterior neck, caudal to the occipital protuberance. Once the catheter has been inserted into the cisterna magna subarachnoid space,

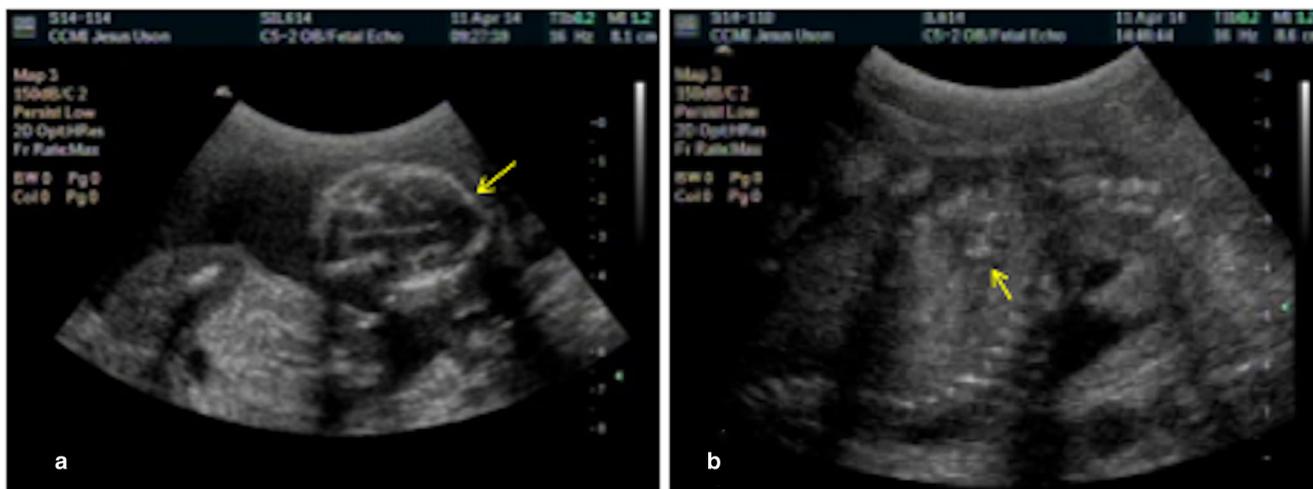


Fig. 2 (a) Ultrasonographic images of the fetal lamb lateral ventricles and cisterna magna in the axial transventricular plane during TA puncture of cisterna magna (arrow), and (b) ultrasonographic demonstration of kaolin

as hyperechoic particles (arrow) passing into the bloodstream on the heart just after injection

we can observe the CSF dropping. In group 3, we had proceeded to exteriorization and opening of the uterus creating a small window in eight fetal lamb. Then, after manual head fixation and by direct palpation, we have introduced a needle in the cisterna magna, entering caudal to the occipital protuberance to place the 22 G catheter into the cisterna magna like it has been previously described [4–6, 13]. The sterile kaolin solution was injected manually, slowly during 1 min into the cisterna magna, after the same volume of CSF was withdrawn. Surgical monitoring has been maintained until the animal has been fully recovered.

Ultrasound prenatal monitoring

We performed gray scale (mode B) prenatal ultrasounds (Philips ATL HDI 5000) on the fetal lambs with a transabdominal convex transducer (C5-2 at 2–5 MHz), by two different maternal-fetal specialists experienced sonographers.

Before and after injections, all the fetuses have been periodically monitored by means of weekly ultrasound scan to rule out complications, check fetal cardiac beating, and determine brain/ventricular findings and measures. The measurement has been done in the true axial plane at the atria of the lateral ventricle and glomus of the choroid plexus. The lateral ventricular diameter was measured from inner margin of the medial ventricular wall to inner margin of the lateral wall, as previously reported [14].

Propofol 1% (6 mg/kg) was used for sheep sedations to perform prenatal ultrasound assessment and document the progression of fetal ventriculomegaly.

At the end of the experimental procedure, the fetus lambs were delivered by C-section and euthanized with potassium chloride. Harvest of samples and necropsy of animals were made following the guidelines of the European Animal Protection Law (Directive 2010/63/EU of the European Parliament).

Statistics

Significant differences intra- and inter-different approaches to inject kaolin in the cisterna magna (control, TA, TU, and EFH) were verified by the Kruskal–Wallis one-way repeated measures analysis. Data was expressed as mean \pm standard deviation and p value ≤ 0.05 was considered statistically significant. All the statistical analysis was performed with GraphPad Prism 7.

Ethical approval

This study was performed according to the European Council Directives (C86/609/EEC and 200/65/EC) and Spanish Guidelines for the Use of Laboratory Animals and the

approval of the official regional governmental IACUC (ES100370001499), and local ethics committee for experimental animal use at the animal facilities of the Jesus Usón Minimally Invasive Surgery Centre (JUMISC) in Spain.

Results

We tested and compared three different methods to induce hydrocephalus in fetal lambs according the feasibility to access the cisterna magna during the injection of kaolin, fetal safety of the injection (mortality rate), and the analysis of the efficacy to generate ventriculomegaly (enlarged lateral ventricles).

Feasibility to access the cisterna magna

Even with different boarding techniques, the difficulty lies in the accuracy of a small space at the fetal lamb, such as the cistern magna. Technically achieving a trans-cerebellar plane (similar to that on humans) we obtained the puncture site.

Analyzing the successful access to the cisterna magna in fetal lambs, we observed 3 failed injections into the cisterna magna in TA percutaneous ultrasound guided access. In these three cases were not possible to access the cisterna magna with the needle even using the guidance of the ultrasound. In 30% of the animals that we tried this approach we failed the injection (Fig. 3A).

Moreover, we observed in one our cases that kaolin passed into the bloodstream just after the injection. In this case, we recognized kaolin on the ultrasound as hyperechoic particles flowing in the heart (Fig. 2B).

In the other two approaches, TU and EFH, we achieved the access to the cisterna magna for kaolin injection (Fig. 3A) (Table 1).

Mortality after procedures

In terms of mortality rates, we considered the abortions after the kaolin injections and fetal death during the subsequent days. We followed up the animals in utero until after 20 days after injection. In the first maternal TA group, we observed two abortions and two fetal losses 10 days after the injections, equivalent to a total of 30% mortality in the TA approach. In the EFH open group, one fetus died 4 days after the injection, which corresponds to a 12.5% of mortality rate. No mortality in the TU group (Fig. 3B) was observed (Table 1).

Analyzing the effectiveness of the different approaches in the kaolin-induced hydrocephalus model, we observed that the TA method had a 60% of total losses considering failure of injection and mortality, compared with the 12.5% in the EFH open group, and 0% for the TU group (Fig. 3C).

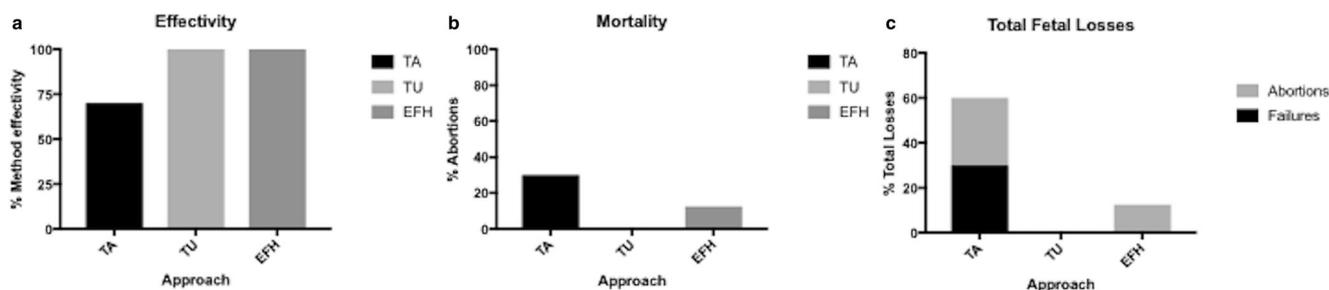


Fig. 3 (a) Diagrams showing the rates of the injection effectivity into the cisterna magna, (b) fetal mortality, and (c) cumulative graph of mortality and technical failures

Hydrocephalus induction—analysis of lateral ventricular diameters

The lateral ventricle diameter progressively increased in the three compared techniques, being significant 4 days after the kaolin injection until the maximum diameter (mm) after 20 days : 6.65 ± 0.96 for TA, 6.43 ± 0.65 for TU, and 6.0 ± 0.97 for EFH when compared with 3.43 ± 0.40 the control group ($*p \leq 0.05$) (Fig. 4). Results of the ventricle dilatation observed are summarized in Table 1.

Discussion

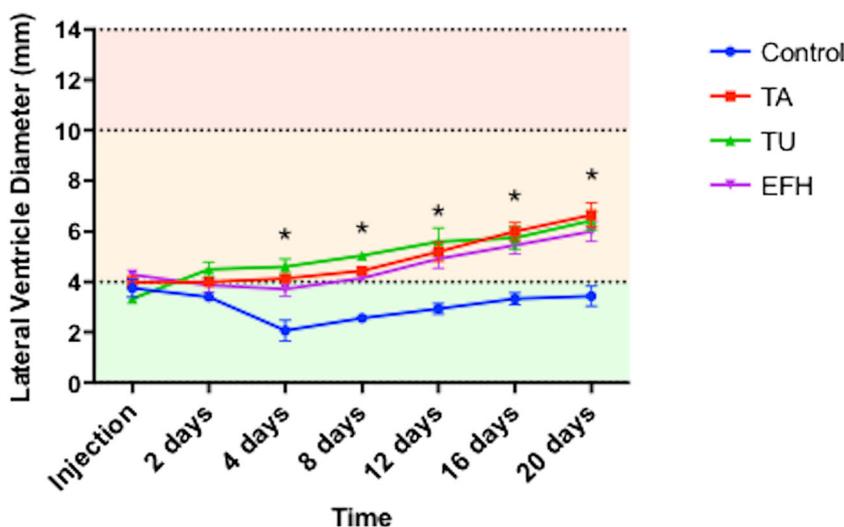
There are many experimental animal models reported in the literature which induce congenital hydrocephalus to reproduce aqueduct stenosis. Most of them use the injection of various compounds into the cisterna magna to block CSF flow [15–22]. Kaolin produces an inflammatory reaction involving the aqueduct of Sylvius, generating its obliteration with secondary mechanical obstruction of the CSF flow. In our comparative study, we used different methods for kaolin injection into the cisterna magna.

In this study, three injection approaches were tested and compared to a normal control group, in terms of accessibility to the cisterna magna, safety of the injection, and also efficacy to create fetal hydrocephalus.

Fetal lambs tolerated the kaolin injections with no intraoperative fetal losses. We observed less mortality and better effectivity rates than previous studies in the same animal model with open surgery (EFH) [6] and by transuterine (TU) injection [10].

Based on our observations, the injection of Kaolin into the cisterna magna of fetal sheep creates ventriculomegaly using any of the three different techniques. However, ultrasound image-guided maternal TA percutaneous method was complex and unsafe, because of the suboptimal fetal head position and difficulties to find cisterna magna in a proper angle to successfully insert the needle and inject kaolin into the subarachnoid space. In this method, we observed two failed injection attempts to the cisterna magna, and two abortions and fetal losses. Moreover, in one case, we observed kaolin into the blood circulation reaching the heart. This is a significant complication in the hydrocephalus model secondary to intravascular injection of the aluminum silicate.

Fig. 4 The diameter of the lateral ventricles progressively increased in the three compared techniques, showing significance (*) 4 days after the kaolin injection



In the TU percutaneous technique, the injection of kaolin into cisterna magna was challenging as well as finding the correct entry point for cisterna magna and even using the palpation due the poor fixation of fetal head and the sliding effect of uterine wall and amniotic membranes over the fetal head. Nevertheless, it was effective and safe at the same level as accessing the cisterna magna with the fetal head exteriorized through a hysterotomy in the EFH technique.

Conclusion

Based on our study, we believe that both, open uterine method, with fetal head exposure, and the TU access, are more effective and safer than the ultrasound-guided transabdominal method. Probably, the method that still offers warranties of success and showed to be easier is the exposed fetal head with complete manual immobilization of the cephalic area on the fetal lamb. The fact of manually fixating the fetal head allows better exposure of the occipital protuberance and easier palpation to successfully access the fetal cisterna magna.

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Authors' contributions Study concept and design: S.D., M.O., and J.L.P. Acquisition of data: M.O., S.A., C.R., L.C., F.V., F. SM., and J.L.P. Analysis and interpretation of data: S.D., M.O., and J.L.P. Drafting of the manuscript: M.O., S.D., and J.L.P. Critical revision of the manuscript for important intellectual content: M.O., S.D., and J.L.P. Statistical analysis: M.O. Obtained funding: J.L.P. Technical or material support: M.O. Study supervision: S.D. and J.L.P.

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Data availability Data supporting the findings of this study are available within the article.

Compliance with ethical standards

Conflict of interest Authors have no competing interests.

Ethical approval This study was performed according to the European Council Directives (C86/609/EEC and 200/65/EC) and Spanish Guidelines for the Use of Laboratory Animals and the approval of the official regional governmental IACUC (ES100370001499), and local ethics committee for experimental animal use at the animal facilities of the Jesus Usón Minimally Invasive Surgery Centre (JUMISC) in Spain.

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