

Treatment of classic mid-trimester preterm premature rupture of membranes (PPROM) with oligo/ anhydramnion between 22 – 26 weeks' gestation by means of continuous amnioinfusion: a randomized multicentric prospective controlled TRIAL

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Abstract

Background: The classic mid-trimester **preterm premature rupture of membranes (PPROM)**, is defined as rupture of fetal membranes prior to 28 weeks' gestation (WG) with oligo/ anhydramnion, complicates approximately 0.4-0.7% of all pregnancies and is associated with very high neonatal mortality and morbidity.

Antibiotics have limited success to prevent bacterial growth, chorioamnionitis and fetal inflammation. The repetitive amnioinfusion doesn't work because of immediately fluid lost after the intervention. The continuous amnioinfusion through the transabdominal port system or catheter in patients with classic PPRM shows promise by flush out of bacteria and inflammatory components from the amniotic cavity, replacing amniotic fluid and thus prolonging PPRM-to-delivery interval.

Aim: This multicenter trial tests the effect of continuous amnioinfusion on the neonatal survival without the typical major morbidities, like severe bronchopulmonary dysplasia, intraventricular hemorrhage, cystic periventricular leukomalacia and necrotizing enterocolitis one year after the delivery.

Methods/Design: randomized multicenter trial; two-arm parallel design. Control group: PPRM patients between 22/0 (20/0) -26/0 WG treated with antibiotics and corticosteroids in accordance to guidelines of German Society of Obstetrics and Gynecology (standard PPRM therapy). In the interventional group the standard PPRM therapy will be complemented by "Amnion Flush Method" with the amnioinfusion of artificial amniotic fluid (up to 100 ml/h, 2400 ml/day).

Subjects: 68 patients with classic PPRM between 22/0 (20/0)-26/0 WG.

TRIAL-registration: ClinicalTrials.gov ID: NCT04696003 and German Clinical Trials Register: DRKS00024503, January 2021.

The trial is approved by the Ethic committee of the Martin-Luther University Halle-Wittenberg (2020-185, January 25, 2021).

Administrative Information

Title {1}	Treatment of classic mid-trimester preterm premature rupture of membranes (PPROM) with oligo/ anhydramnion between 22 – 26 weeks' gestation by means of continuous amnioinfusion: a randomized multicentric prospective controlled TRIAL
Trial registration {2a and 2b}.	ClinicalTrials.gov ID: NCT04696003 and German Clinical Trials Register: DRKS00024503, January 2021.
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Author details {5a}	Michael Tchirikov (scientific input, proof), Christian Haiduk (creation of the protocol related to the medicine product low) Miriam Tchirikov (proof and discussion) Marcus Riemer (proof) Stephan Henschen (proof) Andreas Wienke (statistic) Georg Seliger (proof, correction of the protocol)
Name and contact information for the trial sponsor {5b}	Martin-Luther University Halle-Wittenberg, presented by a Chancellor, represented by a Dean Univ._Prof. Michael Gekle, «Martin-Luther-University Halle-Wittenberg» Magdeburger str. 8, 06112 Halle
Role of sponsor {5c}	The funder is given opportunity to provide feedback but does not have ultimate authority over the study design, data collection and management, analysis, and interpretation of data, writing of the report, and the decision to submit the report for publication. The sponsor assumes the responsibility for the prospective randomized study.

Introduction

Mid-trimester PPRM complicates approximately 0.4-0.7% of all pregnancies and is associated with very high neonatal mortality and severe morbidity [1–4]. The causes of the mid-trimester PPRM are multifactorial [4]. The mechanism of PPRM is discussed as altered membrane morphology including marked swelling and disruption of the collagen network, which can be triggered by bacterial products and pro-inflammatory cytokines and involves the activation of matrix metalloproteinases. [4–7].

The propagation of bacteria is an important contributing factor in adverse neonatal and maternal outcomes after PPRM [1,4,8]. Gomez *et al*/ reported that antibiotics failed to eliminate the amniotic

infection in 83% of PPRM cases [9]. The “classic PPRM” with oligo-/anhydramnion is associated with short latency period and worse neonatal outcome compared to similar gestational aged neonates [4].

In Germany, the number of pregnant women with mid-trimester classic PPRM has been calculated to be about 5-7 thousand per year. Generally, about 40% of very preterm infants who survive the initial NICU die during next 5 years of life and the long-term morbidity of the survivors remain high. Furthermore, more than 40% of surviving neonates following PPRM prior to 25 weeks of gestation develop bronchopulmonary dysplasia (BPD) later on [4,10].

Background And Rationale

The **fetal inflammatory response syndrome (FIRS)**, a systemic inflammation determined by elevated fetal plasma IL-6 concentrations and histological signs of funisitis in the umbilical cord, is an additional independent risk factor for the occurrence of severe neonatal morbidity [11].

The lavage effect of continuous amnioinfusion could prevent the fetus and amniotic cavity from bacterial colonization, reduce the inflammatory response and thus protect the neonate from major complications, such as pulmonary hypoplasia, sepsis, cerebral palsy and joint deformities[4,12–15].

Serial amnioinfusions with standard electrolyte solutions have seen little or no effects onto neonatal outcome probably because of immediately fluid lost after the intervention and possible negative effect of longtime saline solution onto the fetus [4,14,16–19].

Tchirikov et al. published the prolongation of the PPRM-to-delivery interval to 49 days in average of patients with classic PPRM using the intensive lavage of the amniotic cavity with Amnion Flush solution (pump rate 100 ml/h) throughout a transabdominal catheter with an additionally designed anker system [4,12,20,21].

However, in recently published retrospective results from Japan the authors did not improve the neonatal outcome using low rate of continuous amnioinfusion with Ringer’s lactate solution (40 ml/h) [14,15]. In the current German medical guideline to prevention of the preterm birth the amnioinfusion for the treatment of the PPRM is mentioned, but prospective randomized studies must be performed to clarify the positive effect of the continuous amnioinfusion onto the fetal survival [22].

Objectives

Principal research question: Does the intervention (continuous amnioinfusion – “flush out” method) reduce adverse neonatal outcome of patients with PPRM with oligo/anhydramnion and prolong the pregnancy.

Primary hypothesis: “Amnion Flush” Method will improve the neonatal outcome after PPRM between 20/0-26/0 weeks’ gestation.

Trial Design

Methods

The full infrastructure of Coordination Center for Clinical Trials (KKS) of Martin-Luther-University Halle Wittenberg will be involved in ICH-GCP compliant data management and handling, randomization, safety management and monitoring of the study.

Study setting

The study will be performed in 10 tertiary German perinatal centers including six leading German Universities (Fig. 1).

Eligibility criteria

Inclusion criteria: single pregnancy (from 22/0 to 26/0 weeks' gestation), with classic PPROM with oligo/anhydramnion between 20/0 to 26/0 weeks' gestation. From the beginning of 22/0 weeks' gestation the PPROM duration must be lower as of five days till randomization. The patients receive RDS prophylaxis before randomization. The vertical deepest amniotic fluid depot must be <2 cm, and the amniotic fluid Index < 3. The PPROM will be confirmed by positive testing of the placental alpha microglobulin-1 and clearly amniotic fluid lost (Fig 2, Flow diagram).

Exclusion criteria:

The patients with fetal death, high rupture of membranes, pre-PPROM, premature labor, placental abnormalities, placental abruption, evidence of major structural or chromosomal abnormalities, indication for termination of pregnancy (e.g. HELLP syndrome, pathological CTG, fetal bradycardia, eclampsia, umbilical cord prolapse), cervical insufficiency, placenta previa, signs of chorioamnionitis (maternal fever > 37.8° C and two or more of the following criteria: uterine tenderness, malodorous vaginal discharge, maternal leukocytosis > 15000 cells/mm³ (without corticosteroids), maternal tachycardia > 100 beats/min and fetal heart rate > 160 bpm), CRP > 20 mg/dl.

Who will take informed consent?

The authorized physicians of perinatal centers taking part in the trial will identify the potential trial participants with classic PPROM. Eligible women with classic mid-trimester PPROM will be asked for their written informed consent at the time of admission to the hospital - after having received oral and written information. Patients, who are not able to understand the informed consent, will not be included. Additionally for five years all patients undergoing a fetal surgery therapy in hospital had filed a motion for a compassionate use at ethical review committee. Recruitment for "flush out" method via a port-system is ethically sound for PPROM patients with oligo-/anhydramnion based on available research evidence.

In case of complications all equipment for emergency interventions will be easily accessible in the perinatal centers. Additional “safety net” for finalization of the study and induction of delivery has been established to avoid any complications.

Intervention and control groups

Control group

The PPRM patients of the control group receive the standard therapy related to the German Guidelines including antibiotic therapy (Amoxicillin/Clarithromycin therapy or 7 days Amoxicillin and once Azithromycin 1 g per os) or other antibiotics according to recommendations of the physicians from the microbiology department based on the culture results for at least 7 days [22]. Daily blood analysis (Hb, WBC, Th, CRP, IL-6), cervical smear every 5 days. The RDS prophylaxis must be performed before randomization.

Amnion Flush interventional group

The PPRM patients of the Amnion Flush Group receive the standard therapy related to the German Guidelines like the control group [22]. Additionally, under local anaesthesia and ultrasound guiding the perinatal catheter with an anchor system (0.65 mm Diameter, CE 0481, PakuMed GmbH, Essen, Germany) will be inserted into the amniotic cavity for the continuous amnioinfusion with 100 ml/h of Amnion Flush Solution, (Serumwerk Bernburg AG, Germany) until delivery (latest on beginning of 34/0 weeks of gestation) [4,12,13] (Fig. 3).

Detailed description of the perinatal catheter implantation

The catheter implantation will be performed in the operation room in aseptic conditions. Before the catheter implantation the amnioinfusion with 300 ml of Amnion Flush Solution will be performed using 22 gauge needle under ultrasound control. The gynaecologist must have an experience of at least of 100 amniocenteses because of difficult conditions for the puncture related to anhydramnion.

The operation table will be kept for maximum Trendelenburg patients' position avoiding completely lost of infused Amnion flush Solution because of PPRM. If patients will complain about uterine contractions, the single short of Partusisten 25µg will be slowly infused i.v.

The appropriate location for the catheter implantation (preferred area is the uterine fundus without placenta) will be identified using ultrasound. The local anaesthesia with Xylocaine 1% 10 ml will be injected [20]. The 18 gauge needle will be inserted into the amniotic cavity. The anchor catheter will be inserted throughout the needle. The 18 G needle will be removed. The catheter will be fixed up using the sterile transparent foil. The Amnion Flush Solution will be infused using standard medical pumps with the rate of 100 ml/h (+/- 20 ml). Daily ultrasound control must be performed to validate the intraamniotic catheter position and the deepest deport of the amniotic fluid. The deepest pool of amniotic fluid should

be stabilized (target 4 cm). The catheter should be replaced if indicated (e.g. delivery or catheter dislocation or plugging) but latest every 30 days to avoid contamination and/or inflammation.

Primary outcome: survival (event time) without major morbidities (severe bronchopulmonary dysplasia (BPD), and/or grade 3 or 4 intraventricular hemorrhage (IVH 3–4), and/or necrotizing enterocolitis (NEC) indicated for surgical intervention, and/or cystic periventricular leukomalacia (cPVL)) obtained one year after the delivery [23].

Secondary outcomes: Gestational age at delivery, duration of PPRM-to-delivery interval, perinatal mortality, incidence of histological affirmed chorioamnionitis, incidence of FIRS and/or maternal sepsis, cord-artery pH, APGAR scores, neonatal outcome at calculated age 36 weeks of gestation: duration of O₂-supplementation, retinopathy of prematurity. Participants with surviving babies will send a prepaid validated outcome questionnaire at 12 months after the birth of their baby. The independent University Center of Clinical Studies of Martin-Luther-University Halle-Wittenberg will receive the copies of pediatric examinations performing monitoring and final statistical evaluation. Neurodevelopmental assessments as well as the check-up of the surviving children will be performed in tertiary medical centers by trained pediatric specialists.

Participant timeline

The **study site visits** for monitoring the clinical trial will be carried out by chief and assistant monitors. During the clinical phase, the **chief monitor** will visit the medical centers every 6 months (see table 1), thereby being available to staff, supervising the assistant monitors, and in order to address and solve any problems arising. They will review the progress of recruitment and ensure participants' rights and safety, adherence to protocol regulatory compliance. During query process by the KKS, which implies another month of study site visits (beyond the end of recruitment), they will verify unclear data and obtain corrections. Chief monitor will also make sure that plenty of CRF are available to staff, and questionnaires are given to mothers for filling them in prior to discharge from hospital.

Sample size and Statistic

The sample size calculation was based on the primary outcome - survival without major morbidities (severe bronchopulmonary dysplasia (BPD), and/or grade 3 or 4 intraventricular haemorrhage (IVH 3–4), and/or necrotizing enterocolitis (NEC) and/or cystic periventricular leukomalacia (cPVL)).

The intervention is expected to prolong the gestation by around two weeks resulting in an increase of the one year survival rate without major morbidities after 27 gestational weeks of 67% (hazard=0.0334). Based on Chen et al. (2016) the one year survival rate without major morbidities after 25 weeks gestation is around 36% (hazard=0.0851) [23]. To be more conservative sample size calculation was based on a hazard=0.035 for the interventional group. Using a recruiting period of 21 months (four women per month) and an individual follow-up of one year 34 women are needed in each group for a two-sided log-

rank test with 5% significance level, a power of 80% and a loss to follow up rate of 10% per year (hazard=0.0088). Calculations were performed by PASS software.

Assignment of interventions: allocation, concealment mechanism and implementation

The randomization will be performed using the randomizer of the Center of the clinical studies of the Martin-Luther University Halle-Wittenberg, Germany (computer-generated random numbers). Before the randomization the inclusion and exclusion criteria of the study will be printed by investigators into the randomizing table online. Matching criteria will be automatically analyzed and the group of the study and the number will be generated online.

Data collection and management

The trial is registered with full description at the German Clinical Trials Register (Deutsches Register Klinischer Studien, DRKS) and at Clinical Trials Register (ClinicalTrial.gov). The study's protocol is published at the start of the trial.

The Coordination Center for Clinical Trials, University of Halle (Saale), is responsible for data management and archiving. The data will be managed and archived according to existing standards and regulations, in a data format allowing long-term preservation and future reuse.

The data sheet will be completed when the baby will discharge home or after death. Any missing data will be reconciled by the chief investigator and trial administrator by contact with the PIs and examination of the hospital case notes. The data pack will be returned to the trial co-ordination centers after the baby will discharge home or after death.

Post-trial follow-up is at 6 and 12 months postpartum when questionnaires are being sent to mothers' home addresses for the assessment of subjective variables as well as variables pertaining to neonatal medical care for the health-economic evaluation.

CRF will include

data for recruitment and confirmation of eligibility, informed consent, baseline data, process, data during treatment, primary and secondary outcomes, other outcomes, safety variables, prognostic factors, various questionnaires, as well as data of the pediatric evaluations.

The KKS will receive the copies of pediatric examinations. Neurodevelopmental assessments as well as the check-up of the surviving children will be performed in medical centers by trained pediatric specialists.

Monitoring

The coordinating investigator is responsible for implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that the trial is conducted and data generated, documented and reported in compliance with the protocol, ICH-GCP and the applicable regulatory

requirements. Tasks assigned to KKS Halle (see chapter 9, trial supporting facilities) will be fulfilled according to the written SOPs from KKS Halle and German-wide KKS Network. Data from source documents will be transcribed onto the case report forms (CRF) by the clinical research staff of each trial site. All source documents are kept in the trial site and no information in source documents about the identity of the patients will be disclosed. Pre-trial visits and on-site monitoring will be conducted by trained CRAs from KKS Halle. 3-5 pre-trial visits will be performed to allow selection of 3 sites for participation. Based on the pre-trial visit reports the PI and the steering committee will decide on participation of the respective trial site. Because of previous on-site selection visits, for initiation of the study centers conference calls (each site separately) will be considered. Initiation will be supported by .ppt presentations and study materials (investigator's site file, protocol, informed consent, CRFs etc.) earlier supplied to the sites. On-site monitoring will be performed on a risk-based approach and is planned by means of regular visits from the beginning to the end of the trial to check the completeness of patient records, the consistency of entries on the CRFs and the adherence to the protocol and to Good Clinical Practice. Initially the informed consent forms and the adherence to the inclusion and exclusion criteria will be checked. Thereafter, a formal check of captured information for completeness and plausibility will be done followed by a check of the correct transfer of data from the source data. Full (100%) source data verification for the presence of informed consent and adherence to the inclusion/exclusion criteria will be performed. Data used for all primary and safety variables will also be 100% source data verified. The specific extent of the monitoring and the source data verification will be specified in the monitoring manual. Each trial site will be monitored once after the first trial participant has been enrolled and treated. The early monitoring shall ensure prompt intervention by CRA or study center in case of any problems at the trial site such as major inconsistencies in trial conduction and CRF completion. Another 7 (average, individual number depending on recruiting rate and quality of CRF completion) monitoring visits at each trial site will be conducted during the enrolment period. Every trial site will undergo a close-out visit by the CRA after the last participant at that site has finished the follow up. The monitor's access to the trial documents and medical records is ensured by the investigator's agreement and the cooperation agreement between the sponsor and KKS Halle.

Statistical analysis

The collected data will be analyzed by specialists of statistic of the Institute of Medical Epidemiology, Biostatistics and Informatics, Martin-Luther-University Halle-Wittenberg, Germany

Adverse event reporting harms

The Data Safety Monitoring Board (DSMB) will follow the progress of the clinical trial, evaluate the safety and primary efficacy parameters and will propose to the sponsor whether to continue, modify or stop a trial and provide the funding agency with information and advice. The DMSB will set up telephone conferences twice a year to evaluate current safety data. Therefore, the sponsor will provide up-to-date safety data including a summary of AEs, line listing of SAEs and adverse device effects (ADEs). In case of a high number of severe unexpected events in between DMSB meetings or a case of a Suspected

Unexpected Serious Adverse Reaction (SUSAR) or other medically important conditions the DMSB will be informed by the sponsor immediately and may give advice for further procedures if required. An interim analysis is not planned.

Stopping rules

a) for patients:

placental abruption, indication for termination of pregnancy (e.g HELLP syndrome, pathological CTG, fetal bradycardia, eclampsia, umbilical cord prolapse), signs of chorioamnionitis (maternal fever $> 37.8^{\circ}$ C and two or more of the following criteria: uterine tenderness, malodorous vaginal discharge, maternal leukocytosis > 15000 cells/mm³ (without corticosteroids), maternal tachycardia > 100 beats/min, and fetal heart rate > 160 bpm), CRP > 20 mg/dl, increased concentrations of interleukine-6 (> 7 pg/ml), positive bacteriologic examination of the amniotic fluid, pos. Gram-stained sediment or IL-6 concentration > 2600 pg/mL or WBC > 30 mm³.

b) for participating centers:

inability to recruit the patients, complications during the catheter implantation, like placental abruption, damage of the fetus or umbilical cord, fetal bleeding or death (audit after first complication, center ex in case 2 or more complications/year).

for the whole trial:

high range of complications in interventional group compared to the control group (we are going to audit the study every 3 months discussing this point), unexpected adverse reactions to the medical products without correction possibilities, clear statistical benefit of "flush-out" treatment.

Frequency and plans for auditing trial conduct

The medical centers of clinical trial will undergo an audit twice a year by a study manager, KKS, Martin-Luther-University Halle-Wittenberg. To ensure quality of data and study performance the sponsor may conduct site visits by an independent auditor. An audit will only be performed after notification and arrangement with the investigator. An audit certificate will be issued as quality proof and has to be filed in the trial master file and as a copy in the investigator site file.

Ethics approval and consent to participate

The study will be conducted in compliance with the Declaration of Helsinki and all amendments.

The TRIAL has got a permission of the Ethical Committee of the Halle University Medical Center (2020-185, January 25, 2021).

The trial was registered with ClinicalTrials.gov ID: NCT04696003 and German Clinical Trials Register: DRKS00024503.

Written informed consent of the patients as well as the parents is required for enrolment in the study and can be withdrawn at any time without reasons or disadvantages. Eligible women with classic mid-trimester PPRM will be asked for their written informed consent at the time of admission to the hospital - after having received oral and written information. Patients, who are not able to understand the informed consent, will not be included. Recruitment for “flush out” method via a port-system is ethically sound for PPRM patients with oligo-/anhydramnion based on available research evidence.

In case of complications all equipment for emergency interventions will be easily accessible in the perinatal centers. Only hospitals which possess a Level 1 perinatal center in accordance to German law (tertiary perinatal center) will receive permission to participate on this trial.

Any modifications to the protocol will be sent to the ethics committees before they are implemented within the study and communication changes that impact the patients would require signing of a revised consent form.

Discussion

Mid-trimester preterm premature rupture of membranes (PPROM) together with oligohydramnios before 26 weeks affects about 0.4%–0.7% of all pregnancies. A high number of unreported cases should be expected in cases of PPRM prior to 22 weeks of gestation. Mid-trimester PPRM with anhydramnion is associated with a very high neonatal mortality rate as well as an increased risk of long- and short-term severe neonatal morbidities, physical and developmental disabilities, including chronic respiratory disease, neurodevelopmental or behavioral effects (impairment of visual/hearing/executive functioning, global developmental delay and psychiatric/behavioral sequelae) and cardiovascular diseases [3,4,24]. The early birth due to PPRM with anhydramnion during the canalicular stage of the lung development between 16th and 26th weeks' gestation leads to pulmonary hypoplasia [4,25]. Prolonged anhydramnion after PPRM is associated with a four-fold increased risk of composite adverse outcomes, including death, BPD, severe neurological disorders, severe retinopathy, when compared to an age-adjusted control group [8,26].

The causes of the mid-trimester PPRM are multifactorial including local infiltration by bacteria with reaction of pro-inflammatory cytokines, pathologic anatomical remodeling of the amniotic membranes (contribution of MMPs), invasive procedures and fetoscopic surgery, genetic and iatrogenic factors, smoking, vaginal bleeding etc. [4,7,27–33]. Inflammatory mediators likely play a causative role in both disruption of fetal membrane integrity and activation of uterine contraction.

The diagnosis of PPRM is classically established by identification watery leakage from the cervical canal, positive swab assay for placental alpha macroglobulin-1- and indigo carmine-tests [4,34,35]. The management of the PPRM requires balancing the potential neonatal benefits from prolongation of the

pregnancy and reduction of the adverse effects of newborn immaturity with the risk of intra-amniotic infection and its consequences for the mother and infant [2,4,22,36]. Close monitoring for signs of chorioamnionitis (e.g. body temperature, CTG, CRP, leucocytes, IL-6, procalcitonine and amniotic fluid examinations) is necessary to minimize the risk of neonatal and maternal complications.

Despite the fact that broad spectrum antibiotics are routinely used in the therapy of PPRM based on the guidelines' recommendations, it must be considered that the maternally applied drugs only hardly reach the place of bacterial colonization [4,19,22]. The amniotic membranes and the umbilical cord do not have an effective capillary net which would supply the surfaces with the antibiotics from the maternal circulation [37]. The placenta is a selective barrier for foreign substances and the bioavailability among antibiotics is very low for most antibiotic agents [4,38–43]. On the other hand, the healthy fetus without any infection after PPRM probably does not need any longtime trans-placental antibiotic treatment leading to possible change of fetal programming [4,12,13,44].

The anatomical conditions result in the inability to eradicate the colonization of bacteria in the amniotic cavity which means that infectious complications remain a major problem after PPRM. De Santis et al. found out that the patients with PPRM did not appear to demonstrate any benefit from this repetitive amniotic fluid (AF) replacement (250 mL per intervention) as measured by post procedure because fluid loss occurred within 6 hours of instillation [19]. Locatelli et al. found that the serial amnioinfusions could improve the neonatal outcome primarily by prolonging latency. The patients with PPRM and oligohydramnios had a significantly shorter interval to delivery, lower neonatal survival of 20%, 62% of pulmonary hypoplasia and 60% rate of neurological handicap [16].

Roberts et al. concluded that serial transabdominal amnioinfusions showed no significant difference in the outcome of maternal and perinatal outcome. The perinatal mortality was 19/28 vs 19/28 [17,18]. The positive fetal survival effect of serial amnioinfusions was unfortunately counterbalanced by an increased risk of neonatal death: 14 neonates died in AI's group vs. 9 in control group. It is possible that the use of saline solution for AI was inappropriate due to the large deviations from normal human amniotic fluid [45].

Thus, accumulation of sodium and chloride would disturb the sodium potassium pump located in plasma membrane of human cells and could be influencing organs' performance, e.g. cardiac, lung and brain. These adverse effects of the instillation fluid could explain the high mortality rate after AI in this study [4,17,18].

Tchirikov 2018 has summarized literature between 2000 and 2017 on PPRM between 18 and 28 weeks [4]. The positive effect of continuous pump-regulated amnioinfusion of 100 ml/h, 2400 ml/d ("Amnion Flush Method") through the subcutaneously implanted perinatal catheter system with an anker with Amnion Flush Solution in "classic PPRM" less than 28/0 weeks' gestation showing promise since 2008. The continuous amnioinfusion prolongs the mean duration of the PPRM delivery interval for at least 3 weeks improving neonatal outcome [4,12,13]. The patients in whom Jonosteril® (Fresenius Kabi GmbH, Germany), Sterofundin® and isotonic NaCl (B. Braun AG, Melsungen, Germany) or lactated Ringer's

solution (Baxter, Germany) were used, reported significantly increased diuresis, probably, triggered trans-planetary by a fetal response to increased NaCl, fluctuation of osmolality and missing microelements also referred to as 'Salzgurken' effect [4]. The electrolyte solutions for the continuous amnioinfusion were replaced 2012 by artificial amniotic fluid (now Amnion Flush Solution, CE0483, Serumwerk AG, Bernburg, Germany) without any adverse events [4,12,13].

However, two groups from Japan in recently published retrospective studies (2020) were not able improve the neonatal outcome using the low rate amnioinfusion with Ringer's lactate solution (40 ml/h) [14,15]. The authors mentioned the high frequency of catheter dislocation (60%) and lack of pump infusion in many cases [14,15]. The missed positive effect of the prolongation of the PPROM-delivery interval by two weeks onto healthy neonatal survival in these studies could be explained by "Salzgurken-Effekt" of *off-label used* saline solution for a long time with a very high deviation of many parameters compared to human amniotic fluid (Table 1) [4].

Fetal skin in second trimester is still very thin and permeable. It is a matter of common knowledge that the fetus swallows and inspirates/expiration amniotic fluid. Gilbert and Brace published that the fetus swallows 200-250 ml/kg/day amniotic fluid [46]. Continuous amnioinfusion with normal saline solution significantly increased plasma Na^+ and Cl^- concentrations in fetal sheep [47]. The amniotic fluid is a very complex hypoosmotic solution with an alkaline pH, low concentration of the elements Cl^- , K^+ and Na^+ , presence of trace elements, surfactants and many other substances [48]. The change of physiological fetal surroundings for a long period using amnioinfusion with simple electrolyte solutions could destroy in all probabilities the fetal programming. Hyponatremia induced osmotic demyelization disorders, central pontine and extrapontine myelinolysis are well known [49]. Chhabra et al. described the extrapontine myelinolysis induced by hyponatremia [50]. The combination of very immature brain-blood barrier of the fetus with the permeable skin and swallowing of relative large amount of electrolyte solution deteriorates the fluctuations of the NaCl concentration of the fetal brain. This could explain the high neonatal mortality rate after serial amnioinfusion in the Roberts *et al* study and very moderate success of continuous amnioinfusion using Ringer's lactate solution (inappropriate to human amniotic fluid) described in the retrospective Japanese studies [14,15,17,18].

Summarized, the standard treatment options of the classic mid-trimester PPROM with oligo-anhydramnion including RDS prophylaxis and broad spectrum antibiotics hardly improve the neonatal outcome. The extension of the standard therapy by continuous amnioinfusion – "Amnion-Flush" method could improve the neonatal survival without major morbidities. In 2020, the German Federal Ministry of Education and Research funded this prospective multicentre randomized study investigating the effect of continuous amnioinfusion in patients with classic PPROM with oligo/ anhydramnion between 22/0 (20/0) -26/0 WG with Amnion Flush Solution (100 ml/h, 2400 ml/d) throughout transcutaneously placed perinatal catheter with an anchor system compared with the standard PPROM therapy (ClinicalTrials.gov: NTC04696003).

Declarations

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Consent for publication

No individual identifiable person's data is included in this manuscript or will be included in the future publications of the main trial results. A summary of study findings will be made available to participants.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Contributions

All authors contributed to the design of the study and writing of the manuscript. All authors confirm their eligibility as authors and involvement in this study. The authors read and approved the final manuscript.

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None to declare

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References

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. 2008;371(9606):75–84. doi: 10.1016/S0140-6736(08)60074-4.
2. Park GY, Park WS, Sung SI, Kim MS, Lee MH, Jeon GW, et al. Neonatal outcome comparisons between preterm infants with or without early pulmonary hypertension following prolonged preterm premature rupture of membranes before 25 gestational weeks in Korean Neonatal Network. *J Matern Fetal Neonatal Med*. 2020:1–24. doi: 10.1080/14767058.2020.1749590. PubMed PMID: 32228112.

3. Pendse A, Panchal H, Athalye-Jape G, Campbell C, Nathan E, Rao S, et al. Neonatal outcomes following prelabour rupture of membranes before 23 weeks of gestation- a retrospective cohort study. *J Neonatal Perinatal Med.* 2020. doi: 10.3233/NPM-190366. PubMed PMID: 32224534.
4. Tchirikov M, Schlabritz-Loutsevitch N, Maher J, Buchmann J, Naberezhnev Y, Winarno AS, et al. Mid-trimester preterm premature rupture of membranes (PPROM): etiology, diagnosis, classification, international recommendations of treatment options and outcome. *J Perinat Med.* 2018;46(5):465–88. doi: 10.1515/jpm-2017-0027. PubMed PMID: 28710882.
5. Athayde N, Edwin SS, Romero R, Gomez R, Maymon E, Pacora P, et al. A role for matrix metalloproteinase-9 in spontaneous rupture of the fetal membranes. *Am J Obstet Gynecol.* 1998;179(5):1248–53. PubMed PMID: 9822510.
6. Wang H, Ogawa M, Wood JR, Bartolomei MS, Sammel MD, Kusanovic JP, et al. Genetic and epigenetic mechanisms combine to control MMP1 expression and its association with preterm premature rupture of membranes. *Hum Mol Genet.* 2008;17(8):1087–96. doi: 10.1093/hmg/ddm381. PubMed PMID: 18178580.
7. Romero R, Chaiworapongsa T, Espinoza J, Gomez R, Yoon BH, Edwin S, et al. Fetal plasma MMP-9 concentrations are elevated in preterm premature rupture of the membranes. *Am J Obstet Gynecol.* 2002;187(5):1125–30. PubMed PMID: 12439489.
8. Soylyu H, Jefferies A, Diambomba Y, Windrim R, Shah PS. Rupture of membranes before the age of viability and birth after the age of viability: comparison of outcomes in a matched cohort study. *J Perinatol.* 2010;30(10):645–9. doi: 10.1038/jp.2010.11. PubMed PMID: 20220762.
9. Gomez R, Romero R, Nien JK, Medina L, Carstens M, Kim YM, et al. Antibiotic administration to patients with preterm premature rupture of membranes does not eradicate intra-amniotic infection. *J Matern Fetal Neonatal Med.* 2007;20(2):167–73. doi: 10.1080/14767050601135485. PubMed PMID: 17437216.
10. Williams O, Michel B, Hutchings G, Debauche C, Hubinont C. Two-year neonatal outcome following PPRM prior to 25 weeks with a prolonged period of oligohydramnios. *Early Hum Dev.* 2012;88(8):657–61. doi: 10.1016/j.earlhumdev.2012.01.012. PubMed PMID: 22682720.
11. Muraskas J, Astrug L, Amin S. FIRS: Neonatal considerations. *Semin Fetal Neonatal Med.* 2020;25(4):101142. doi: 10.1016/j.siny.2020.101142. PubMed PMID: 32912755.
12. Tchirikov M, Zhumadilov Z, Winarno AS, Haase R, Buchmann J. Treatment of Preterm Premature Rupture of Membranes with Oligo-/Anhydramnion Colonized by Multiresistant Bacteria with Continuous Amnioinfusion and Antibiotic Administrations through a Subcutaneously Implanted Intrauterine Port System: A Case Report. *Fetal Diagn Ther.* 2015. doi: 10.1159/000438483. PubMed PMID: 26447923.

13. Tchirikov M, Bapayeva G, Zhumadilov ZS, Dridi Y, Harnisch R, Herrmann A. Treatment of PPRM with anhydramnion in humans: first experience with different amniotic fluid substitutes for continuous amnioinfusion through a subcutaneously implanted port system. *J Perinat Med*. 2013;41(6):657–63. doi: 10.1515/jpm-2012-0296. PubMed PMID: 23774012.
14. Ono T, Tsumura K, Kawasaki I, Ikeda M, Hideshima M, Tsuda S, et al. Continuous amnioinfusion for treatment of mid-trimester preterm premature rupture of membranes with oligoamnios. *J Obstet Gynaecol Res*. 2020;46(1):79–86. doi: 10.1111/jog.14151. PubMed PMID: 31650672.
15. Esaki M, Maseki Y, Tezuka A, Furuhashi M. Continuous amnioinfusion in women with PPRM at periviable gestational ages. *J Matern Fetal Neonatal Med*. 2020;33(7):1151–6. doi: 10.1080/14767058.2018.1517307. PubMed PMID: 30149736.
16. Locatelli A, Andreani M, Ghidini A, Verderio M, Pizzardi A, Vergani P, et al. Amnioinfusion in preterm PROM: effects on amnion and cord histology. *J Perinatol*. 2008;28(2):97–101. doi: 10.1038/sj.jp.7211876. PubMed PMID: 18046340.
17. Roberts D, Vause S, Martin W, Green P, Walkinshaw S, Bricker L, et al. Amnioinfusion in very early preterm prelabor rupture of membranes (AMIPROM): pregnancy, neonatal and maternal outcomes in a randomized controlled pilot study. *Ultrasound Obstet Gynecol*. 2014;43(5):490–9. doi: 10.1002/uog.13258. PubMed PMID: 24265189.
18. Roberts D, Vause S, Martin W, Green P, Walkinshaw S, Bricker L, et al. Amnioinfusion in preterm premature rupture of membranes (AMIPROM): a randomised controlled trial of amnioinfusion versus expectant management in very early preterm premature rupture of membranes—a pilot study. *Health Technol Assess*. 2014;18(21):1–135. doi: 10.3310/hta18210. PubMed PMID: 24713309.
19. Santis M de, Scavo M, Noia G, Masini L, Piersigilli F, Romagnoli C, et al. Transabdominal amnioinfusion treatment of severe oligohydramnios in preterm premature rupture of membranes at less than 26 gestational weeks. *Fetal Diagn Ther*. 2003;18(6):412–7. PubMed PMID: 14564111.
20. Tchirikov M, Oshovskyy V, Steetskamp J, Falkert A, Huber G, Entezami M. Neonatal outcome using ultrathin fetoscope for laser coagulation in twin-to-twin-transfusion syndrome. *J Perinat Med*. 2011;39(6):725–30. doi: 10.1515/JPM.2011.091. PubMed PMID: 21867454.
21. Tchirikov M, Steetskamp J, Hohmann M, Koelbl H. Long-term amnioinfusion through a subcutaneously implanted amniotic fluid replacement port system for treatment of PPRM in humans. *Eur J Obstet Gynecol Reprod Biol*. 2010;152(1):30–3. doi: 10.1016/j.ejogrb.2010.04.023. PubMed PMID: 20488612.
22. Berger R, Abele H, Bahlmann F, Bedei I, Doubek K, Felderhoff-Müser U, et al. Prävention und Therapie der Frühgeburt. Leitlinie der DGGG, OEGGG und SGGG (S2k-Niveau, AWMF-Registernummer 015/025, Februar 2019) – Teil 2 mit Empfehlungen zur tertiären Prävention der Frühgeburt und zum Management

- des frühen vorzeitigen Blasensprungs. *Z Geburtshilfe Neonatol.* 2019;223(6):373–94. doi: 10.1055/a-1008-8730. PubMed PMID: 31801169.
23. Chen F, Bajwa NM, Rimensberger PC, Posfay-Barbe KM, Pfister RE. Thirteen-year mortality and morbidity in preterm infants in Switzerland. *Arch Dis Child Fetal Neonatal Ed.* 2016;101(5):F377-83. doi: 10.1136/archdischild-2015-308579. PubMed PMID: 27059074.
24. Crane JMG, Magee LA, Lee T, Synnes A, Dadelszen P von, Dahlgren L, et al. Maternal and perinatal outcomes of pregnancies delivered at 23 weeks' gestation. *J Obstet Gynaecol Can.* 2015;37(3):214–24. PubMed PMID: 26001868.
25. Burri PH. Fetal and postnatal development of the lung. *Annu Rev Physiol.* 1984;46:617–28. doi: 10.1146/annurev.ph.46.030184.003153. PubMed PMID: 6370120.
26. Manuck TA, Varner MW. Neonatal and early childhood outcomes following early vs later preterm premature rupture of membranes. *Am J Obstet Gynecol.* 2014;211(3):308.e1-6. doi: 10.1016/j.ajog.2014.05.030. PubMed PMID: 24858202.
27. The royal womens hospital. Rupture of the Membranes - Preterm Premature (PPROM): Policy, Guideline and Procedure Manual [cited 2016 Dec 11]. Available from: <https://thewomens.r.worldssl.net/images/uploads/downloadable-records/clinical-guidelines/rupture-of-membranes-preterm-premature-pprom.pdf>.
28. Romero R, Miranda J, Chaemsaitong P, Chaiworapongsa T, Kusanovic JP, Dong Z, et al. Sterile and microbial-associated intra-amniotic inflammation in preterm prelabor rupture of membranes. *J Matern Fetal Neonatal Med.* 2015;28(12):1394–409. doi: 10.3109/14767058.2014.958463. PubMed PMID: 25190175.
29. Romero R, Friel LA, Velez Edwards DR, Kusanovic JP, Hassan SS, Mazaki-Tovi S, et al. A genetic association study of maternal and fetal candidate genes that predispose to preterm prelabor rupture of membranes (PROM). *Am J Obstet Gynecol.* 2010;203(4):361.e1-361.e30. doi: 10.1016/j.ajog.2010.05.026. PubMed PMID: 20673868.
30. Joyce EM, Moore JJ, Sacks MS. Biomechanics of the fetal membrane prior to mechanical failure: review and implications. *Eur J Obstet Gynecol Reprod Biol.* 2009;144 Suppl 1:S121-7. doi: 10.1016/j.ejogrb.2009.02.014. PubMed PMID: 19303191.
31. Helmig BR, Romero R, Espinoza J, Chaiworapongsa T, Bujold E, Gomez R, et al. Neutrophil elastase and secretory leukocyte protease inhibitor in prelabor rupture of membranes, parturition and intra-amniotic infection. *J Matern Fetal Neonatal Med.* 2002;12(4):237–46. doi: 10.1080/jmf.12.4.237.246. PubMed PMID: 12572592.

32. George RB, Kalich J, Yonish B, Murtha AP. Apoptosis in the chorion of fetal membranes in preterm premature rupture of membranes. *Am J Perinatol.* 2008;25(1):29–32. doi: 10.1055/s-2007-1004828. PubMed PMID: 18075963.
33. Dutta EH, Behnia F, Boldogh I, Saade GR, Taylor BD, Kacerovsky M, et al. Oxidative stress damage-associated molecular signaling pathways differentiate spontaneous preterm birth and preterm premature rupture of the membranes. *Mol Hum Reprod.* 2016;22(2):143–57. doi: 10.1093/molehr/gav074. PubMed PMID: 26690900.
34. Beckmann MW, Wiegratz I, Dereser MM, Baier P, Born HJ. Diagnostik des Blasensprungs: Vergleich des vaginalen Nachweises von fetalem Fibronectin und der intraamnialen Injektion von Indigo Carmine. *Geburtshilfe Frauenheilk.* 1993;53(2):86–91. doi: 10.1055/s-2007-1023643. PubMed PMID: 8462836.
35. Sosa CG, Herrera E, Restrepo JC, Strauss A, Alonso J. Comparison of placental alpha microglobulin-1 in vaginal fluid with intra-amniotic injection of indigo carmine for the diagnosis of rupture of membranes. *J Perinat Med.* 2014;42(5):611–6. doi: 10.1515/jpm-2013-0245. PubMed PMID: 24698819.
36. Pristauz G, Bauer M, Maurer-Fellbaum U, Rotky-Fast C, Bader AA, Haas J, et al. Neonatal outcome and two-year follow-up after expectant management of second trimester rupture of membranes. *Int J Gynaecol Obstet.* 2008;101(3):264–8. doi: 10.1016/j.ijgo.2007.12.007. PubMed PMID: 18289539.
37. Benirschke K, Burton GJ, Baergen RN. *Pathology of the Human Placenta.* 6th ed. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012.
38. Polachek H, Holcberg G, Sapir G, Tsadkin-Tamir M, Polachek J, Katz M, et al. Transfer of ciprofloxacin, ofloxacin and levofloxacin across the perfused human placenta in vitro. *Eur J Obstet Gynecol Reprod Biol.* 2005;122(1):61–5. doi: 10.1016/j.ejogrb.2004.11.031. PubMed PMID: 16154040.
39. Park HS, Ahn B-J, Jun JK. Placental transfer of clarithromycin in human pregnancies with preterm premature rupture of membranes. *J Perinat Med.* 2012;40(6):641–6. doi: 10.1515/jpm-2012-0038. PubMed PMID: 23095192.
40. Onwuchuruba CN, Towers CV, Howard BC, Hennessy MD, Wolfe L, Brown MS. Transplacental passage of vancomycin from mother to neonate. *Am J Obstet Gynecol.* 2014;210(4):352.e1-4. doi: 10.1016/j.ajog.2014.01.019. PubMed PMID: 24679944.
41. Muller AE, Oostvogel PM, DeJongh J, Mouton JW, Steegers EAP, Dorr PJ, et al. Pharmacokinetics of amoxicillin in maternal, umbilical cord, and neonatal sera. *Antimicrob Agents Chemother.* 2009;53(4):1574–80. doi: 10.1128/AAC.00119-08. PubMed PMID: 19164154.
42. Hnat M, Bawdon RE. Transfer of meropenem in the ex vivo human placenta perfusion model. *Infect Dis Obstet Gynecol.* 2005;13(4):223–7. doi: 10.1080/10647440500147992. PubMed PMID: 16338783.

43. Heikkinen T, Laine K, Neuvonen PJ, Ekblad U. The transplacental transfer of the macrolide antibiotics erythromycin, roxithromycin and azithromycin. *BJOG*. 2000;107(6):770–5. PubMed PMID: 10847234.
44. Meeraus WH, Petersen I, Gilbert R. Association between antibiotic prescribing in pregnancy and cerebral palsy or epilepsy in children born at term: a cohort study using the health improvement network. *PLoS One*. 2015;10(3):e0122034. doi: 10.1371/journal.pone.0122034. PubMed PMID: 25807115.
45. Underwood MA, Gilbert WM, Sherman MP. Amniotic fluid: not just fetal urine anymore. *J Perinatol*. 2005;25(5):341–8. doi: 10.1038/sj.jp.7211290. PubMed PMID: 15861199.
46. Gilbert WM, Brace RA. Amniotic fluid volume and normal flows to and from the amniotic cavity. *Semin Perinatol*. 1993;17(3):150–7. PubMed PMID: 8378799.
47. Shields LE, Moore TR, Brace RA. Fetal electrolyte and acid-base responses to amnioinfusion: lactated Ringer's versus normal saline in the ovine fetus. *J Soc Gynecol Investig*. 1995;2(4):602–8. PubMed PMID: 9420864.
48. Fitzsimmons ED, Bajaj T. *StatPearls: Embryology, Amniotic Fluid*. Treasure Island (FL); 2020.
49. Brown WD. Osmotic demyelination disorders: central pontine and extrapontine myelinolysis. *Curr Opin Neurol*. 2000;13(6):691–7. doi: 10.1097/00019052-200012000-00014. PubMed PMID: 11148672.
50. Chhabra A, Kaushik R, Kaushik RM, Goel D. Extra-pontine myelinolysis secondary to hypernatremia induced by postpartum water restriction. *Neuroradiol J*. 2017;30(1):84–7. doi: 10.1177/1971400916678246. PubMed PMID: 28059675.

Tables

Due to technical limitations, Tables 1 and 2 are only available as a download in the Supplemental Files section.

Figures

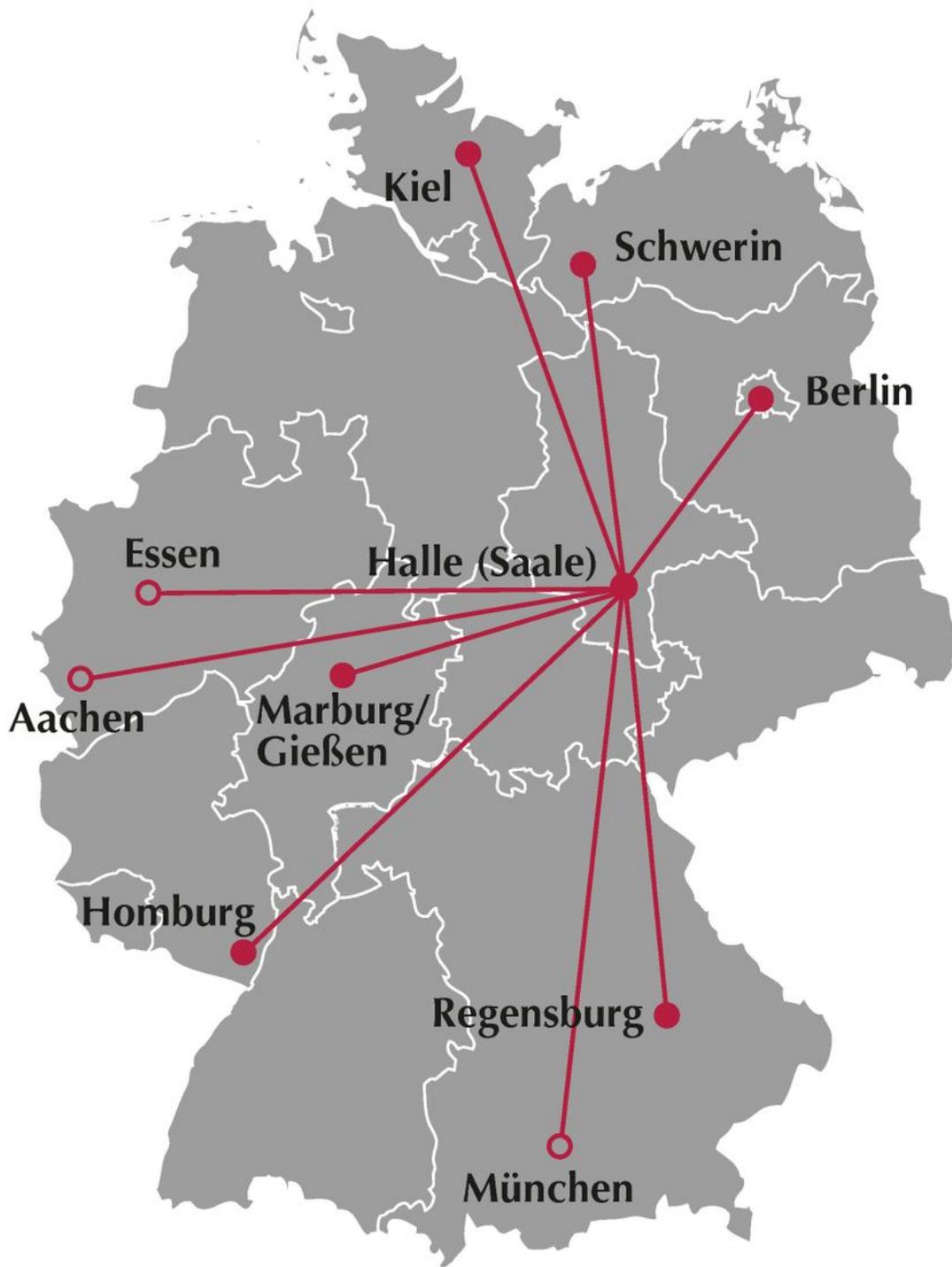


Figure 1

German tertiary perinatal centers taking part in the TRIAL ClinicalTrials.gov: NTC04696003

(ClinicalTrials.gov ID: NCT04696003)

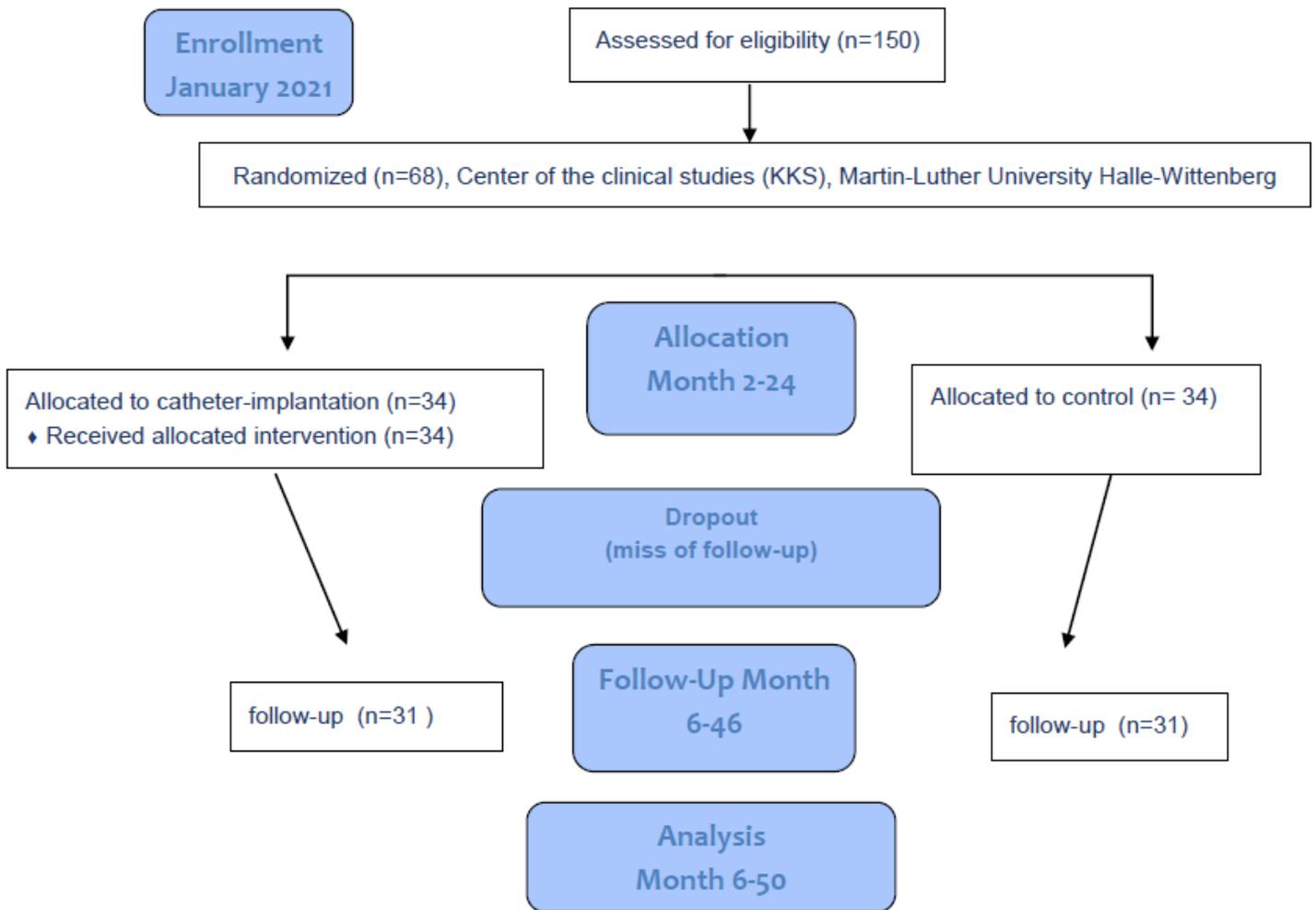


Figure 2

Flow diagram of the TRIAL Comparison between the Amniotic Flush Solution and standard saline solutions in relation to the human amniotic fluid.

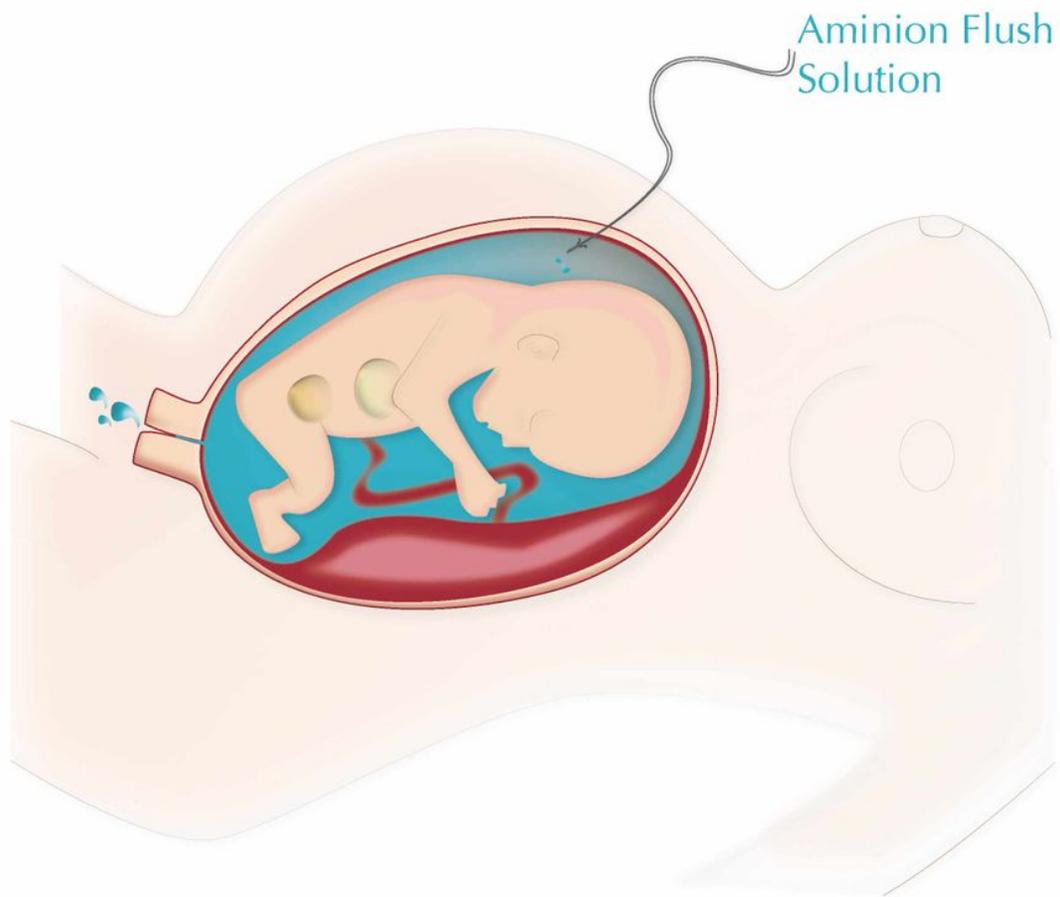


Figure 3

The schema of Amnion Flush Method

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