Statistical Analysis Plan

FINAL

AEPC study on Effect of fetal aortic valvuloplasty on outcomes. A prospective observational cohort study with a comparison cohort

Version 2021-03-18

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List of abbreviations

Abbreviation	Definition
BMI	Body mass index
BSA	Body surface area
BV	Biventricular circulation
CI	Confidence interval
EDD	Estimated date of delivery
EFE	Endocardial fibroelastosis
eCRF	Electronic case report form
FAS_{BIRTH}	Full analysis set at birth population
FAS_{ENR}	Full analysis set enrolled population
FAV	Fetal aortic valvuloplasty
GA	Gestational age
GW	Gestational weeks
IUD	Intrauterine death
LA	Left atrium
MR	Mitral regurgitation
OR	Odds ratio
PHT	Pulmonary hypertension
PP	Per protocol
PP_{BIRTH}	Per protocol at birth population
PP_{ENR}	Per protocol enrolled population
SDS	Standard deviation score
ToP	Termination of pregnancy
UV	Univentricular circulation

List of definitions

Term	Definition
Gestational age	Gestational Age = (280 - (Estimated Delivery Date - Reference Date)) / 7. Reported as weeks and days of gestational age.
Biventricular circulation	The systemic circulation supported only by the left ventricle and the pulmonary by the right ventricle (+/- PFO/ASD)
Univentricular circulation	The systemic circulation supported completely or partly by the right ventricle
Absence of pulmonary hypertension	A TR max velocity ≤ 2.8 m/s with no other echocardiographic signs of pulmonary hypertension and/or catheter data showing a mean pulmonary arterial pressure <25 mmHg

1 STUDY DETAILS

1.1 Study Objectives

Primary objective:

To evaluate if fetal valvuloplasty in aortic stenosis improves outcomes up to 2 years after birth compared with no fetal valvuloplasty, measured with transplantation-free survival from fetal diagnosis to 2 years postnatal age with a biventricular circulation without pulmonary hypertension at that time.

Secondary objective:

To evaluate fetal and maternal safety of fetal valvuloplasty.

- Intervention-related fetal death (defined as fetal death within 24 hours of procedure).
- Fetal death not directly related to the intervention, except termination of pregnancy.
- Maternal complications to procedure (requiring intensive care or resulting in maternal death).
- Preterm delivery < 37 weeks.
- Fetal left heart growth from the point of study inclusion until just before the first postnatal catheter or surgical intervention

1.2 Study Design

The study is an international multicenter prospective observational cohort study with a comparison cohort. Included are fetuses with a diagnosis of aortic stenosis who satisfy inclusion/exclusion criteria between 23+0 and 31+6 weeks of gestation. The decision whether a fetal balloon dilatation shall be attempted is not part of the study protocol. The number of examinations of mother/fetus/infant in this study is not different from the number of examinations that will be recommended for someone choosing not to be part of this study. The participation in the study is not affecting the treatment mothers and fetuses are receiving during pregnancy, nor how the infant is examined and treated after birth. The aim of the study is to, in a prospective and organized way, collect and evaluate multi-center data in order to reduce the risk for selection bias, missing data and inter-variability between participating centers.

Cases will be recruited from centers offering fetal aortic valvuloplasty and and from centers not offering this treatment. All intervention cases will be recruited from high volume

intervention centres which have performed at least 20 fetal cardiac interventions during the last five years before including the first case in the study. Fetal and postnatal echocardiographic examinations will be reviewed and remeasured by a core laboratory to confirm eligibility for inclusion, and identify potential measurement errors.

Data from two fetal echoes and one postnatal will be collected and each contains a comprehensive set of two-dimensional and Doppler measurements which will enable analysis of cardiac growth and development during pregnancy. Analysis of change of dimensions of the left heart structures and selected hemodynamic variables from the point of study inclusion until just before the first postnatal catheter or surgical procedure provides an opportunity to make a comparison between the groups that is unbiased with respect to centerspecific postnatal treatment policies. Three echocardiographic examinations and a 2-year follow-up regarding patient outcome will be performed as per Figures 1-3 below.

Figure 1. Study Overview – Fetal Protocol

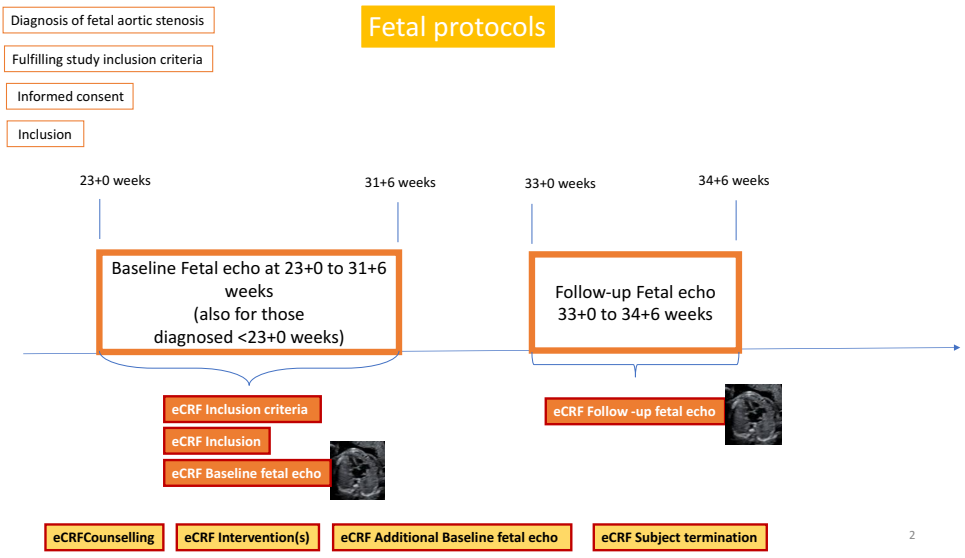


Figure 2. Study Overview – Neonatal Protocol

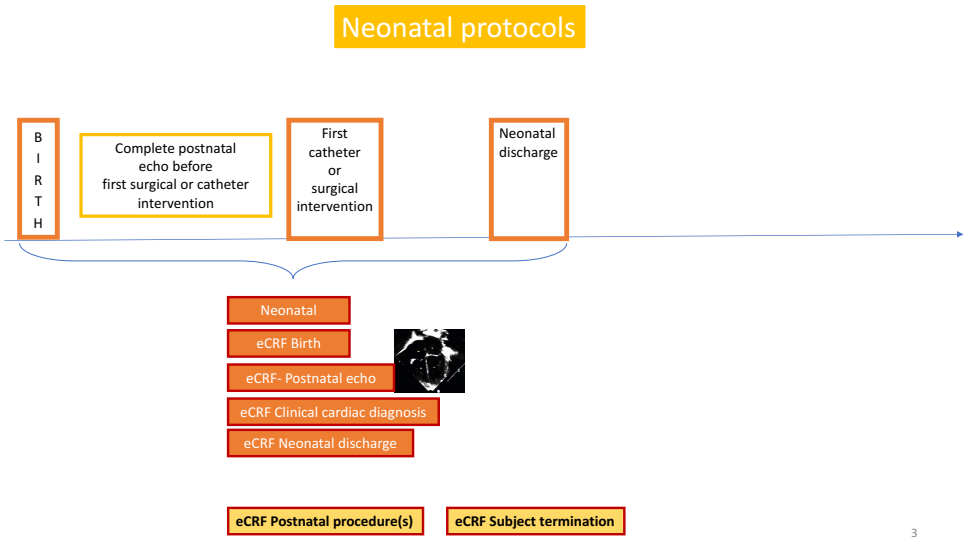
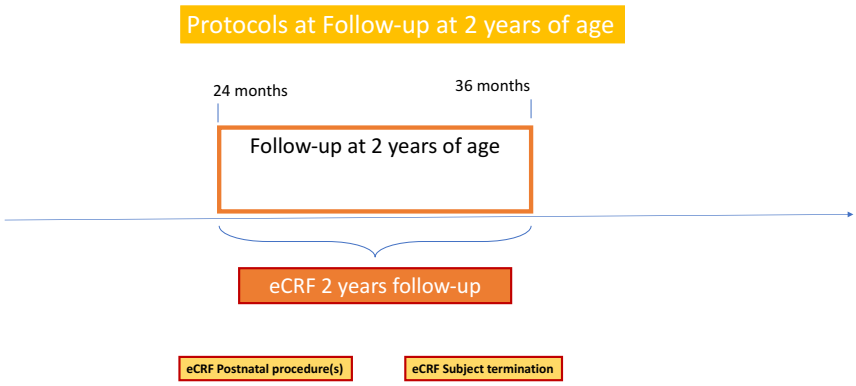


Figure 3. Study Overview – Protocol at Follow-up at 2 Years of Age



1.3 Inclusion and Exclusion Criteria

1.3.1 Inclusion Criteria

Inclusion criteria represent currently used criteria for fetal intervention in aortic stenosis. They are identical in the intervention and the non-intervention groups.

A. All of the following echocardiographic criteria need to be satisfied between 23+0 and 31+6 weeks (z-scores* according to schneider):

1 Aortic valve stenosis with antegrade flow through the valve

2 Predominantly left-to-right shunt at the atrial level

3 Predominantly retrograde flow in the aortic arch between the first two brachiocephalic vessels

4 Qualitatively depressed left ventricular function

5 LV end-diastolic diameter z-score* > +/-0

6 Left ventricular inlet length in diastole, z-score* according to gestational age

a. GA ≤ 24+6: Z-score > ±0
b. GA 25+0 to 27+6: Z-score > −0.75
c. GA ≥ 28+0: Z-score > −1.50

7 Mitral valve diameter in diastole z-score* > −2.0

B. All of the following postnatal treatment options need to be available:

1 Surgical or catheter based aortic valvotomy

2 Ross-Konno surgery

3 Norwood or hybrid stage-one surgery

1.3.2 Exclusion criteria

1. Any associated cardiac defect except persistent left superior vena cava and coarctation of the aorta.
2. Any significant (i.e. that might influence outcome) extracardiac anomaly and/or known chromosomal aberration. Also, if such a condition is present at inclusion but diagnosed only after birth the case will be retrospectively excluded.

1.4 Study Groups

The main analysis comparing intervention versus non-intervention will be on an intention-to-treat, i.e. intention to perform fetal aortic valvuloplasty, basis. Intention to perform fetal valvuloplasty is defined as being present if the needle has been introduced through the maternal abdominal wall even if the fetal thorax has not been touched. Subjects will be

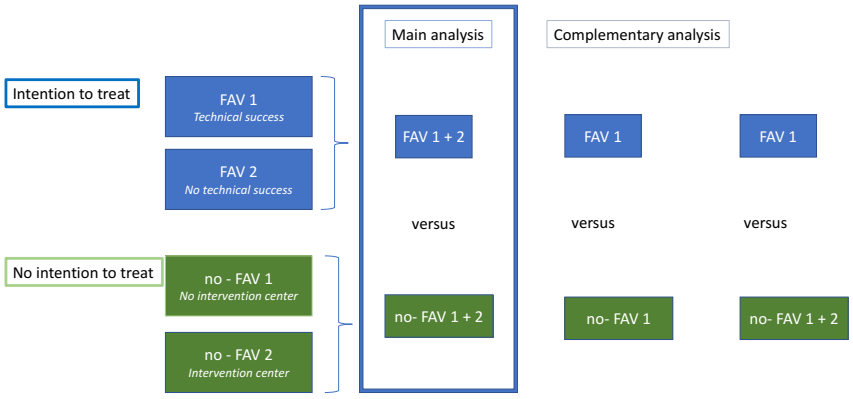
identified as belonging to the fetal aortic valvuloplasty (FAV) or the no-FAV groups as listed below.

The following groups will be identified:

- Subjects that undergo a technically successful fetal aortic valvuloplasty at a high volume intervention centre (FAV 1).
- Subjects with intention-to-treat (according to the above definition) at a high volume intervention centre but with no technical success (FAV 2).
- Subjects counselled at a center not performing or referring for fetal aortic valvuloplasty (no-FAV 1).
- Subjects counselled at a high- or low-volume intervention center, or at a center offering referrals to a center performing interventions, and the parental decision is to not perform fetal aortic valvuloplasty (no-FAV 2).

The main analyses will be to compare FAV 1+2 versus no-FAV 1+2. The expected proportion of enrolled fetuses/infants will be 2 [FAV] : 1 [no-FAV]. Complementary analyses will be comparing FAV 1 versus no-FAV 1, and FAV 1 versus no-FAV 1+2.

Figure 4. Main and complementary analysis



1.5 Sample Size

The duration of this observational study will depend on the rate of inclusion. Based on our previous experience from a European retrospective study, and on the increasing prenatal detection rate of cardiac defects, it is estimated that 10-20 intervention cases can be included each year. Inclusion rate of non-intervention cases will depend on how many non-intervention

centers decide to join the study and on the proportion of subjects offered intervention who choose to refrain. It is projected that at least half as many non-intervention cases can be included each year. The study plans to end when 100 FAV cases are enrolled and at least 50 no-FAV cases, or 5 years from the first included patient, whichever occurs first.

The power calculations below are performed applying two-sided Fisher's exact test. The main analyses, however, will require adjusted analyses due to the study design being observational and not randomized. The confounders and their relation to the outcome and the main exposure variable are not completely known at the time of the study planning. Therefore, simplified power calculations were performed in order to assure that the study has potential to answer the main posed questions.

Inclusion of 100 intervention cases and 50 controls

The aim is to include at least 100 fetuses in the FAV 1+2 groups and 50 in the comparison group no-FAV 1+2. The largest numbers will likely be in FAV 1 and no-FAV 1 while the numbers in groups FAV 2 and probably also in no-FAV 2 will be smaller.

Assuming number of fetuses included as per above, 150 in total, and 30% success rate for the primary endpoint (=transplantation-free survival from fetal diagnosis to 2 years postnatal age, having a biventricular circulation without pulmonary hypertension at that time) in the no-FAV 1+2 group and 60% success rate in the FAV 1+2 group, using alpha 0.05 and two-sided Fisher's exact test, a power of 91.8% would be able to be achieved. For a power of at least 80%, and all other assumptions unchanged, a success rate of 56% vs 30% in the FAV and no-FAV group, respectively, would be needed to be observed.

Inclusion of 100 intervention cases and 100 controls

Assuming number of fetuses included as per above, 200 in total, and 30% success rate for the primary endpoint in the no-FAV 1+2 group and 60% success rate in the FAV 1+2 group, using alpha 0.05 and two-sided Fisher's exact test, a power of 98.7% would be able to be achieved. For a power of at least 80%, and all other assumptions unchanged, a success rate of 51% vs 30% in the FAV and no-FAV group, respectively, would be needed to be observed.

2 STUDY POPULATIONS

2.1 Definition of Study Populations

2.1.1 Full Analysis Set for All Enrolled

Fetuses with severe MR and severe LA enlargement and/or intact or highly restrictive inter-atrial communication and/or cases with hydrops present at inclusion are all high-risk cases regarding survival, but may have a rather good prognosis if they survive the first critical period. It is likely they will be overrepresented in the FAV group to such a degree that it will be difficult to control for through propensity score matching. Therefore this subpopulation will be excluded from the full analysis set and analysed separately. Fetuses developing any of these conditions after inclusion will not be excluded from the full analysis set.

All enrolled fetuses that have satisfied inclusion/exclusion criteria and belong to FAV 1+2 and no-FAV 1+2 groups and not listed as belonging to the high-risk population described above will be included in the Full Analysis Set enrolled (FAS_{ENR}) population. Infants wrongly included in the study (not fulfilling all inclusion or fulfilling any of the exclusion criteria) will not be part of the FAS_{ENR} population, but will be described in the safety analyses. As detailed under 1.3.2, also subjects with exclusion criteria present at inclusion but diagnosed only after birth will be retrospectively excluded from the FAS_{ENR} population.

2.1.2 Full Analysis Set for Live Births Intended for Postnatal Treatment

All enrolled fetuses that have satisfied inclusion/exclusion criteria and belong to FAV 1+2 and no-FAV 1+2 groups and not listed as the high-risk population above that also are live at birth will be included in the Full Analysis Set at birth (FAS_{BIRTH}) population. Infants wrongly included in the study will not be part of the FAS_{BIRTH} population, but will be described in the safety analyses.

2.1.3 Per-Protocol Population for All Enrolled

All enrolled fetuses with no major protocol violations will be included in the Per Protocol enrolled (PP_{ENR}) population. The final decisions regarding the PP_{ENR} population will be taken at the Clean File meeting before the database lock.

2.1.4 Per-Protocol Population for Live Births Intended for Postnatal Treatment

All enrolled fetuses that are *live at birth and intended for treatment* with no major protocol violations will be included in the Per Protocol at birth (PP_{BIRTH}) population. The final decisions

regarding the PP_{BIRTH} population will be taken at the Clean File meeting before the database lock.

2.1.5 High-Risk Population

The high-risk population will include all subjects with any of the following conditions at inclusion: Severe MR and severe LA enlargement and/or intact or highly restrictive inter-atrial communication and/or hydrops. Fetuses developing any of these conditions after inclusion will not be analysed as belonging to the high-risk population.

2.1.6 Safety Population

All enrolled fetuses will be included in the safety population, also those wrongly included if such cases will exist.

Figure 5. Full analysis sets (FAS)

ToP=Termination of Pregnancy; IUD=Intrauterine Death; GW=Gestational Weeks; FAS=Full Analysis Set; FAV=Fetal Aortic Valvuloplasty

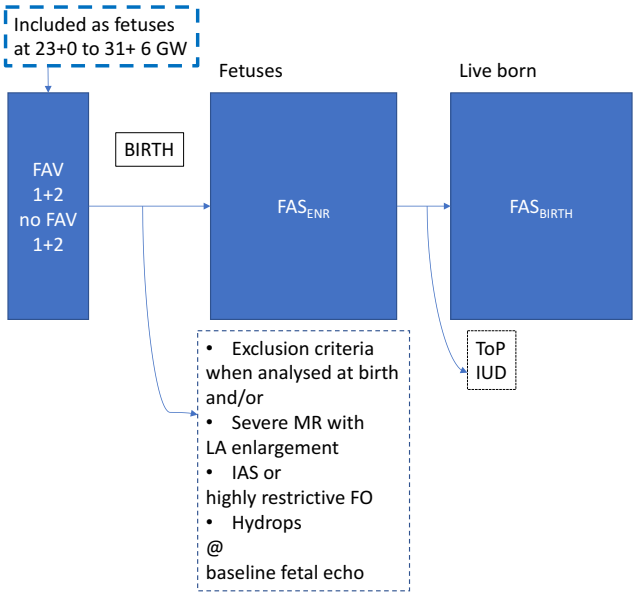


Figure 6. Per protocol populations (PP)

ToP=Termination of Pregnancy; IUD=Intrauterine Death; GW=Gestational Weeks; PP=Per Protocol; FAV=Fetal Aortic Valvuloplasty

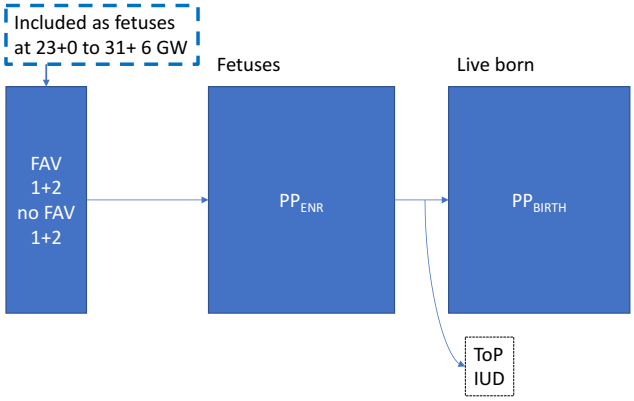
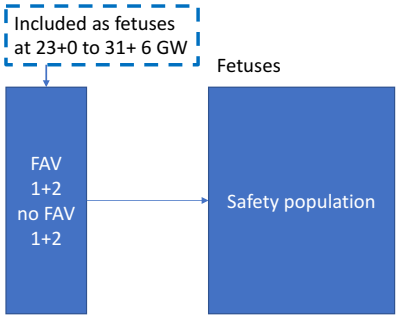


Figure 7. Safety population

GW=Gestational Weeks; FAV=Fetal Aortic Valvuloplasty



3 STUDY VARIABLES

3.1 Fetal and Pregnancy Variables

The following characteristics regarding the pregnancy and the fetus will be collected at inclusion and baseline fetal echo:

- Gestational age at primary diagnosis (weeks and days) (potential confounder)
- Year of diagnosis (potential confounder)
- Primary diagnostic hospital, city and country

- Average yearly case volume of center performing fetal intervention - (potential confounder)
- Gestational age at baseline fetal echo (weeks and days) (potential confounder)
- Hospital, city and country where each echo was performed
- Maternal age
- Parity
- Multiple pregnancy (potential confounder)
- Mother's body mass index (BMI) (potential confounder)
- Maternal comorbidities
- Maternal contraindications to fetal intervention
- Echo data (baseline fetal echo). Only those considered as potential confounders listed here (for a full set of variables for all three echoes, 2 fetal and 1 neonatal, please see the annotated eCRF):
    - Left ventricular end-diastolic diameter (mm and z-score) (potential confounder)
    - Left ventricular inlet length in diastole (mm and z-score) (potential confounder)
    - Mitral valve diameter, 4 ch view in end diastole (potential confounder)
    - Aortic valve annulus diameter (mm) (potential confounder)
    - Mitral valve inflow pattern (Biphasic; Fused; Monophasic; No inflow) (potential confounder)
    - LV:RV inlet length ratio (potential confounder)

3.2 Neonatal Variables

The following characteristics will be collected for the neonate in addition to the neonatal echo (for details please see annotated CRF):

- Gestational age at birth (weeks and days)
- Gender
- Mode of delivery (vaginal, caesarean)
- Apgar score (at 1 min and 5 min)

- Birth weight (g)
- Birth weight standard deviation score (SDS)
- Birth height (cm)
- Birth height SDS
- Body surface area (BSA) (m^2)
- Head circumference (cm)
- Head circumference SDS
- Intention to treat
- Emergency procedures before 24 hrs of age
- Prostaglandin treatment
- Inotropic drugs
- Diagnostic details of the left heart before postnatal intervention
- Other clinical cardiac diagnoses before intervention
- Initial surgical pathway
- Clinical cardiac diagnoses at neonatal discharge
- Non-cardiac diagnoses at neonatal discharge

3.3 Follow-up at Two Years Variables

The following variables will be collected at two years follow-up.

- Age at follow-up (months)
- Weight (kg)
- Weight SDS
- Height (cm)
- Height SDS
- Type of circulation (univentricular or biventricular)
- Pulmonary hypertension if biventricular

- Detailed cardiac diagnosis
- Non-cardiac diagnoses
- Neurological sequelae
- List of all cardiac surgical and catheter procedures performed

3.4 Efficacy Variables

3.4.1 Primary Efficacy Variable

The primary efficacy variable is transplantation-free survival until 2 years postnatal age, having a biventricular circulation without pulmonary hypertension at that time. Diagnosis of pulmonary hypertension will be based on catheter/echo/clinical data and graded as mild, moderate or severe. This means that death, transplantation and biventricular circulation with pulmonary hypertension or no biventricular circulation will be part of this composite endpoint coded as yes/no. This variable cannot be analysed as time to event variable due to the fact that biventricular circulation and pulmonary hypertension are evaluated at 2 years of age. Early discontinuations for other reasons than death, without transplantation and with biventricular circulation without pulmonary hypertension at the time of early discontinuation will be counted in no event group.

The primary variable will be analysed on FAS_{ENR} (main analysis), FAS_{BIRTH}, PP_{ENR} and PP_{BIRTH}, overall for FAV 1+2 vs no-FAV 1+2 (main analysis), and descriptively FAV 1 vs no-FAV 1 and FAV 1 vs no-FAV 1+2.

3.4.2 Secondary Efficacy Variables

Secondary efficacy variables in this study are as following:

- Transplantation-free survival from fetal diagnosis to 2 years of age (analysed as time to event on FAS_{ENR} (main analysis), FAS_{BIRTH} (followed from birth), PP_{ENR} and PP_{BIRTH} (followed from birth), overall for FAV 1+2 vs no-FAV 1+2 (main analysis), and descriptively FAV 1 vs no-FAV 1 and FAV 1 vs no-FAV 1+2).
- Survival from fetal diagnosis to live birth (analysed as time to event on FAS_{ENR} (main analysis) and PP_{ENR}, overall for FAV 1+2 vs no-FAV 1+2 (main analysis), and descriptively FAV 1 vs no-FAV 1 and FAV 1 vs no-FAV 1+2).

- Proportion of included patients who have a biventricular circulation at neonatal hospital discharge (analysed on FAS_{ENR} (main analysis), FAS_{BIRTH} , PP_{ENR} and PP_{BIRTH}, overall for FAV 1+2 vs no-FAV 1+2 (main analysis), and descriptively FAV 1 vs no-FAV 1 and FAV 1 vs no-FAV 1+2).
- Proportion of included patients who have a biventricular circulation at 2 years of age (analysed on FAS_{ENR} (main analysis), FAS_{BIRTH} , PP_{ENR} and PP_{BIRTH}, overall for FAV 1+2 vs no-FAV 1+2 (main analysis), and descriptively FAV 1 vs no-FAV 1 and FAV 1 vs no-FAV 1+2).
- Number of postnatal catheter and/or surgical procedures before 2 years of age (analysed on FAS_{BIRTH} (main analysis) and PP_{BIRTH}, overall for FAV 1+2 vs no-FAV 1+2 (main analysis), and descriptively FAV 1 vs no-FAV 1 and FAV 1 vs no-FAV 1+2).

3.5 Safety Variables

3.5.1 Fetal Left Heart Growth

Change of dimensions of left heart structures and hemodynamics from the baseline fetal echo to the neonatal echo performed before the first postnatal intervention. The following variables will be analysed.

- Mitral valve diameter in diastole (z-score)
- Left ventricular inlet length (z-score)
- Direction of flow in the aortic arch (antegrade/retrograde)
- Left ventricular diastolic dimension (z-score)
- Left ventricular function
- Shunt direction through foramen ovale (left-to-right, right-to-left, bidirectional)

3.5.2 Fetal aortic valvuloplasty - technical details

The following details about the fetal cardiac intervention are among those that will be recorded:

- Type of maternal anesthesia

- Pre- and procedural fetal medication
- Fetal resuscitation drugs used
- Size of needle
- Number of cardiac punctures
- Diameter of balloon
- Balloon to aortic valve diameter ratio
- Inflation pressure
- Number of inflations
- Technical success by criteria

3.5.3 Fetal and maternal complications of fetal intervention

Safety of the prenatal procedure will be evaluated through the following variables:

- Intervention-related fetal death (defined as fetal death within 24 hours of procedure)
- Fetal death not directly related to the intervention, except termination of pregnancy
- Maternal complications to procedure (requiring intensive care or resulting in maternal death)
- Preterm delivery < 37 weeks
- Reasons for fetal or postnatal death

3.5.4 Events

All postnatal cardiac surgical or catheter interventions from birth to 2 years of age will be listed.

4 STATISTICAL METHODOLOGY

4.1 General Methodology

Continuous variables will be described by means, standard deviation (SD), median and range, and categorical by number and percentages.

Main analyses will be performed comparing FAV 1+2 versus no-FAV 1+2 groups on FAS_{ENR} population. Complementary analyses will be performed on FAS_{BIRTH}, PP_{ENR} and PP_{BIRTH}. Separate descriptive comparisons between FAV 1 vs no-FAV 1 and FAV 1 vs no-FAV 1+2 groups will also be performed.

Comparisons between the two groups with respect to demographics and baseline characteristics and safety data will be performed using Fisher's exact test for dichotomous variables, Mantel-Haenszel Chi-square test for ordered categorical variables, Chi-square test for non-ordered categorical variables, and Fisher's non-parametric permutation test for continuous variables.

Due to the non-randomised design, all analyses will be adjusted for confounders. It is important that all known confounders at the time of inclusion are defined. The main confounders will be gestational age at first diagnosis and at inclusion, morphology and hemodynamics of the fetal heart at the time of study inclusion and possibly some data about the mother's health which could have an impact on the chance of success with fetal intervention, such as BMI. Although the study is designed to minimize bias by using strict echocardiographic inclusion criteria, it will be necessary to analyse the groups at baseline carefully with respect to a number of selected echocardiographic variables describing the size and function of the left heart. If this analysis shows that, in spite of the strict inclusion criteria, there are significant differences between the groups regarding data that are at the same time risk factors for the studied outcomes the analyses will be adjusted for those additionally defined confounders besides the known ones. A sensitivity analysis will also be performed for the primary variable applying propensity score matching procedure (1:1 or 2:1 if possible) including all variables that are of importance ($p<0.10$ using logistic regression) for discrimination of FAV vs no-FAV group and those identified/known confounders in the adjusted analyses.

Known confounders are:

- Gestational age at primary diagnosis (earlier gestational age could indicate more severe disease, and might differ between countries and centres due to different

screening policies) and gestational age at inclusion. Most probably they are highly correlated and in that case the more influential of the two will be used.

Variables listed under 3.1 as potential confounders will be investigated whether they are important for discriminating FAV vs no-FAV and whether they are confounders (associated $p<0.10$ both to the group and the outcome).

Dichotomous outcome variables will be analysed using logistic regression, adjusted for identified confounders available at the time of study inclusion. Odds-ratios (OR) with 95% confidence intervals (CI) will be presented.

Time-to-event analyses will be performed using Cox proportional hazards models adjusting for identified confounders. Proportional hazards assumption will be checked by visually reviewing log(-log(survival)) vs log(time) graphs, and by adding interaction between treatment group and log(time) in the model, where significant interaction indicates non-proportionality. Other methods will be applied, e.g. Poisson regression for time varying data, allowing study of changes in treatment effects over follow-up time, in case non-proportionality of hazards occurs.

Time-to-event data will be described by using Kaplan-Meier technique.

The primary analyses for the FAV vs no-FAV group conducted on FAS_{ENR} population will be considered as confirmatory analyses and a total significance mass of 0.05 will be applied. The tests will be performed on the primary variable and the most important secondary variables using fix sequential testing procedure according to the following order:

1. Primary variable: Transplantation-free survival from fetal diagnosis to 2 years postnatal age, having a biventricular circulation without pulmonary hypertension at that time

2. Secondary variable: Transplantation-free survival from fetal diagnosis to 2 years of age

3. Secondary variable: Survival from fetal diagnosis to live birth at term (≥37 weeks)

4. Secondary variable: Proportion of included fetuses who have a biventricular circulation at neonatal hospital discharge

5. Secondary variable: Proportion of included fetuses who have a biventricular circulation at 2 years of postnatal age

This means that first test must be significant on 0.05 significance level in order to be able to further confirm the tests 2 to 5, and the second test must be significant on 0.05 level in order to be able to confirm tests number 3 to 5, etc.

Analysis of longitudinal change of left heart size and function, will be conducted in an exploratory manner. This will be investigated using mixed models for repeated data, with normal distribution for z-scores and binomial distribution for binary variables, handling time in study both as distinct visits and continuous (gestational age) as found most appropriate. Linear and non-linear models will be investigated. Model selection will be based on lowest goodness of fit statistics, e.g. Akaike's Information Criterion. Even though a $p<0.05$ the tests will not be considered confirmed, since not included in the multiplicity corrections above. Estimates, 95% CI, and p-values will be evaluated for descriptive purpose.

All tests will be two-tailed. All analyses will be performed by using SAS software version 9.4 or later (SAS Institute Inc., Cary, NC, USA).

4.2 Patient Disposition and Data Sets Analysed

The number of patients included in each of the FAS_{ENR}, FAS_{BIRTH}, PP_{ENR}, PP_{BIRTH}, and safety populations will be summarized for each treatment group. Patients who completed the study and who withdrew from study prematurely will also be presented with a breakdown of the reasons for withdrawal by treatment group.

4.3 Protocol Violations/Deviations

Major protocol deviations are those that are considered to have an effect on the analysis. The protocol violations will be continuously collected during the study. The clinical monitors of the study will review the list and the finalisation of the major protocol deviations will be done at the clean file meeting.

The number of patients with major protocol deviations will be summarized per treatment group.

4.4 Fetal, Pregnancy, Neonatal and Follow-up at 2 Years Variables

Fetal, pregnancy, neonatal and follow-up at 2 years variables will be summarized by treatment group for the FAS and PP populations and analysed according to the methods described in section General Methodology above.

4.5 History of cardiac surgical procedures and catheter interventions

This will be described per study group for the FAS populations.

4.6 Efficacy Analyses

4.6.1 Primary Efficacy Analysis

Primary efficacy analysis, test between FAV vs no-FAV regarding transplantation-free survival from fetal diagnosis to 2 years postnatal age, having a biventricular circulation without pulmonary hypertension at that time, will be performed using logistic regression adjusted for confounders identified according to the section General Methodology above. OR with 95% CI will be presented. The primary analysis will be performed on FAS_{ENR} population and will compare FAV 1+2 versus no-FAV 1+2. This analysis will be included in the evaluation of the confirmatory tests. Complementary analyses will be performed on FAS_{BIRTH}, PP_{ENR}, PP_{BIRTH} populations, and on all populations comparing FAV 1 with no–FAV 1 and FAV 1 vs no-FAV 1+2.

Sensitivity analyses will be performed using Fisher's exact test comparing the FAV group with the matched no-FAV group obtained applying propensity score matching according to the description in General Methodology above, both on FAS and PP populations.

Vertical bar charts will be presented for graphical purpose.

4.6.2 Secondary Efficacy Analyses

Secondary variables will be analysed according to described methods in General Methodology above.

4.6.3 Subgroup Analyses

Descriptive data on primary and secondary outcomes will be performed on following subgroups for FAV 1+2 vs no-FAV 1+2, FAS_{ENR} and FAS_{BIRTH} populations:

- Subgroup 1: Fetuses with diagnosis of fetal aortic stenosis that undergo technically successful FAV and additional fetal cardiac intervention(s) such as atrial septal balloon dilatation or stent placement.
- Subgroup 2 (part of the High-risk population): Fetuses with diagnosis of fetal aortic stenosis and hydrops.
- Subgroup 3 (equals to High-risk population): Fetuses with diagnosis of fetal aortic stenosis, severe mitral regurgitation and left atrial enlargement with or without restrictive or intact atrial communication and with or without hydrops.

4.7 Safety Analyses

4.7.1 Fetal Left Heart Growth

Fetal left heart growth will be described longitudinally and analysed in exploratory manner as specified in the General Methodology above.

4.7.2 Intervention and Technical Details

Information about intervention and its technical details will be summarized on safety population per treatment groups.

4.7.3 Complications of fetal intervention and maternal complications

Data regarding complications and reasons for death will be described for safety population per treatment groups. No formal tests will be performed.

4.7.4 Events

Events will be described per study group on safety population, as number of events and number of infants with a certain event.

5 INTERIM ANALYSES

No interims analyses are planned.

6 CHANGES OF ANALYSIS FROM PROTOCOL

There are no significant changes from the original protocol which was submitted together with the application for ethical approval, except that the statistical analysis plan has been elaborated in more detail. However this has not resulted in any change of the protocol which could have ethical consequences.

7 LISTING OF TABLES AND FIGURES

7.1 Listing of Tables

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7.2 Listing of Figures

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