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REVIEW ARTICLE

Sildenafil for Antenatal Treatment of Congenital Diaphragmatic Hernia: From Bench to Bedside

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Abstract: ***Background***: Persistent pulmonary hypertension (PPH) is one of the main causes of mortality and morbidity in infants affected by congenital diaphragmatic hernia (CDH). Since the structural changes that lead to PPH take place already in utero, a treatment starting in the prenatal phase may prevent the occurrence of this complication.

***Objective*:** To summarize the development process of antenatal sildenafil for CDH.

***Methods*:** The pharmacokinetics and efficacy of sildenafil have been assessed in the rat and the rabbit model. The transfer of the drug through the human placenta has been measured with the *ex-vivo* placenta perfusion model. Results from this experiment are being incorporated in a pregnancy-physiologically based pharmacokinetic (p-PBPK) model. A phase I-IIb placental transfer and safety study is ongoing.

***Results*:** Sildenafil administration to pregnant rats and rabbits led to therapeutic foetal drug levels without maternal and foetal toxicity, although it was associated with impaired vascular development in foetuses with non-hypoplastic lungs. Peak concentrations and 24-hour exposure were higher in pregnant rabbits compared to non-pregnant ones. In rat and rabbit foetuses with CDH, sildenafil rescued the lung vascular anomalies and partially improved parenchymal development. Sildenafil crossed the human placenta at a high rate *ex-vivo*, independently from the initial maternal concentration.

***Conclusion*:** There is preclinical evidence that maternally administered sildenafil prevents the vascular changes that lead to PPH in CDH newborns. The phase I/IIb clinical study together with the p-PBPK model will define the maternal dose needed for a therapeutic effect in the foetus. Foetal safety will be investigated both in the clinical study and in the sheep. The final step will be a multicentre, randomized, placebo-controlled trial.

Keywords: Foetal therapy, congenital diaphragmatic hernia, pulmonary hypertension, sildenafil, transplacental transfer, lung development.

1. THE DISEASE: PULMONARY HYPERTENSION ASSOCIATED WITH CONGENITAL DIAPHRAGMATIC HERNIA

Congenital diaphragmatic hernia (CDH) is a rare disease, occurring in 1 every 2,000–4,000 live births and accounting for 8% of all major congenital anomalies [1]. The classical hypothesis is that failure to fully form the diaphragm in the first trimester of pregnancy results in a diaphragmatic defect. As a consequence, abdominal organs herniate into the thorax, disturb lung development and eventually cause pulmonary hypoplasia. However, studies in rodents have demonstrated that pulmonary hypoplasia in CDH occurs prior to diaphragm development [2, 3]. These results led Keijzer and co-workers to formulate the "dual-hit hypothesis" [4]. According to this theory, CDH is a primary lung pathology, whose major features occur as a result of two independent developmental insults. The first insult affects both lungs and occurs before the diaphragm has fully developed, in a background of genetic and environmental factors. The second hit affects diaphragm formation and further impairs the development of the ipsilateral lung through compression of this lung by the herniated abdominal organs.

CDH lungs are characterized by a lower number of generation of airways, less and smaller alveoli, thickened alveolar walls and an increased amount of interstitial tissue [5], so that there is less alveolar airspace and gas exchange surface area. Parallel to airway changes, there is an equal reduction in arteries, resulting in a hypoplastic vascular bed. Morphologically, the thickening of the vascular wall is determined by the increase in arterial media and adventitia, by neomuscularisation of the small pulmonary arteries, which are normally partially or non-muscularised [6, 7] and by hypermuscularisation of the midsized and large vessels (>100μm) [8]. The structural remodeling of the small pulmonary arteries reduces their ability to dilate, in order to increase the vascular bed capacity and reduce the pressure in the pulmonary circulation during perinatal transition [9]. After birth, further muscularisation of this "immature" pulmonary vasculature occurs, leading to abnormal response to mechanical and chemical stimuli [10]. These structural changes in the lung vascular compartment lead to persistent pulmonary hypertension (PPH), which cannot simply be solved by surgically repairing the diaphragm. During perinatal transition, the elevated pulmonary vascular resistance in the abnormally developed lung leads to right-to-left shunting of de-oxygenated blood across to the systemic circulation. This results in hypoxaemia and ultimately, right ventricular failure with systemic hypotension and shock [11]. PPH, especially in the context of CDH, is frequently lethal [1] and non-responsive to the most commonly used vasodilatory agents, such as inhaled nitric oxide (NO) [12, 13]. In the retrospective series, the presence and severity of PPH has been proven to predict pulmonary morbidity and death [14, 15]. Also, beyond neonatal pulmonary hypertension, late (months after birth) and chronic (years after birth) pulmonary hypertension deeply affect the quality of life in CDH survivors [16].

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2. PRENATAL INTERVENTIONS FOR CDH: RATIONALE AND SELECTION OF CANDIDATES

Despite the optimization of postnatal strategies, the average mortality of CDH remains 30%, and is in many cases related to PPH [17]. Although PPH only becomes clinically evident after birth, the structural changes that lead to it occur already *in utero* [6, 9]. An ideal therapeutic strategy would, therefore, be to act already in the prenatal phase, in order to prevent such changes. In foetal lungs, in contrast to adults, both the vascular endothelium and the smooth muscle cells exist in a "synthetic" or "primed" state and their growth and differentiation can be modulated by exogenous stimuli [18, 19]. Therefore, in foetuses the potential for vascular cell growth is increased, provided an intervention can target this.

Obviously, any antenatal intervention requires a method for adequate case selection.

Today, prenatal ultrasound screening allows identifying 70% of CDH cases by the second trimester of pregnancy [20]. In isolated cases, the combination of ultrasound markers (side of the defect, the presence of liver herniation and lung size) allows to personalize prognosis and to stratify CDH foetuses into groups with increasing pulmonary hypoplasia and corresponding mortality rates [21]. The literature on prediction of PPH is more limited (systematically reviewed in [22]). Although several candidate parameters have been suggested in individual case series, including measurements of lung size [23-26], as well as herniation of abdominal organs into the chest [24, 27-29], the latter systematic review did not identify a strong predictor for PPH. New studies are currently being performed to validate the previously suggested indices and to identify new and better predictors. Among these, direct assessment of the pulmonary vasculature [30-32] and assessment of left ventricle dimensions [33] may provide additional information.

The foetuses with the worst prognosis could be offered intrauterine treatment. Foetal lung growth can be stimulated by tracheal occlusion (TO), which is currently offered as a percutaneous procedure (FETO). Although promising outcomes have been reported [34], FETO remains investigational hence is evaluated in a randomized clinical trial in foetuses with left-sided CDH and severe and moderate hypoplasia (www.totaltrial.eu) [35]. The moderate TOTAL-trial has already recruited more than 85% of the sample size (158 patients analysed at the fourth interim analysis in 12/2018). The arm for severe CDH started later [36], and the first interim analysis was done in 10/2017 (46 patients).

Independently from the outcome of that trial, FETO remains a complex, not widely available invasive procedure with an inherent risk for preterm delivery, partly offsetting the beneficial effects of foetal therapy [37]. The maximum post-FETO survival reported in severe cases is 50–60%, which in part is caused by insufficient airway growth and, above all, limited improvement of vascular development. Indeed, whereas FETO increases lung size, it does not seem to solve the problem of PPH [38]. Alternative prenatal strategies are therefore required to also address the problem of PPH. Preferentially, these would be medical rather than surgical, to overcome the risks and limitations of foetal procedures. The best solution would be a treatment for PPH already proven safe and effective postnatally. Ideally, the drug should be effective after maternal intake, and should have no significant adverse effects on the mother or foetus.

3. THE STUDY DRUG: SILDENAFIL

Sildenafil is a selective inhibitor of phosphodiesterase type 5 (PDE 5), which specifically degrades cyclic guanosine monophosphate and is found in high concentrations in the pulmonary arteries and the corpora cavernosa. Normally, endothelium-derived NO stimulates the production of cyclic guanosine monophosphate (cGMP), which then mediates smooth muscle relaxation. By inhibiting PDE5, sildenafil inhibits the breakdown of cGMP to GMP, thus prolongs the actions of cGMP. Higher cellular concentrations of cGMP stimulate the formation of cGMP-dependent protein kinase (PKG). Competitive inhibition of PDE3 by sildenafil also inhibits the breakdown of cyclic adenosine monophosphate (cAMP), which results in increased production of cAMP-dependent protein kinase (PKA) [39]. Vasodilation results from modulation of ion channel activity by cGMP and, to a minor extent, cAMP. Furthermore, PKA and PKG modulate smooth muscle proliferation. Therefore, in contrast with conventional vasodilators, which improve haemodynamics but do not inhibit the histological progression of the disease, sildenafil may prevent or even reverse vascular remodelling [40] (Fig. **1**). Sildenafil is administered orally, is well tolerated with few drug interactions, and does not require intensive monitoring, making it an attractive alternative to other drugs for the treatment of PPH. Although PDE 5 is expressed in all visceral and vascular smooth muscle cells, sildenafil has only a modest effect on systemic blood pressure [41]. In the context of pulmonary hypertension, sildenafil is currently approved for the adult and paediatric population [42]. There is a growing interest for the use of sildenafil for the treatment of PPH of various aetiologies in the newborn [43], including CDH [44-46], in particular when other therapies such as inhaled NO fail. The efficacy of postnatal sildenafil treatment in CDH newborns is currently evaluated in a randomized controlled trial within the "ERNICA" European Reference Network (CoDiNOS trial, EudraCT number: 2017-000421-13). The drug is also approved for the treatment of erectile dysfunction.

4. ANIMAL STUDIES

4.1. Efficacy of Sildenafil in Animal Models for CDH

Luong et al (2011) were the first to evaluate the efficacy on transplacental sildenafil *in the nitrofen rat* model for CDH [47]. When administered subcutaneously to the dams from gestational day (GD) 11.5 to 20.5 (term= GD21), sildenafil increased pulmonary vessel density, reduced vessel wall thickness and right ventricular hypertrophy and improved postnatal pulmonary artery relaxation in CDH foetuses. This observation has been confirmed in the same model by several subsequent studies with different experimental designs [48, 49], even when sildenafil was given at a more relevant time point in pregnancy, mimicking administration at a later stage of lung development [50, 51]. The nitrofen rodent is an 'early' model, which mimics disturbances of lung and diaphragm development from early gestation. However, foetal lung development in rats differs from humans, since alveolarisation only occurs after birth.

We therefore investigated the effects of antenatal sildenafil in a larger animal model (*the rabbit*), because it has a lung development similar to man. When CDH is surgically induced in healthy foetal rabbits in their pseudoglandular phase (GD23 out of 31), it reproduces the clinical phenotype in various aspects [52, 53]. The rabbit model also permits a more detailed assessment of foeto-maternal safety, because placental transfer in the latter half of pregnancy mirrors that in humans [54]. The rabbit mid-gestational and end-gestational placenta are functionally positioned between rodents (haemotrichorial) and man (haemomonochorial) [55]. Also, pregnancy-induced haemodynamic changes are comparable with those present in women, that is, with a steady increase in maternal blood pressure in the latter half of gestation [54].

After surgical induction of CDH, does were randomized to receive a daily subcutaneous injection of either sildenafil (10 mg/kg) or placebo from GD24 to GD30. At term, does were euthanized to harvest the foetuses [56]. The proportionate medial thickness of peripheral lung vessels was significantly higher in CDH pups compared with controls, yet fell in the normal range when CDH foetuses were exposed to sildenafil. These vessels were also more often muscularised in CDH foetuses than in controls, whereas sildenafil treatment was associated with a degree of peripheral ves-

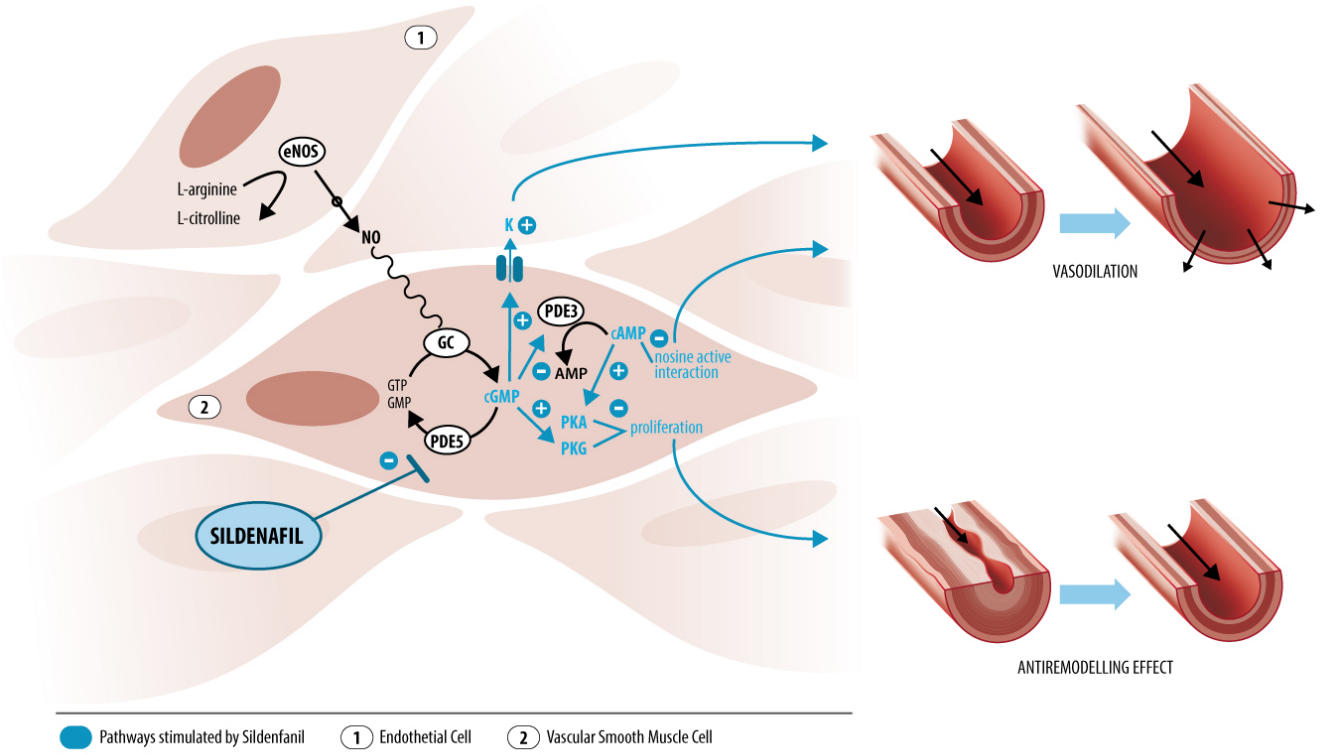

Fig. (1). Schematic representation of the mechanism of action of sildenafil on the pulmonary vasculature. eNOS, endothelial nitric oxide synthase; NO, nitric oxide; GC, guanylate cyclase; GTP, guanosine triphosphate; GMP, guanosine triphosphate; cGMP, cyclic GMP; PDE, phosphodiesterase; ATP, adenosine triphosphate; AMP, adenosine monophosphate; cAMP, cyclic AMP; PKG, cGMP-dependent protein kinase; PKA, cAMP-dependent protein kinase. Reproduced with permission from UZ Leuven, Leuven, Belgium. Drawing: Myrthe Boymans.

sels muscularisation in the normal range. Sildenafil was also associated with an increase in immunoreactivity for vascular endothelial growth factor (VEGF) and its receptor Flk-1 in the lung parenchyma, both in CDH foetuses and in controls. VEGF is of paramount importance in lung development, as it contributes to pulmonary angiogenesis and vessel growth, with subsequent alveolar maturation and adequate matching of ventilation and perfusion [57, 58]. The decreased VEGF expression in the lung parenchyma is a consistent finding in CDH, both in humans [59] and in animal models [47, 56, 60, 61].Sildenafil might upregulate VEGF expression by activation of endothelial nitric oxide synthase (eNOS) [62]. The analysis of vessel branching on reconstructed 3D images acquired with micro-CT demonstrated that the placebo-exposed CDH foetuses had significantly fewer vessels of the fifth order or higher (further called 'peripheral') compared with controls. Again, the number of peripheral vessels of sildenafil-exposed CDH foetuses was in the normal range (Fig. **2**). Of interest, the proportion of peripheral vessels was reduced in control foetuses treated with sildenafil. The mechanism behind this finding remains unclear but may be related to a reduction in pulmonary blood pressure [63]. This could be beneficial in lungs with increased vascular resistance but might lead to adverse effects, such as hypoperfusion and impaired vessel growth in normal lungs. Finally, we could demonstrate reduced *in utero* pulmonary vascular resistance by micro-ultrasound in both CDH and control foetuses treated with sildenafil. Sildenafil also had an effect on the lung parenchyma. At birth, sildenafil exposed foetuses had a normal mean terminal bronchiolar density. Functionally, sildenafil improved postnatal lung mechanics as demonstrated by Flexivent ventilation.

In summary, in the rabbit model, sildenafil reversed the pathological changes in peripheral vessels, improved vascular branching and also improved airway morphometry in CDH. All these changes might be expected to prevent the development of PPH.

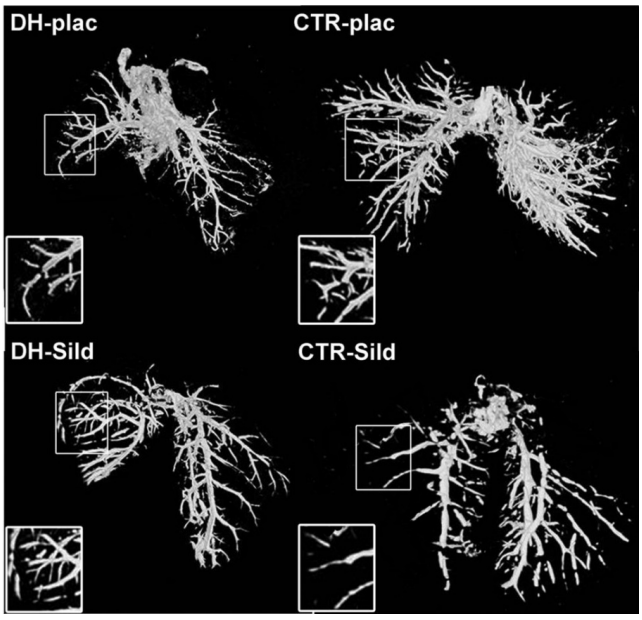

Fig. (2). Effect of sildenafil on lung vasculature in the CDH rabbit model. Representative 3D surface rendered images showing impaired vascular branching in CDH-foetuses, which is restored by exposure to sildenafil**.** From Russo et al [89]. Reproduced with permission from BMJ Publishing Group Ltd.

4.2. Transplacental Transfer of Sildenafil: Preclinical Pharmacokinetic and Pharmacodynamics

In the study by Luong et al [47], sildenafil was administered subcutaneously to the dams at a dose of 50 mg/Kg/d, and was detected in foetal blood with a peak at 6 hours. CDH was associated with a significant decrease in lung cGMP concentration and increased activated PDE 5 expression. Maternal sildenafil treatment produced a marked increase in lung cGMP in CDH foetuses and in controls and a significant attenuation in active PDE 5 expression, indicating that sildenafil is biologically active in the foetal lung. The expression on VEGF was also increased by sildenafil.

In the rabbit, a dose of 10 mg/Kg/d led to therapeutic foetal plasma concentrations for at least 22 h/day [56]. The target therapeutic interval in the foetus was between 47 and 500 ng/mL, according to previous studies on pulmonary hypertension. Peak concentrations in excess of 500 ng/mL in humans have been previously shown to cause a 40% incidence of visual disturbances and 25% of vascular events [64]. A target of 47 ng/mL was selected so that the concentration of unbound sildenafil would produce a 53% inhibition of PDE 5 activity [65]. This was also the target concentration used in the trial for FDA approval of sildenafil to treat pulmonary arterial hypertension in children [66]. Sildenafil kinetics was similar in the maternal and foetal circulation, with a peak at 30 minutes and concentrations above the maximal target (500 ng/ml) for the first hour. Of note, plasmatic sildenafil concentrations in foetal and maternal plasma 22 hours after the third day of therapy did not differ from those 22 hours after the first injection, indicative that the drug is not accumulating.

We also investigated the effect of pregnancy on the pharmacokinetics of sildenafil in rabbits [67]. Using NONMEM, we performed population pharmacokinetic modelling based on plasma samples from pregnant and non-pregnant rabbits. All animals received a single subcutaneous sildenafil administration at the same dose used in the efficacy study (10 mg/kg). One sample was obtained per rabbit at different time-points up to 22 hours after the injection. Compared to non-pregnant rabbits, the central and peripheral volume of distribution and inter-compartmental clearance of sildenafil were lower in pregnant rabbits. The formation clearance from sildenafil to its metabolite desmethylsildenafil was also reduced during pregnancy. Therefore, pregnancy leads to an increased peak concentration and 24-hour exposure for sildenafil. The pregnancy-related alterations in the pharmacokinetic profiles of sildenafil in rabbits demonstrate that extrapolation from non-pregnant to pregnant subjects might be inappropriate, or even hazardous, and should be validated in pregnant women.

4.3. *Ex-vivo* Study: Transfer of Sildenafil Through the Human Placenta

It was therefore essential to determine clinically which dose would need to be given to a mother in order to obtain therapeutic foetal sildenafil levels. This information is at present missing. We therefore first evaluated the transfer of sildenafil through the human placenta, by using an *ex-vivo* placenta perfusion model [68]. Dual perfusion of a single placental lobule is a validated experimental model to study human placental transfer of substances and reliably predict foetal exposure to maternally administered drugs [69]. Placentas were collected after term delivery from healthy volunteers, cannulated and dually perfused. Sildenafil citrate was added to the maternal circulation at two different concentrations: the maximum tolerated concentration (MC, 500 ng/mL) and the therapeutic concentration (TC, 50 ng/mL), respectively. Samples were collected from both the foetal and the maternal reservoir at different time-points for three hours and the concentration of sildenafil was determined. The ratio between foetal and maternal concentrations (FM ratio) was calculated for each time-point.

Sildenafil crossed the placenta at both initial maternal concentrations. Both maternal and foetal levels reached a plateau at 90-120 minutes. At steady state, the FM ratio was above 0.9 for both MC and TC placentas, indicating that sildenafil crosses the placenta at relatively high rates independently from the maternal concentration. This suggests that there is sufficient placental transfer to reach target foetal drug levels at non-toxic maternal doses.

4.4. Phase I-IIb Trial: *In-vivo* Transplacental Transfer and Safety

Based on the *ex-vivo* data, and on the information on the use of sildenafil in pregnancy coming from other studies, we have designed and initiated recruitment for a phase I-IIb clinical trial (EudraCT number 2016-002619-17) [70]. This is a randomized, investigator blinded, double armed parallel groups study with the primary objective to measure the *in-vivo* transfer of sildenafil through the placenta in women at two gestational ages: the second and early third trimester (women undergoing termination of pregnancy at weeks 20.0 to 32.6) and term (women with CDH foetuses at weeks 36.6 to 40). These correspond to the period when sildenafil treatment would start (i.e. after diagnosis and prediction of prognosis, which is most accurate after 20 weeks, but ideally before the end of the canalicular stage [71]), and end. Participants are randomized to two different sildenafil doses (25 or 75 mg), both already used in clinical studies in the pregnant population [72, 73]. In the second trimester study, a single dose of the investigational product is administered before termination of pregnancy, and maternal and foetal blood samples are collected at the time of foeticide for determination of sildenafil concentration. In the study in term women, sildenafil is administered three times daily from three days before planned delivery until actual delivery, following which maternal and umbilical cord samples are collected. Indices of short term maternal and foetal tolerance and of the vasodilatory effect of sildenafil on the foetal pulmonary circulation are also measured.

4.5. *In silico* Model: A Pregnancy-Physiologically-Based Pharmacokinetic Model to Predict Human Foetal Exposure After Maternal Administration of Sildenafil

Transplacental parameters estimated from the *ex-vivo* model can be implemented in pregnancy–physiologically based pharmacokinetic (p-PBPK) models. This approach has special relevance in relation to recent guidance from regulatory agencies on drug development during pregnancy and lactation. p-PBPK modelling and simulation can complement clinical trials for this vulnerable patient population [74].

After building and validating a PBPK model for sildenafil in the adult population, we are now creating a p-PBPK model by implementing physiological parameters (e.g. gestational age-dependent changes in maternal weight, individual organ volumes/blood flows, cardiac output, glomerular filtration rate, and drug-metabolizing enzyme activities), data from the placenta transfer experiment and data on the foetal and amniotic fluid compartment. The present approach will enable a basic prediction of foetal pharmacokinetic prior to drug administration to the mother. This will be a useful tool to integrate the results of our phase I-IIb dose-finding clinical study, and to help elucidate appropriate dosing information.

4.6. Additional Preclinical Studies: The Lamb Model

Sheep are a higher species, size-wise more comparable to humans, and their foetal physiology is very similar to that of humans. Surgical induction of CDH at GD65 (term = 145 days) leads to pulmonary hypoplasia with airway and vascular changes similar to clinical autopsy specimens [75]. The main advantage of this model is that foetal/newborn lambs can undergo catheterization, therefore pulmonary resistances and flow can be measured directly [76, 77]. Evidence in the sheep model is already present for tadalafil, another PDE 5 inhibitor [78], which had a biological effect on the foetal lung. However, there is little clinical experience on the use of tadalafil during pregnancy, and use of this drug in the neonatal and

infant population is considered contraindicated by some authors due to lack of maturation of the glucuronidation pathway [79]. Sildenafil is therefore a more logic choice.

We are now conducting with the group of Prof Hooper at the Ritchie Centre (Melbourne, Australia) studies on transplacental sildenafil administration to foetal lambs with CDH.

Furthermore, together with the group of Prof Flake at Children's Hospital of Philadelphia, we have designed an experiment on a unique lamb model to further investigate the effects of sildenafil in CDH foetuses. This group has recently developed an extra-uterine support system that mimics the uterine environment and reproduces foetal physiology in foetal lambs [80]. The system permits to keep extremely preterm foetal lambs (GD106) surviving for up to four weeks, maintain physiological hemodynamics, integrity of the foetal circulation, organ growth, and lung maturation, and transition to extra-uterine life. This "Extracorporeal Support of the Premature Infant" (ESPI) model offers a unique opportunity to investigate foetal pharmacologic therapy by removing the confounding effects of the maternal-placental axis. Furthermore, this is the only setting allowing continuous foetal monitoring (both invasive and with ultrasound) and is therefore ideal to assess the effect of sildenafil on the foetal (pulmonary) circulation and other hemodynamic effects on the organs. This will provide a unique set of data on foetal efficacy and safety of sildenafil treatment. It will be the first application of ESPI for studying pharmacologic effects on the foetus.

Results from both those sheep studies will further support clinical translation. Since lambs can be ventilated and monitored for several hours after birth, these experiments will provide additional information on foetal and short-term neonatal safety.

4.7. Maternal and Foetal Safety: Available Data from Other Clinical Studies

Sildenafil has already been used in pregnant women. Several case reports on the chronic use of sildenafil for treatment of maternal pulmonary hypertension demonstrated efficacy without apparent maternal or foetal side effects [81-83]. The drug has also been used in case series for treatment of early onset pre-eclampsia [73] and intrauterine growth restriction (IUGR) [84] and in randomized clinical trials for the treatment of IUGR (STRIDER trial) [72] and oligohydramnios [85]. Its use during labor to reduce foetal distress is being assessed in an ongoing randomized controlled trial [86]. A systematic review demonstrated that sildenafil administration is safe for the mother [87]. However, the Dutch arm of the STRIDER trial on maternal sildenafil administration for intrauterine growth restriction was recently suspended, because of the lack of benefit yet "potential signal of harm relating to an increased incidence of persistent pulmonary hypertension" [88]. This finding mandates additional studies on foetal safety and stresses the concept that the drug should only be prescribed in the context of high-quality clinical trials.

However, we believe that this suspicion should not lead to the immediate suspension of all studies on sildenafil in pregnancy. There are several arguments for that. First, the increased incidence of PPH in the Dutch trial is not consistent with results of the other STRIDER-studies in the UK and New Zealand/Australia (data not yet published), nor with other studies on antenatal sildenafil administration. Second, assuming there would be an association between sildenafil and PPH in IUGR foetuses, this should not automatically be extrapolated to other clinical conditions, such as women with CDH-foetuses. In the case of CDH, foetal lung development is impaired hence not comparable to that in case of growth restriction. The effect of sildenafil on the lung vasculature is therefore expected to be different. The different effects of sildenafil on normal and abnormal lungs have already been shown in animal studies, including our study in the rabbit model [50, 89].

In conclusion, antenatal sildenafil still represents a promising strategy for CDH, but confirming foetal safety is of utmost importance. Useful information on short term foetal and neonatal tolerance will be provided by our phase I-IIb clinical trial as well by the ongoing studies in the sheep model. After those, a phase IV randomized, placebo-controlled trial will allow to investigate also long and medium term safety.

CONCLUSION

In the past years, we and others have explored the potential of repurposing sildenafil as a transplacental therapy to prevent the occurrence of PPH in CDH foetuses. This review summarizes the

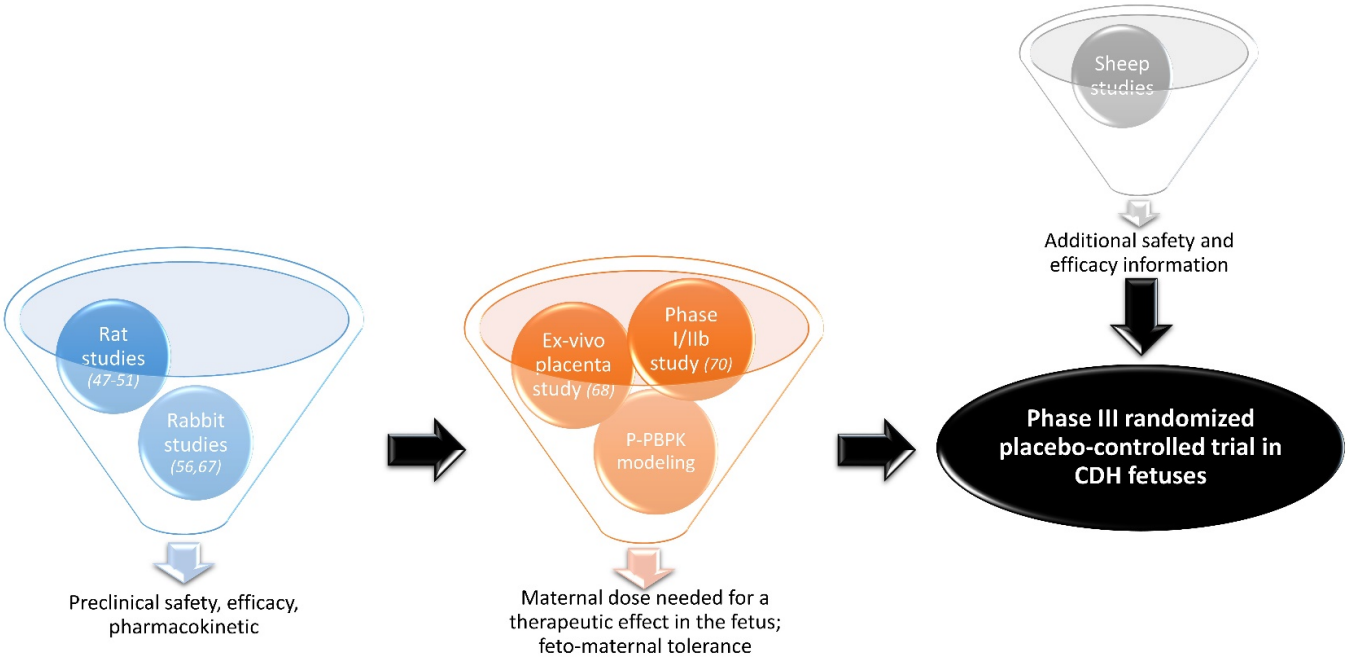

Fig. (3). Schematic representation of the developmental plan for sildenafil as a foetal treatment in CDH.

drug development process up to now and the ongoing steps to clinical application (Fig. **3**). Given the results of preclinical studies, we were granted orphan designation of this drug for the prenatal prevention of PPH in CDH from the European Medical Agency [90]. If foetal safety and efficacy are confirmed by the current studies, the final step will be a multicentre, randomized, placebo-controlled trial to demonstrate efficacy of sildenafil in reducing the occurrence of PPH in CDH infants. Recently, the European Commission has supported the creation of European virtual thematic networks (European Reference Networks), aimed at sharing knowledge, exchanging information and facilitating collaborations between specialized centres. "ERNICA" is the European reference network on rare diseases of the foregut (including CDH) grouping skills from 20 centres in 11 countries. Such network will facilitate randomized clinical trials on CDH foetuses, despite the rarity of the condition.

Further research is ongoing both in humans and in animal models to improve the understanding of the pathophysiology of the foetal pulmonary circulation and the mechanisms causing PPH. All this might allow individualisation of antenatal treatments and optimisation of their timing, dosing and duration."

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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REFERENCES

[1] Kotecha S, Barbato A, Bush A, *et al.* Congenital diaphragmatic hernia. Eur Respir J 2012; 39 (4): 820-9.

[2] Cilley RE, Zgleszewski SE, Krummel TM, Chinoy MR. Nitrofen dose-dependent gestational day-specific murine lung hypoplasia and left-sided diaphragmatic hernia. Am J Physiol 1997; 272 (2 Pt 1): L362-71.

[3] Allan DW, Greer JJ. Pathogenesis of nitrofen-induced congenital diaphragmatic hernia in fetal rats Journal of applied physiology (Bethesda, Md : 1985) 1997; 83 (2): 338-47.

[4] Keijzer R, Liu J, Deimling J, Tibboel D, Post M. Dual-hit hypothesis explains pulmonary hypoplasia in the nitrofen model of congenital diaphragmatic hernia. Am J Pathol 2000; 156 (4): 1299-306.

[5] Kitagawa M, Hislop A, Boyden EA, Reid L. Lung hypoplasia in congenital diaphragmatic hernia. A quantitative study of airway, artery, and alveolar development. Br J Surg 1971; 58 (5): 342-6.

[6] Rottier R, Tibboel D. Fetal lung and diaphragm development in congenital diaphragmatic hernia. Semin Perinatol 2005; 29 (2): 86-93.

[7] Shehata SM, Sharma HS, van der Staak FH, van de Kaa-Hulsbergen C, Mooi WJ, Tibboel D. Remodeling of pulmonary arteries in human congenital diaphragmatic hernia with or without extracorporeal membrane oxygenation. J Pediatr Surg 2000; 35 (2): 208-15.

[8] Kool H, Mous D, Tibboel D, de Klein A, Rottier RJ. Pulmonary vascular development goes awry in congenital lung abnormalities. Birth Defects Res C Embryo Today 2014; 102 (4): 343-58.

[9] Shehata SM, Tibboel D, Sharma HS, Mooi WJ. Impaired structural remodelling of pulmonary arteries in newborns with congenital diaphragmatic hernia: A histological study of 29 cases. J Pathol 1999; 189 (1): 112-8.

[10] Peacock A. Vasodilators in pulmonary hypertension. Thorax 1993; 48 (12): 1196-9.

[11] Storme L, Aubry E, Rakza T, *et al.* Pathophysiology of persistent pulmonary hypertension of the newborn: impact of the perinatal environment. Arch Cardiovasc Dis 2013; 106 (3): 169-77.

[12] Bagolan P, Morini F. Long-term follow up of infants with congenital diaphragmatic hernia. Semin Pediatr Surg 2007; 16 (2): 134-44.

[13] Inhaled nitric oxide and hypoxic respiratory failure in infants with congenital diaphragmatic hernia. Pediatrics 1997; 99 (6): 838-45.

[14] Lusk LA, Wai KC, Moon-Grady AJ, Steurer MA, Keller RL. Persistence of pulmonary hypertension by echocardiography predicts short-term outcomes in congenital diaphragmatic hernia J Pediatr 2015; 166 (2 25): 1-6.

[15] Vanamo K, Rintala R, Sovijärvi A, *et al.* Long-term pulmonary sequelae in survivors of congenital diaphragmatic defects. J Pediatr Surg 1996; 31 (8): 1096-9.

[16] Behrsin J, Cheung M, Patel N. Sildenafil weaning after discharge in infants with congenital diaphragmatic hernia. Pediatr Cardiol 2013; 34 (8): 1844-7.

[17] van den Hout L, Schaible T, Cohen-Overbeek TE, *et al.* Actual outcome in infants with congenital diaphragmatic hernia: the role of a standardized postnatal treatment protocol. Fetal Diagn Ther 2011; 29 (1): 55-63.

[18] Gao Y, Cornfield DN, Stenmark KR, Thébaud B, Abman SH, Raj JU. Unique aspects of the developing lung circulation: structural development and regulation of vasomotor tone. Pulm Circ 2016; 6 (4): 407-25.

[19] Owens GK. Molecular control of vascular smooth muscle cell differentiation and phenotypic plasticity Novartis Foundation symposium 2007; 283: 174-91. discussion 91-3, 238-41

[20] Gallot D, Boda C, Ughetto S, Perthus I, Robert-Gnansia E, Francannet C, *et al.* Prenatal detection and outcome of congenital diaphragmatic hernia: A French registry-based study Ultrasound in obstetrics & gynecology : The official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2007; 29 (3): 276-83.

[21] Jani J, Nicolaides KH, Keller RL, Benachi A, Peralta CF, Favre R, *et al.* Observed to expected lung area to head circumference ratio in the prediction of survival in fetuses with isolated diaphragmatic hernia Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2007; 30 (1): 67-71.

[22] Russo FM, Eastwood MP, Keijzer R, Al-Maary J, Toelen J, Van Mieghem T, *et al.* Lung size and liver herniation predict need for extracorporeal membrane oxygenation but not pulmonary hypertension in isolated congenital diaphragmatic hernia: systematic review and meta-analysis Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2017; 49 (6): 704-13.

[23] Heling KS, Wauer RR, Hammer H, Bollmann R, Chaoui R. Reliability of the lung-to-head ratio in predicting outcome and neonatal ventilation parameters in fetuses with congenital diaphragmatic hernia Ultrasound in obstetrics & gynecology : The official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2005; 25: 112-8.

[24] Lusk LA, Wai KC, Moon-Grady AJ, Basta AM, Filly R, Keller RL. Fetal ultrasound markers of severity predict resolution of pulmonary hypertension in congenital diaphragmatic hernia. Am J Obstet Gynecol 2015; 213 (2): 216.e1-8.

[25] Ruano R, Takashi E, da Silva MM, Campos JA, Tannuri U, Zugaib M. Prediction and probability of neonatal outcome in isolated congenital diaphragmatic hernia using multiple ultrasound parameters Ultrasound in obstetrics & gynecology : The official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2012; 39 (1): 42-9.

[26] Ruano R, Takashi E, da Silva MM, Haeri S, Tannuri U, Zugaib M. Quantitative lung index, contralateral lung area, or lung-to-head ratio to predict the neonatal outcome in isolated congenital diaphragmatic hernia? Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine 2013; 32 (3): 413-7.

[27] Basta AM, Lusk LA, Keller RL, Filly RA. Fetal Stomach Position Predicts Neonatal Outcomes in Isolated Left-Sided Congenital Diaphragmatic Hernia. Fetal Diagn Ther 2015.

[28] Done E, Gratacos E, Nicolaides KH, Allegaert K, Valencia C, Castanon M, *et al.* Predictors of neonatal morbidity in fetuses with severe isolated congenital diaphragmatic hernia undergoing fetoscopic tracheal occlusion Ultrasound in obstetrics & gynecology :

The official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2013; 42 (1): 77-83.

[29] Jani JC, Benachi A, Nicolaides KH, Allegaert K, Gratacos E, Mazkereth R, *et al.* Three-dimensional ultrasonographic measurements of the fetal lungs for prediction of perinatal outcome in isolated congenital diaphragmatic hernia The journal of obstetrics and gynaecology research 2009; 35 (6): 1031-41.

[30] Ruano R, Aubry MC, Barthe B, Dumez Y, Benachi A. Three-dimensional ultrasonographic measurements of the fetal lungs for prediction of perinatal outcome in isolated congenital diaphragmatic hernia. J Obstet Gynaecol Res 2009; 35 (6): 1031-41.

[31] Ruano R, Aubry MC, Barthe B, Mitanchez D, Dumez Y, Benachi A. Quantitative analysis of fetal pulmonary vasculature by 3-dimensional power Doppler ultrasonography in isolated congenital diaphragmatic hernia. Am J Obstet Gynecol 2006; 195 (6): 1720-8.

[32] Ruano R, Aubry MC, Barthe B, Mitanchez D, Dumez Y, Benachi A. Predicting perinatal outcome in isolated congenital diaphragmatic hernia using fetal pulmonary artery diameters. J Pediatr Surg 2008; 43 (4): 606-11.

[33] Kinsella JP, Steinhorn RH, Mullen MP, *et al.* The Left Ventricle in Congenital Diaphragmatic Hernia: Implications for the Management of Pulmonary Hypertension. J Pediatr 2018; 197: 17-22.

[34] Al-Maary J, Eastwood MP, Russo FM, Deprest JA, Keijzer R. Fetal Tracheal Occlusion for Severe Pulmonary Hypoplasia in Isolated Congenital Diaphragmatic Hernia: A Systematic Review and Meta-analysis of Survival. Ann Surg 2016; 264 (6): 929-33.

[35] Dekoninck P, Gratacos E, Van Mieghem T, *et al.* Results of fetal endoscopic tracheal occlusion for congenital diaphragmatic hernia and the set up of the randomized controlled TOTAL trial. Early Hum Dev 2011; 87 (9): 619-24.

[36] Rodrigues HC, Deprest J, v d Berg PP. When referring physicians and researchers disagree on equipoise: the TOTAL trial experience. Prenat Diagn 2011; 31 (6): 589-94.

[37] Deprest J, Gratacos E, Nicolaides KH, Group FT. Fetoscopic tracheal occlusion (FETO) for severe congenital diaphragmatic hernia: evolution of a technique and preliminary results Ultrasound in obstetrics & gynecology : The official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2004; 24 (2): 121-6.

[38] Done E, Gratacos E, Nicolaides K, Allegaert K, Valencia C, Castanon M, *et al.* Predictors of neonatal morbidity in fetuses with severe isolated congenital diaphragmatic hernia undergoing fetoscopic tracheal occlusion Ultrasound in obstetrics & gynecology : The official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2013.

[39] Barnett CF, Machado RF. Sildenafil in the treatment of pulmonary hypertension. Vasc Health Risk Manag 2006; 2 (4): 411-22.

[40] Tantini B, Manes A, Fiumana E, *et al.* Antiproliferative effect of sildenafil on human pulmonary artery smooth muscle cells. Basic Res Cardiol 2005; 100 (2): 131-8.

[41] Tunks RD, Barker PC, Benjamin DK Jr, Cohen-Wolkowiez M, Fleming GA, Laughon M, *et al.* Sildenafil exposure and hemodynamic effect after Fontan surgery Pediatric critical care medicine : A journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies 2014; 15 (1): 28-34.

[42] Wharton J, Strange JW, Møller GM, *et al.* Antiproliferative effects of phosphodiesterase type 5 inhibition in human pulmonary artery cells. Am J Respir Crit Care Med 2005; 172 (1): 105-13.

[43] Steinhorn RH, Kinsella JP, Pierce C, *et al.* Intravenous sildenafil in the treatment of neonates with persistent pulmonary hypertension. J Pediatr 2009; 155 (6): 841-847.e1.

[44] Sanchez Luna M, Franco ML, Bernardo B. Therapeutic strategies in pulmonary hypertension of the newborn: where are we now? Curr Med Chem 2012; 19 (27): 4640-53.

[45] Kipfmueller F, Schroeder L, Berg C, Heindel K, Bartmann P, Mueller A. Continuous intravenous sildenafil as an early treatment in neonates with congenital diaphragmatic hernia. Pediatr Pulmonol 2018; 53 (4): 452-60.

[46] Bialkowski A, Moenkemeyer F, Patel N. Intravenous sildenafil in the management of pulmonary hypertension associated with congenital diaphragmatic hernia. European journal of pediatric surgery : official journal of Austrian Association of Pediatric Surgery [et al] =. Z Kinderchir 2015; 25 (2): 171-6.

[47] Luong C, Rey-Perra J, Vadivel A, *et al.* Antenatal sildenafil treatment attenuates pulmonary hypertension in experimental congenital diaphragmatic hernia. Circulation 2011; 123 (19): 2120-31.

[48] Yamamoto Y, Thebaud B, Vadivel A, Eaton F, Jain V, Hornberger LK. Doppler parameters of fetal lung hypoplasia and impact of sildenafil American journal of obstetrics and gynecology 2014; 211 (3): 263 e1-8.

[49] Burgos CM, Pearson EG, Davey M, *et al.* Improved pulmonary function in the nitrofen model of congenital diaphragmatic hernia following prenatal maternal dexamethasone and/or sildenafil. Pediatr Res 2016; 80 (4): 577-85.

[50] Mous DS, Kool HM, Burgisser PE, *et al.* Treatment of rat congenital diaphragmatic hernia with sildenafil and NS-304, selexipag's active compound, at the pseudoglandular stage improves lung vasculature. Am J Physiol Lung Cell Mol Physiol 2018; 315 (2): L276-85.

[51] Mous DS, Kool HM, Buscop-van Kempen MJ, *et al.* Clinically relevant timing of antenatal sildenafil treatment reduces pulmonary vascular remodeling in congenital diaphragmatic hernia. Am J Physiol Lung Cell Mol Physiol 2016; 311 (4): L734-42.

[52] Wu J, Yamamoto H, Gratacos E, *et al.* Lung development following diaphragmatic hernia in the fetal rabbit. Hum Reprod 2000; 15 (12): 2483-8.

[53] Flemmer AW, Jani JC, Bergmann F, *et al.* Lung tissue mechanics predict lung hypoplasia in a rabbit model for congenital diaphragmatic hernia. Pediatr Pulmonol 2007; 42 (6): 505-12.

[54] McArdle AM, Denton KM, Maduwegedera D, Moritz K, Flower RL, Roberts CT. Ontogeny of placental structural development and expression of the renin-angiotensin system and 11beta-HSD2 genes in the rabbit. Placenta 2009; 30 (7): 590-8.

[55] Fischer B, Chavatte-Palmer P, Viebahn C, Navarrete Santos A, Duranthon V. Rabbit as a reproductive model for human health. Reproduction 2012; 144 (1): 1-10.

[56] Russo FM, Toelen J, Eastwood MP, *et al.* Transplacental sildenafil rescues lung abnormalities in the rabbit model of diaphragmatic hernia. Thorax 2016; 71 (6): 517-25.

[57] Voelkel NF, Vandivier RW, Tuder RM. Vascular endothelial growth factor in the lung. Am J Physiol Lung Cell Mol Physiol 2006; 290 (2): L209-21.

[58] Aman J, Bogaard HJ, Vonk Noordegraaf A. Why vessels do matter in pulmonary disease. Thorax 2016; 71 (8): 767-9.

[59] van der Horst IW, Rajatapiti P, van der Voorn P, van Nederveen FH, Tibboel D, Rottier R, *et al.* Expression of hypoxia-inducible factors, regulators, and target genes in congenital diaphragmatic hernia patients Pediatric and developmental pathology : The official journal of the Society for Pediatric Pathology and the Paediatric Pathology Society 2011; 14 (5): 384-90.

[60] Sanz-López E, Maderuelo E, Peláez D, *et al.* Changes in the expression of vascular endothelial growth factor after fetal tracheal occlusion in an experimental model of congenital diaphragmatic hernia. Crit Care Res Pract 2013; 2013: 958078.

[61] Chang R, Andreoli S, Ng YS, Truong T, Smith SR, Wilson J, *et al.* VEGF expression is downregulated in nitrofen-induced congenital diaphragmatic hernia Journal of pediatric surgery 2004; 39 (6): 825-. discussion -8

[62] Zhao X, Lu X, Feng Q. Deficiency in endothelial nitric oxide synthase impairs myocardial angiogenesis. Am J Physiol Heart Circ Physiol 2002; 283 (6): H2371-8.

[63] Reffelmann T, Kloner RA. Therapeutic potential of phosphodiesterase 5 inhibition for cardiovascular disease. Circulation 2003; 108 (2): 239-44.

[64] Goldenberg MM. Safety and efficacy of sildenafil citrate in the treatment of male erectile dysfunction. Clin Ther 1998; 20 (6): 1033-48.

[65] Ballard SA, Gingell CJ, Tang K, Turner LA, Price ME, Naylor AM. Effects of sildenafil on the relaxation of human corpus cavernosum tissue in vitro and on the activities of cyclic nucleotide phosphodiesterase isozymes. J Urol 1998; 159 (6): 2164-71.

[66] Barst RJ, Ivy DD, Gaitan G, *et al.* A randomized, double-blind, placebo-controlled, dose-ranging study of oral sildenafil citrate in treatment-naive children with pulmonary arterial hypertension. Circulation 2012; 125 (2): 324-34.

[67] Russo FM, Mian P, Krekels EH, Van Calsteren K, Tibboel D, Deprest J, *et al.* Pregnancy affects the pharmacokinetics of sildenafil and its metabolite in the rabbit Xenobiotica; the fate of foreign compounds in biological systems 2018; 1-8.

[68] Russo FM, Conings S, Allegaert K, Van Mieghem T, Toelen J, Van Calsteren K, *et al.* Sildenafil crosses the placenta at therapeutic levels in a dually perfused human cotyledon model American journal of obstetrics and gynecology 2018; 219 (6 :619): e1-e10.

[69] Hutson JR, Garcia-Bournissen F, Davis A, Koren G. The human placental perfusion model: A systematic review and development of a model to predict in vivo transfer of therapeutic drugs. Clin Pharmacol Ther 2011; 90 (1): 67-76.

[70] Russo FM, Benachi A, Van Mieghem T, *et al.* Antenatal sildenafil administration to prevent pulmonary hypertension in congenital diaphragmatic hernia (SToP-PH): study protocol for a phase I/IIb placenta transfer and safety study. Trials 2018; 19 (1): 524.

[71] Hislop A. Developmental biology of the pulmonary circulation. Paediatr Respir Rev 2005; 6 (1): 35-43.

[72] Ganzevoort W, Alfirevic Z, von Dadelszen P, *et al.* STRIDER: Sildenafil Therapy In Dismal prognosis Early-onset intrauterine growth Restriction--a protocol for a systematic review with individual participant data and aggregate data meta-analysis and trial sequential analysis. Syst Rev 2014; 3: 23.

[73] Samangaya RA, Mires G, Shennan A, *et al.* A randomised, double-blinded, placebo-controlled study of the phosphodiesterase type 5 inhibitor sildenafil for the treatment of preeclampsia. Hypertens Pregnancy 2009; 28 (4): 369-82.

[74] Dallmann A, Pfister M, van den Anker J, Eissing T. Physiologically Based Pharmacokinetic Modeling in Pregnancy: A Systematic Review of Published Models. Clin Pharmacol Ther 2018; 104 (6): 1110-24.

[75] Adzick NS, Outwater KM, Harrison MR, *et al.* Correction of congenital diaphragmatic hernia in utero. IV. An early gestational fetal lamb model for pulmonary vascular morphometric analysis. J Pediatr Surg 1985; 20 (6): 673-80.

[76] Jaillard S, Elbaz F, Bresson-Just S, *et al.* Pulmonary vasodilator effects of norepinephrine during the development of chronic pulmonary hypertension in neonatal lambs. Br J Anaesth 2004; 93 (6): 818-24.

[77] Larrue B, Jaillard S, Lorthioir M, *et al.* Pulmonary vascular effects of sildenafil on the development of chronic pulmonary hypertension in the ovine fetus. Am J Physiol Lung Cell Mol Physiol 2005; 288 (6): L1193-200.

[78] Shue EH, Schecter SC, Gong W, *et al.* Antenatal maternally-administered phosphodiesterase type 5 inhibitors normalize eNOS expression in the fetal lamb model of congenital diaphragmatic hernia. J Pediatr Surg 2014; 49 (1): 39-45. [discussion].

[79] Vorhies EE, Ivy DD. Drug treatment of pulmonary hypertension in children. Paediatr Drugs 2014; 16 (1): 43-65.

[80] Partridge EA, Davey MG, Hornick MA, *et al.* An extra-uterine system to physiologically support the extreme premature lamb. Nat Commun 2017; 8: 15112.

[81] Lacassie HJ, Germain AM, Valdés G, Fernández MS, Allamand F, López H. Management of Eisenmenger syndrome in pregnancy with sildenafil and L-arginine. Obstet Gynecol 2004; 103 (5 Pt 2): 1118-20.

[82] Streit M, Speich R, Fischler M, Ulrich S. Successful pregnancy in pulmonary arterial hypertension associated with systemic lupus erythematosus: A case report. J Med Case Reports 2009; 3: 7255.

[83] Molelekwa V, Akhter P, McKenna P, Bowen M, Walsh K. Eisenmenger's syndrome in a 27 week pregnancy--management with bosentan and sildenafil. Ir Med J 2005; 98 (3): 87-8.

[84] von Dadelszen P, Dwinnell S, Magee LA, *et al.* Sildenafil citrate therapy for severe early-onset intrauterine growth restriction. BJOG 2011; 118 (5): 624-8.

[85] Maher MA, Sayyed TM, Elkhouly N. Sildenafil Citrate Therapy for Oligohydramnios: A Randomized Controlled Trial. Obstet Gynecol 2017; 129 (4): 615-20.

[86] Dunn L, Flenady V, Kumar S. Reducing the risk of fetal distress with sildenafil study (RIDSTRESS): A double-blind randomised control trial. J Transl Med 2016; 14 (1): 15.

[87] Dunn L, Greer R, Flenady V, Kumar S. Sildenafil in Pregnancy: A Systematic Review of Maternal Tolerance and Obstetric and Perinatal Outcomes. Fetal Diagn Ther 2016.

[88] Groom KM, Ganzevoort W, Alfirevic Z, Lim K, Papageorghiou AT. Clinicians should stop prescribing sildenafil for fetal growth restriction (FGR): comment from the STRIDER Consortium Ultrasound in obstetrics & gynecology : The official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2018.

[89] Russo FM, Toelen J, Eastwood MP, *et al.* Transplacental sildenafil rescues lung abnormalities in the rabbit model of diaphragmatic hernia. Thorax 2016; 71 (6): 517-25.

[90] Available from. http://www.ema.europa.eu/docs/en_GB/document_library/Orphan_designation/2017/ 07/WC500231579.pdf