ORIGINAL RESEARCH ARTICLE

Prophylactic Sildenafil in Preterm Infants at Risk of Bronchopulmonary Dysplasia: A Pilot Randomized, Double-Blinded, Placebo-Controlled Trial

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Abstract

Background Bronchopulmonary dysplasia (BPD) is the need for oxygen therapy at 36 weeks postmenstrual age (PMA). Sildenafil has been shown to enhance the lung alveolarization and vascularization in newborn animal models after lung injury and has possible therapeutic potential for the prevention of BPD.

Objective To perform a proof-of-concept, Phase II, pilot randomized, double-blind, clinical trial to study the efficacy of sildenafil in preventing BPD, in postnatal (< 24 h), extremely and very preterm infants.

Methods This Phase II, pilot randomized, double-blind, clinical trial was conducted in the Neonatal Intensive Care Unit of Women's Wellness and Research Center, Doha, Qatar during 2012–2014. Infants of $24^{0/7}$–$29^{6/7}$ weeks' gestation were eligible if they needed respiratory or oxygen support ≥ 25% at randomization, and if they were at a postnatal age of < 24 h at randomization. Forty preterm infants were randomly assigned to receive off-label oral sildenafil (0.5 mg/kg every 6 h) or a placebo solution, for one week. The primary endpoints were the incidence of BPD and death at 36 weeks PMA, and the side effects. Secondary outcomes included the incidence of BPD and the respiratory support at day 28 of life, duration of oxygen use, fraction of inspired oxygen use at 36 weeks and 28 days of life, duration of hospitalization, and the incidence of significant retinopathy of prematurity, severe intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, patent ductus arteriosus, and late sepsis.

Results No significant differences were observed between the sildenafil and placebo study groups in mortality at 36 weeks PMA (10% vs 20%, $p = 1$), respiratory support at 36 weeks (30% vs 25%, $p = 0.57$), and side effects (0% vs 0%). For all other secondary outcomes, no significant differences were detected.

Conclusions While not associated with side effects, off-label oral sildenafil did not demonstrate benefits in the prevention of BPD or death in the extreme and very preterm infants. Future studies of dosing and efficacy that target different regimens of sildenafil are warranted before sildenafil is recommended for the prevention of BPD.

Key Points

This trial had a small sample size and short duration of therapy. While sildenafil treatment was not associated with side effects, it did not demonstrate benefit as a preventative therapy against bronchopulmonary dysplasia (BPD) in very preterm infants.

Future dosing and efficacy trials that target varying dosing regimens of sildenafil are needed to show benefit for the off-label use of sildenafil in the prevention of BPD.

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1 Introduction

Preterm birth is a major cause of morbidity and mortality worldwide. Complications in the premature neonates are in fact responsible for over one-third of the 3.1 million deaths worldwide in a year, second only to pneumonia as a primary cause of mortality in children aged < 5 years [1]. Bronchopulmonary dysplasia (BPD) is among the most common adverse outcomes in very preterm neonates with an incidence of 5–68%, which increases significantly with decreasing gestational age and birth weight, affecting 30–40% of infants weighing < 1500 g at birth [2, 3]. BPD also develops as a result of lung injury caused by factors include maternal intrauterine growth restriction, lack of antenatal corticosteroids, chorioamnionitis, postnatal ventilation, hyperoxia, and/or inflammation, leading to

long-term consequences that include bronchospastic disease, poor growth and development, and pulmonary artery hypertension (PAH) [2, 4, 5].

Originally, BPD was defined based on lung injury due to mechanical ventilation and oxygen therapy and was found mostly in premature neonates born at 26–30 weeks postmenstrual age (PMA). Advances in neonatology and obstetric care such as antenatal corticosteroids and surfactant replacement therapy resulted in a changed disease phenotype that was seen in preterm babies that could survive at younger gestational ages (24–26 weeks PMA) [6]. Therefore, a new BPD definition was introduced based on impaired alveolar and capillary development of the immature lungs as the need for oxygen therapy at 36 weeks PMA or treatment with oxygen supplement for more than 28 days [6], which then, at 36 weeks corrected age, is further classified as mild (no oxygen supplementation), moderate (oxygen supplementation < 30%) or severe (oxygen supplementation ≥ 30% and/or requirement for positive pressure support) [7]. Despite advances in both perinatal and neonatal care, BPD remains a common cause of morbidity with a substantial burden on individuals and health resources [8]. With increasing survival of very premature neonates, efforts are needed to limit the burden associated with BPD. Management of BPD remains a major challenge to clinicians, and established BPD is associated with poorer long-term neurodevelopmental outcome and an increased readmission risk due to respiratory conditions. Survivors of BPD often have respiratory health injuries as adults [8–12].

Sildenafil is a modulator of the cyclic guanosine monophosphate (cGMP) pathway, elevating its levels in the body. The elevated cGMP eventually leads to reduced intracellular calcium levels and the suppression of smooth muscle proliferation. Hence, in newborn animal models, sildenafil was found to preserve angiogenesis, enhance alveolarization, and reduce right-ventricular hypertrophy, medial wall thickness, pulmonary vascular resistance and the inflammatory response, with the latter particularly making sildenafil an attractive potential option for BPD-associated PAH [13, 14].

Here, in a recent rat model experiment, sildenafil was suggested to have possible therapeutic potential for the prevention of BPD, attributable to its activation of the hypoxia-inducible factor signaling pathway [15]. Several cohort and case-control studies evaluated the use of sildenafil for the treatment of BPD-induced PAH in infants, where these mostly reported an improved echocardiographic diagnosis of PAH [16–20]. Also, in relation to the BPD-induced PAH in infants, four clinical trials evaluated the therapeutic effect of sildenafil [21–24]. In these, term and near-term infants were included, with all reporting an improved oxygenation index

and survival. Within the context of the current research, regarding the use of the sildenafil in the early prevention of BPD, there is only one randomized clinical trial (RCT) in literature, by Konig et al [8], in 20 extremely preterm infants receiving sildenafil (n = 10) versus placebo (n = 10). No beneficial BPD preventive effect could be demonstrated, and except one severe case of hypotension, no differences in side effects were observed between study groups.

Since the 2005 FDA approval for adult PAH, and despite a 2012 black box warning against long-term use in 1- to 7-year-old children due to increased risk of death at high doses, there has been an increasing trend of utilizing the off-label preparation of sildenafil in infants [25, 26].

The objective of this proof-of-concept, Phase II, pilot randomized double-blinded clinical trial was to assess the efficacy and safety of oral sildenafil in < 24 h postnatal, extremely to very preterm infants for reducing the incidence of BPD.

2 Patients and Methods

2.1 Design

Phase II, pilot randomized, double-blind placebo-controlled clinical trial.

2.2 Setting

The study was conducted between 2012 and 2014 in the neonatal intensive care unit (NICU) of Women's Wellness and Research Center (WWRC), the tertiary hospital at Hamad Medical Corporation (HMC), in Qatar.

The study was appropriately approved by the institutional human research ethics committee at the Medical Research Center of HMC (reference #11087/11). The study was conducted in full conformance with principles of the "Declaration of Helsinki", Good Clinical Practice and within the laws and regulations of Ministry of Public Health in Qatar. Prior to the randomization of an enrolled infant, written informed consent was obtained from parents.

2.3 Patients

Preterm infants were eligible for study inclusion if they had a gestational age of $24^{0/7}$–$29^{6/7}$ weeks, postnatal age of < 24 h at randomization, and a need of respiratory support or oxygen ≥ 25% at randomization. Excluded, were infants who were not considered viable by the attending clinical staff, had congenital malformation that would adversely affect life expectancy or neurodevelopment, had known or strongly suspected congenital heart disease, had severe hemodynamic

instability (more than one inotrope support) at randomization, and had liver failure (elevated alanine aminotransferase and aspartate aminotransferase enzymes > 200).

2.4 Study Groups

Study neonates were randomly assigned to either oral sildenafil (0.5 mg/kg every 6 h) or a placebo solution, for one week. There are limited data available to recommend dosage regimens in infant populations. Due to the paucity of data, we followed a cautious approach, in which a 0.5 mg/kg test dose was used as a start dose. The start dose proved to be effective in the treatment of persistent pulmonary hypertension of the newborns in previous literature [16, 27–31]. The dose was then increased in increments of 0.5 mg/kg six hourly until a target maintenance daily dose of 2 mg/kg was reached. A 20-mg tablet was crushed into a fine powder that was mixed as 1:1 with a solution of simple syrup (50%) and methylcellulose (50%), with a final concentration of 2.5 mg/mL. For the placebo solution, a 1:1 mixture of simple syrup and methylcellulose was used and also administered every 6 h for 1 week. To ensure safety, shorter duration of one week was used for the duration of medications. The infant's response was observed, and the medication was discontinued if no clear response or improvement was attained. Both solutions were stored in amber glass bottles and kept refrigerated in the bedside refrigerator. The solutions were given as appropriate via an orogastric tube and then flushed with normal saline (0.9% sodium chloride in sterile water). The dosing protocol was 0.2 mL/kg solution (0.5 mg/kg) every 6 h, administered for one week. Treatment was continued even if the patient was weaned to room air at any age of life. Treatment was discontinued when the parents refused continuation of treatment. Supportive therapy was similar in both study groups which, as per the local NICU guideline in the WWRC, included total parenteral nutrition, respiratory support, fluid intake, and developmental care.

2.5 Randomization and Data Collection

Eligible infants were randomized within 24 h. The initial dose of study therapies was also administered within 24 h of randomization. Multiple birth infants were randomized independently and received the same therapy group. A simple random allocation was performed through the use of codes in pre-concealed envelopes. A pharmacist prepared the study bottles, with each having a sealed code for identification. The randomization was stratified according to gestational age and birth weight. Gestational age strata were stratum A: $24^{0/7}$–$27^{0/7}$ weeks and stratum B: $27^{1/7}$–$29^{6/7}$ weeks. Birth weight strata were stratum A: 500–999 g, stratum B: 1000–1499 g, and stratum C: 1500–2500 g. Bedside

clinicians and nurses were unaware of study group assignment. Data collection was performed during the treatment period and until discharge to home, using a daily record data sheet as all participants were in-patients during primary and secondary outcome measures. The data collection was carried out by an expert research nurse and nursing staff supervised by the principal investigator, co-investigators, and neonatologists at the NICU.

2.6 Safety Monitoring

During the study, all patients were carefully monitored for side effects by a Safety Monitoring Data Committee, constituting three consultants, the research nurse coordinator, and the NICU nursing staff under the neonatologist supervision. Heart rate and blood pressure were continuously monitored in neonates during the administration of sildenafil. Gastrointestinal symptoms (nausea, vomiting and diarrhea), erythema, skin pyrexia, and irritability were observed by the nursing staff every 4 h.

2.7 Outcome Measures

Primary outcomes were the incidence of BPD and death at 36 weeks PMA, in addition to side effects that are associated with sildenafil during the study period. Secondary outcomes were the incidence of BPD and the respiratory support at Day 28 of life, duration of oxygen therapy, fraction of inspired oxygen use at 36 weeks, and 28 days of life, duration of hospitalization until discharge to home, and the incidence of significant retinopathy of prematurity (stage II plus disease or stage ≥ III), severe intraventricular hemorrhage (grade III and IV), periventricular leukomalacia, necrotizing enterocolitis, patent ductus arteriosus, and late sepsis. The association of each of retinopathy of prematurity, severe intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, patent ductus arteriosus, and late sepsis with the incidence of BPD and death at 36 weeks PMA was also examined.

2.8 Definition of the Outcome Measures

- BPD was defined as the need for supplemental oxygen at a postmenstrual age of 36 weeks [32].
- Side effects were the relevant events that were associated with sildenafil during the study period until hospital discharge. Side effects included tachycardia, arrhythmia, hypotension, erythema, skin pyrexia, seizure, irritability, nausea/vomiting, and diarrhea [8, 25].
- Retinopathy of prematurity (stage II plus disease or stage ≥ III) was defined as vascularization of the ridge with extraretinal fibrovascular proliferation, partial or

total retinal detachment from 31 corrected weeks until hospital discharge [33].

- Intraventricular hemorrhage was defined as grade III: intraventricular hemorrhage with ventricular dilation or grade IV: parenchymal hemorrhage from birth until hospital discharge [34].
- Periventricular leukomalacia was defined as multifocal areas of necrosis presented in the cortical white substance at 36 weeks PMA [35].
- Necrotizing enterocolitis was defined according to Bell's staging criteria from birth until hospital discharge [36].
- Patent ductus arteriosus was when the ductus arteriosus failed to close within 72 h after birth from birth until hospital discharge [37]. All neonates with patent ductus arteriosus were managed as per the local clinical guideline in the NICU, WWRC, HMC, that includes:

  – Ibuprofen IV as first-line therapy (initial dose of 10 mg/kg IV followed by two additional doses of 5 mg/kg IV given at 24-h intervals).
  – Second course of ibuprofen IV is given if the duct fails to close after the first course.
  – Indomethacin may be given if the duct fails to close after the second course of ibuprofen, if not contraindicated (initial dose of 0.2 mg/kg IV followed by):

    - If infants were < 2 day: 0.1 mg/kg/dose IV, 12-hourly for two doses.
    - If infants were between 2–7 days: 0.2 mg/kg/dose IV, 12-hourly for two doses.
    - If infants were > 7 days: 0.25 mg/kg/dose IV, 12-hourly for two doses.

  – Surgical ligation was performed if the patient remained symptomatic after two courses of ibuprofen or indomethacin or if the treatment contraindicated.

- Late sepsis definition is from > 3 days of birth until discharge [38].

2.9 Statistical Analysis

Baseline patient characteristics were described as categorical variables using number and percentage and as continuous data using mean and standard deviation. Chi-square and Fisher's exact tests were used for categorical data, the Student's t test, and one-way analysis of variance test were used for continuous normally distributed data, and Mann–Whitney and Kruskal–Wallis tests were used for continuous not normally distributed data using an alpha of 0.05. IBM SPSS Statistics version 22 was used for all statistical analyses. The risk ratios of the study clinical outcomes were calculated and

reported with 95% confidence intervals. A logistic regression analysis was used to estimate association of each of retinopathy of prematurity, intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, patent ductus arteriosus, and late sepsis with the incidence of BPD and death at 36 weeks PMA. Study analysis was by intention to treat.

2.10 Sample Size

Phase II clinical trials are generally studies investigating the therapeutic efficacy and toxicity of a medication in a relatively small number of patients (generally 40–70 patients) [39, 40]. Therefore, 40 neonates were recruited for the purpose of the study.

3 Results

3.1 Demographic Characteristics of Study Participants

Throughout the study period, of 105 preterm infants admitted to the NICU,15 infants did not meet the inclusion criteria: 4 were associated with intrauterine growth restriction, 2 with congenital anomalies, 2 delivered at home, 5 delivered before 24 weeks, and 2 were very large for gestational age. Of the 90 eligible infants, 40 were successfully included and completed the study procedures, while 10 were not approached and 40 parents refused to participate. Figure 1 is a chart of the study flow, including patients who were not admitted to the study. Table 1 is a comprehensive list of the baseline infant characteristics, which were statistically not different between the two study groups.

3.2 Clinical Outcomes

3.2.1 Primary Outcomes

Surviving infants until 36 weeks had similar rates of BPD between the two groups [6 (30%) in the sildenafil group vs 5 (25%)], risk ratio 1.2, 95% confidence interval (0.49–3.63), p-value 0.57. BPD was present in 11 infants including: severe BPD in 5 infants (3 in sildenafil vs 3 in placebo), moderate BPD in 5 infants (3 in sildenafil vs 2 in placebo). Twice as many infants died in the placebo group compared with the sildenafil group, but this was not statistically different [2 (10%) vs 4 (20%)], risk ratio 1, 95% confidence interval (0.77–1.30), p-value 1. Three patients in the placebo group died after completing the study therapy at 12, 14, and 174 days of hospital stay, while one died after receiving the second dose of placebo. In the sildenafil group, two neonates died after the study treatment at 23 and 88 days of hospital

Fig. 1 Flowchart of patient recruitment
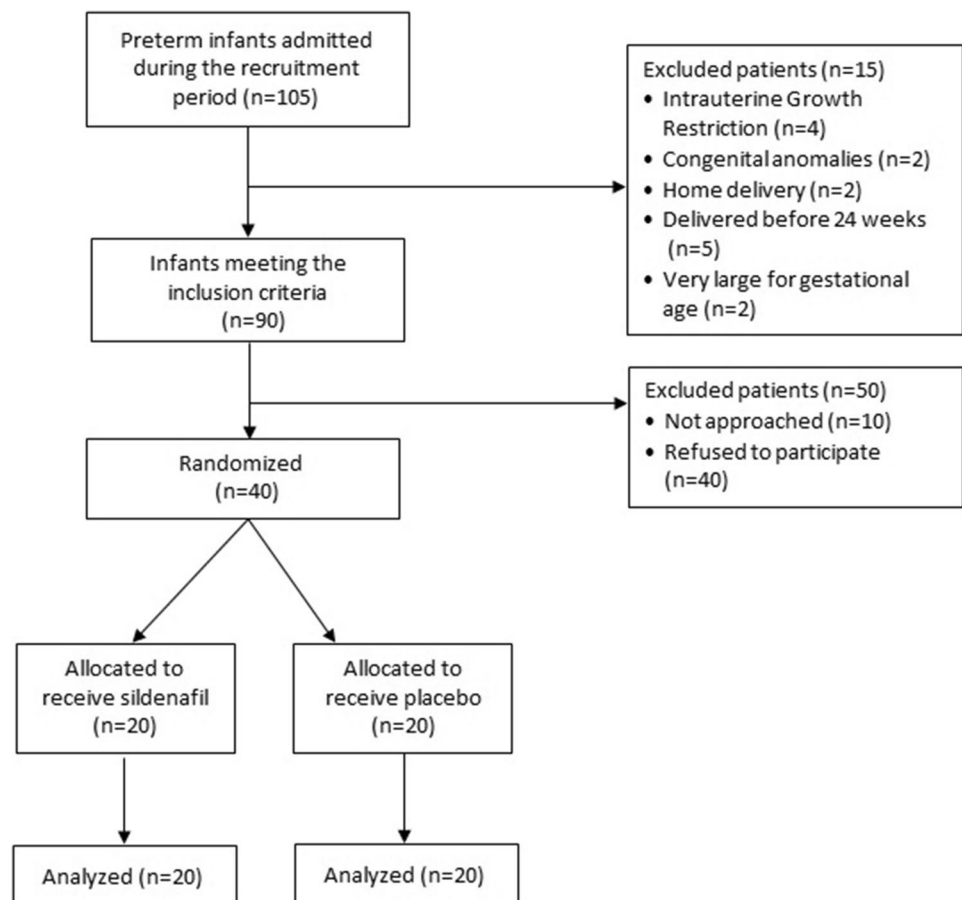
stay. In relation to the safety outcome, no side effects were reported in either group (Table 2).

3.2.2 Secondary Outcomes

Overall, both sildenafil and placebo were similar in relation to all of the primary and secondary study outcomes, as shown in Table 2. More infants in the placebo group needed respiratory support at 28 days than in the sildenafil group (65% vs 60%, $p = 0.78$). The placebo group had a 15% longer average respiratory support duration than the sildenafil group, but this did not reach the statistical significance $p = 0.23$. The need for fraction of inspired oxygen at 36 weeks and 28 days were higher in neonates receiving placebo compared with sildenafil ($p = 0.57$ and $p = 0.58$, respectively). The length of hospital stay was 10% longer with placebo than with sildenafil, but this was not statistically different ($p = 0.14$). Apart from the retinopathy of prematurity, there was a general trend of more cases of intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, patent ductus arteriosus, and late sepsis reported with sildenafil than with placebo in this study. Apart from periventricular leukomalacia, differences in the number of cases were relatively minor. But none of the differences were statistically significant between the two groups.

In relation to the correlation between study outcomes and patient characteristics, the mortality rate at 36 weeks PMA was statistically negatively associated with each of the gestational age at delivery and the maternity care provided during pregnancy. The respiratory support provided by 36 weeks PMA was statistically positively associated with each of the occurrences of intraventricular hemorrhage, necrotizing enterocolitis, gestational age, and with receiving antenatal care or postnatal steroids. The use of fraction of inspired oxygen at 36 weeks was statistically positively related to the presence of retinopathy of prematurity, necrotizing enterocolitis, as well as with higher gestational age and receiving postnatal steroids. Table 3 summarizes the correlation between each study outcome and the patient characteristics and comorbidities.

3.2.3 Singleton and Multiple Infants

The study population consisted of 22 singletons, 10 twins, 5 triplets and 3 quadruplets. Triplets were more likely to be male patients and of African ethnicity compared with singletons, twins, and quadruplets, which were mostly of Asian origin. There was an inverse relation between

Table 1 Main baseline patient demographics

Characteristic	Sildenafil (n=20) No. (%)	Placebo (n=20) No. (%)	p value
Sex			1
Male	13 (65)	11 (55)	
Female	7 (35)	9 (45)	
Gestational age (weeks)			
Extremely preterm (<28)	10 (50)	10 (50)	1
Very preterm (≥28 and <30)	10 (50)	10 (50)	
Extremely preterm (<28)	26±1.08	25.5±1.31	0.5
Very preterm (≥28 and <30)	28.57±0.53	28.5±0.53	
All gestational ages	26.9±1.55	26.7±1.84	0.42
Birth weight (g)			
<2500 and ≥1500	2 (10)	1 (5)	0.11
<1500 and ≥1000	12 (60)	7 (35)	
<1000	6 (30)	12 (60)	
<2500 and ≥1500	1655±148.49	1530±0	0.07
<1500 and ≥1000	1173.2±131.52	1155.71±99.47	
<1000	763.33±113.43	819.17±93.17	
All birth weights	1089.1±291.12	972.5±227.43	0.08
Current birth weight (at 36 weeks postmenstrual age)			1
Appropriate for gestational age	20 (100)	20 (100)	
Large for gestational age	0 (0)	0 (0)	
Small for gestational age	0 (0)	0 (0)	
Ethnicity			0.16
Asian	16 (80)	15 (75)	
African	4 (20)	5 (25)	
Type of delivery			0.37
Vaginal	7 (35)	11 (55)	
Caesarean	13 (65)	9 (45)	
Number of pregnancies			0.62
Singleton	10 (50)	12 (60)	
Twin	6 (30)	4 (20)	
Triplet	3 (15)	2 (10)	
Quadruplet	1 (5)	2 (10)	
Chorioamnionitis			1
Yes	3 (15)	1 (5)	
No	17 (85)	19 (95)	
Antenatal care			0.07
Yes	15 (75)	15 (75)	
No	5 (25)	5 (25)	
Antenatal steroid			1
Yes	18 (90)	17 (85)	
No	2 (10)	3 (15)	
Antenatal steroid dose (dexamethasone)			0.47
0	2 (10)	3 (15)	
1	2 (10)	6 (30)	
2	14 (70)	9 (45)	
3	0 (0)	0 (0)	
4	2 (10)	2 (10)	

Table 1 (continued)

Characteristic	Sildenafil (n = 20) No. (%)	Placebo (n = 20) No. (%)	p value
Maternal disease			0.74
Premature rupture of membranes + Group B streptococcus	7 (35)	6 (30)	
Premature rupture of membranes	3 (15)	2 (10)	
Group B streptococcus	2 (10)	2 (10)	
Pre-eclampsia toxemia	3 (15)	2 (10)	
Gestational diabetes	1 (5)	1 (5)	
Anemia	1 (5)	4 (20)	
Antepartum hemorrhage	3 (15)	1 (5)	
Others	0 (0)	2 (10)	
One-min score of APGAR (Appearance, Pulse, Grimace, Activity, and Respiration)			0.07
Critically low (0–3)	1 (5)	2 (10)	
Fairly low (4–6)	11 (55)	9 (45)	
Generally normal (7–10)	8 (40)	9 (45)	
Five-min APGAR score			1
Critically low (0–3)	0 (0)	1 (5)	
Fairly low (4–6)	1 (5)	1 (5)	
Generally normal (7–10)	19 (95)	18 (90)	
History of resuscitation			0.05
Intubation	12 (60)	9 (45)	
Continuous positive airway pressure	8 (40)	9 (45)	
Intubation + chest compression	0 (0)	1 (5)	
Intubation + chest compression + epinephrine	0 (0)	1 (5)	
Intubation time			0.5
At birth	12 (60)	11 (55)	
Later	8 (40)	9 (45)	
Respiratory support at randomization			0.24
Nasal cannula	0 (0)	0 (0)	
Continuous positive airway pressure	4 (20)	7 (35)	
Bi-level positive airway pressure	7 (35)	4 (20)	
Mechanical ventilation	7 (35)	8 (40)	
High frequency ventilation	2 (10)	1 (5)	
Blank air room	0 (0)	0 (0)	
FIO_2 at randomization	19 (95)	20 (100)	0.37
FIO_2 at randomization	33.59 ± 19	33.59 ± 19	0.27
Time of randomization	17.45 ± 6.41	14.1 ± 6.96	0.09
Surfactant			0.5
Yes	17 (85)	16 (80)	
No	3 (15)	4 (20)	
Number of doses of surfactant			0.6
0	3 (15)	4 (20)	
1	10 (50)	5 (25)	
2	3 (15)	8 (40)	
3	3 (15)	1 (5)	
4	1 (5)	2 (10)	
Postnatal steroid			1
Yes	2 (10)	5 (25)	
No	18 (90)	15 (75)	

Table 1 (continued)

Characteristic	Sildenafil ($n=20$) No. (%)	Placebo ($n=20$) No. (%)	p value
Inhaled nitric oxide			1
Yes	2 (10)	5 (25)	
No	18 (90)	15 (75)	
Fluid intake			1
Yes	20 (100)	19 (95)	
No	0 (0)	1 (5)	
Diuretics			0.56
Yes	5 (25)	5 (25)	
No	15 (75)	15 (75)	
Patent ductus arteriosus			0.64
Yes	15 (75)	13 (65)	
No	5 (25)	7 (35)	
Patients with patent ductus arteriosus who received treatment (pharmacological or surgical therapy as per the local NICU guideline in the WWRC, HMC)			1
Yes	11 (55)	10 (50)	
No	9 (45)	10 (50)	
Late sepsis			1
Yes	10 (50)	7 (35)	
No	10 (50)	13 (65)	

Frequency (%) for categorical data, mean ± SD for continuous data

FIO_2 fraction of inspired oxygen, *HMC* Hamad Medical Corporation, *WWRC* Women's Wellness and Research Center

Table 2 Clinical outcomes of the study

Clinical outcome	Sildenafil	Placebo	p value	Risk ratio	95% confidence interval
Mortality at 36 weeks	2 (10)	4 (20)	1	1	(0.77–1.30)
Respiratory support at 36 weeks	6 (30)	5 (25)	0.57	1.2	(0.49–3.63)
Respiratory support at 28 days	12 (60)	13 (65)	0.78	1	(0.65–1.62)
Respiratory duration	47.5 ± 31.44	55.9 ± 51.57	0.23		
FIO_2 at 36 weeks	23.71 ± 11.40	30.25 ± 7.80	0.57		(0.53–11.3)
BPD classification according to FIO_2 at 36 weeks (NICHD criteria)					
Mild (FIO_2 at 36 weeks)	14	15			
Moderate (FIO_2 at 36 weeks)	3	2			
Severe (FIO_2 at 36 weeks)	3	3			
FIO_2 at 28 days	26.83 ± 11.83	27.50 ± 18.72	0.58		(0.8–10.99)
Length of hospital stay	67.05 ± 30.09	74.3 ± 55.06	0.14		
Retinopathy of prematurity	5 (25)	7 (35)	0.4	1	(0.27–1.88)
Intraventricular hemorrhage	2 (10)	1 (5)	1	1.06	(0.05–5.08)
Periventricular leukomalacia	4 (20)	0 (0)	0.11	0.8	(0.64–1)
Patent ductus arteriosus	14 (70)	11 (55)	0.73	1.15	(0.77–1.74)
Late sepsis	10 (50)	7 (35)	1	0.89	(0.43–1.83)
Necrotizing enterocolitis	3 (15)	2 (10)	1	0.67	(0.12–3.57)

Frequency (%) for categorical data, mean ± SD for continuous data

BPD bronchopulmonary dysplasia, FIO_2 fraction of inspired oxygen

Table 3 The impact of comorbidities on the study outcomes

Variable	p value	Strength of association
Mortality at 36 weeks		
Retinopathy of prematurity	1	
Intraventricular hemorrhage	0.39	
Periventricular leukomalacia	0.6	
Necrotizing enterocolitis	0.15	
Gender	0.37	
Gestational age	0.02	− 0.42
Birth weight	0.64	
Ethnicity	0.58	
Chorioamnionitis	1	
Antenatal care	0.03	− 0.4
Antenatal steroid	1	
Antenatal steroid doses	0.34	
Surfactant use	0.57	
Postnatal steroid	0.22	
Patent ductus arteriosus	1	
Late sepsis	1	
Respiratory support at 36 weeks		
Retinopathy of prematurity	0.2	
Intraventricular hemorrhage	0.04	0.58
Periventricular leukomalacia	0.59	
Necrotizing enterocolitis	0.03	0.64
Gender	0.28	
Gestational age	0.008	0.56
Birth weight	0.23	
Ethnicity	0.5	
Chorioamnionitis	1	
Antenatal care	0.03	0.5
Antenatal steroid	0.54	
Antenatal steroid doses	0.05	
Surfactant use	0.92	
Postnatal steroid	0.002	0.73
Patent ductus arteriosus	0.74	
Late sepsis	0.08	
FIO$_2$ at 36 weeks		
Retinopathy of prematurity	0.004	0.99
Intraventricular hemorrhage	0.13	
Periventricular leukomalacia	0.7	
Necrotizing enterocolitis	0.03	0.68
Gender	0.52	
Gestational age	0.02	0.53
Birth weight	0.26	
Ethnicity	0.84	
Chorioamnionitis	0.48	
Antenatal care	0.24	
Antenatal steroid	0.33	
Antenatal steroid doses	0.49	
Surfactant use	0.8	
Postnatal steroid	0.02	0.71

Table 3 (continued)

Variable	p value	Strength of association
Patent ductus arteriosus	1	
Late sepsis	0.25	

FIO_2 fraction of inspired oxygen

plurality and birth weight and gestational age except in quadruplet infants. Triplets were on average 825.84 g smaller and 24.67 gestational age weeks younger than twins and singletons. The mothers of twins were more likely to have chorioamnionitis than mothers of singletons. Additionally, the mothers of singletons, triplets and quadruplets were more likely to have received antenatal care (defined as 1 or more antenatal visit) during pregnancy than mothers of twins. Moreover, mothers of multiples were more likely to have received antenatal steroids (defined as at least 1 dose), and to have delivered by caesarean section except in triplet group. Maternal diseases were less common in multiple pregnancies. Singletons and twins were almost similar in relation to the 1-min APGAR score of < 3 and only singletons achieved a 5-min APGAR score of < 3 with 8.33%, and no infants were small for gestational age. No difference was found between the groups in any of the characteristics except in the gestational age $p = 0.007$ (Table 4).

There were no significant differences between the infant groups in the incidence of each of the outcome variables. Twins were at increased risk of mortality, intraventricular hemorrhage, and periventricular leukomalacia. Singletons required more respiratory support at 36 weeks, oxygen at 36 weeks and 28 days, were at increased risk of retinopathy of prematurity, and needed a longer hospital stay. While triplets required more respiratory support at 28 days, were at increased risk of patent ductus arteriosus, late sepsis, and necrotizing enterocolitis, and needed longer duration of respiratory support (Table 5).

4 Discussion

This pilot study was conducted to investigate the efficacy and safety of early administration of oral sildenafil in < 24 h postnatal, extremely to very preterm infants for prevention of BPD. The study showed that prophylactic use of sildenafil for 7 days failed to improve the likelihood of survival or demonstrate clinical benefit without the need for respiratory support at 36 weeks' PMA. No side effects were reported in either group; therefore, sildenafil safety needs to be questioned. As compared with placebo, infants

Table 4 Singleton and multiple infants' characteristics

Characteristic	Singletons (n=22)	Twins (n=10)	Triplets (n=5)	Quadruplets (n=3)	p value
Sex (male)	59%	58.34%	83.34%	50%	0.69
Gestational age (weeks), mean ± SD	27.28 ± 1.69	26.29 ± 1.41	24.67 ± 0.58	28 ± 0	0.007
Birth weight (gram), mean ± SD	1084.5 ± 295.99	1028.75 ± 251.76	825.84 ± 108.37	988.5 ± 31.82	0.35
Small for gestational age	0%	0%	0%	0%	NA
Ethnicity					0.23
Asian	83%	83.34%	33.34%	100%	
African	18%	16.67%	66.67%	0%	
Type of delivery					0.42
Vaginal	50%	45.84%	66.67%	0%	
Caesarean	50%	54.17%	33.34%	100%	
Chorioamnionitis	20%	20.84%	0%	0%	0.71
Antenatal care	78%	58.34%	83.34%	100%	0.67
Antenatal steroid	78%	100%	100%	100%	0.88
Maternal disease					0.64
Premature rupture of membranes + Group B streptococcus	8.33%	0%	0%	0%	
Premature rupture of membranes	20%	0%	66.67%	0%	
Group B streptococcus	14%	0%	0%	0%	
Pre-eclampsia toxemia	8.33%	33.33%	0%	0%	
Gestational diabetes	0%	20.84%	0%	0%	
Anemia	10%	50%	0%	0%	
Antepartum hemorrhage	14%	0%	0%	0%	
Others	8.33%	0%	0%	100%	
One-min APGAR score ≤ 3	9.09%	10%	0%	0%	1
Five-min APGAR score ≤ 3	8.33%	0%	0%	0%	1

NA not applicable

Table 5 Outcomes of singleton and multiple infants

Outcome	Singletons (n=22)	Twins (n=10)	Triplets (n=5)	Quadruplets (n=3)	p value
Mortality at 36 weeks	9.09%	30%	20%	0%	0.36
Respiratory support at 36 weeks	36.36%	20%	10%	0%	0.74
Respiratory support at 28 days	68.18%	50%	100%	0%	0.22
Respiratory duration, mean ± SD	61.29 ± 50.55	34.1 ± 31.8	67.4 ± 17.69	23 ± 5.29	0.09
FIO2 at 36 weeks, mean ± SD	30.14 ± 6.23	23 ± 2.83	30 ± 0	0 ± 0	0.57
FIO2 at 28 days, mean ± SD	29.21 ± 18.43	25.6 ± 11.05	23 ± 2.35	0 ± 0	0.1
Length of hospital stay, mean ± SD	81.32 ± 50.9	47.9 ± 38.36	74.8 ± 12.3	49.67 ± 7.57	0.16
Retinopathy of prematurity	40.91%	20%	20%	0%	0.52
Intraventricular hemorrhage	4.55%	20%	0%	0%	0.38
Periventricular leukomalacia	9.1%	20%	0%	0%	0.71
Patent ductus arteriosus	72.73%	50%	100%	33.33%	0.25
Late sepsis	36.36%	10%	80%	33.33%	0.34
Necrotizing enterocolitis	9.1%	0%	60%	0%	0.06

FIO$_2$ fraction of inspired oxygen

who received sildenafil required less respiratory support at 28 days and for a shorter period, required less fraction of inspired oxygen at 36 weeks and 28 days, were hospitalized for a shorter duration, but were associated with more cases of intraventricular hemorrhage, periventricular leukomalacia, late sepsis, necrotizing enterocolitis, and patent ductus arteriosus.

We limited the recruitment to infants who required respiratory support or oxygen therapy ≥ 25% at randomization as these infants are at a higher risk of respiratory distress syndrome, BPD, and death, based on the neonatologists' opinions at the NICU of WWRC [41, 42]. Although the inclusion criteria included infants born between 24 and 29 gestational age weeks, weighing less than 2500 g, both study groups had almost equal average of very and extremely preterm infants and had more very low birth-weight infants, making both groups more prone to respiratory morbidity and mortality.

This trial differed in design from the study by Konig et al., (the only RCT) in literature, which was conducted in 20 extremely preterm infants born at less than 28 weeks gestational age receiving 1 mg/kg/dose three times daily of sildenafil after 7 days of mechanical ventilation ($n = 10$) versus placebo ($n = 10$) [8]. In our study, sildenafil was started earlier (within 24 h of randomization). We assumed that early administration of sildenafil might be needed to prevent irreversible lung injury, attenuate hyperoxic lung injury, and to improve angiogenesis and alveolarization as has been reported in animal model experiment [15].

The first use of sildenafil in infants was reported in 1999 to facilitate weaning from inhaled nitrous oxide after corrective cardiac surgery [43]. In the STARTS-2 trial by Barst et al., the children taking high doses of sildenafil (over 3 mg/kg/day) were more associated with mortality than those who received lower doses [25]. As a result, and as discussed already above, the US FDA issued an alert warning against the use of sildenafil in children aged 1–7 years [26]. By design, the Barst et al study provides no information about the efficacy and safety of sildenafil in infants with BPD as it was conducted in children aged > 1 year without BPD [25]. Furthermore, there are several concerns about extrapolating children's results to infants. One concern is that infants with BPD have complex pathophysiology and are commonly on several concurrent medications. Another matter is regarding the response to dose. This oral absorption is unpredictable in smaller infants, and even in older children the dose-response relationship is still not well identified [3].

Several observational studies demonstrated that infants treated with sildenafil due to BPD-induced PAH reported an improved echocardiographic diagnosis of PAH [16–20]. Moreover, Baquero et al., Vargas-Origel et al., Soliz et al., and Herrera et al studies reported oxygenation index improvement and survival without associated side effects with sildenafil use in infants with BPD-induced PAH [21–24]. Indeed, the only RCT in literature by Konig et al., reported no beneficial BPD prevention effect with sildenafil, and no differences in drug side effects were observed between study groups [8]. This study, however, was limited by the small sample size and, more importantly, by the fact that neonates started sildenafil after 7 days of mechanical ventilation. It is highly possible that after a week of mechanical ventilation, it is too late for prevention to work. An irreversible lung injury might have already occurred, and this is also supported by results from animal studies, where shorter mechanical ventilation duration of as little as 12 h caused lung injuries that were irreversible [8]. In the current RCT, a larger sample size was investigated, with sildenafil given to neonates as early as < 24 h postnatal.

The study groups have identical demographics at baseline. Unfortunately, sildenafil did not reduce the rate of BPD, and while it was associated with less mortality than placebo, this was not statistically significant. The causes of death in both study groups were not examined and are not an objective in the current trial. In a recent population-based study conducted by Schindler et al in ten NICUs in Australia, to explore the causes of mortality in extremely and very preterm neonates, acute respiratory failure was the most common cause of death among neonates born at 22–25 weeks of gestation, followed by intraventricular hemorrhage, and late sepsis. In infants born at 26–28 gestational weeks, intraventricular hemorrhage was the most common cause of mortality, while perinatal asphyxia was more associated with infants born at 29–31 weeks of gestation [44].

Similar trends were observed with the secondary outcomes. The placebo group had a 15% longer average respiratory support duration and a 10% longer length of hospital stay than the sildenafil group, but these were not statistically different. Premature infants are vulnerable to many complications such as intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, patent ductus arteriosus, and late sepsis due to their biological and physiological immaturity [45]. Apart from the retinopathy of prematurity, there was a general trend of more cases of intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, patent ductus arteriosus, and late sepsis reported with sildenafil than with placebo in this study. Apart from periventricular leukomalacia, differences in the number of cases were relatively minor but none were statistically significant between the two groups. Similar findings were reported by Backes et al., who observed no significant differences in the occurrence of retinopathy of prematurity, intraventricular hemorrhage, necrotizing enterocolitis, and patent ductus arteriosus in neonates with BPD-PAH between those patients who received sildenafil as compared to those not receiving sildenafil [46].

No side effects were reported with sildenafil in this study. This is anticipated as significant side effects would not be possible to detect in small number of patients and shorter duration of sildenafil. The finding of the side effects, however, is are consistent with the findings from the relevant trial by Konig et al., in the extremely preterm infants. Nevertheless, case reports and observational literature studies have reported some potentially serious side effects with sildenafil, including hypotension, pulmonary hemorrhage, and cerebral hemorrhage [20, 47–49]. Sildenafil was previously linked to interference with retinal function [50–52]. Recent literature data on retinopathy of prematurity, however, consistent with the results in this study, noted no evidence to support the removal of sildenafil in very preterm infants due to concerns over retinopathy of prematurity [8, 53].

In relation to the correlation between study outcomes and patient characteristics, this was not evaluated in any of the literature RCTs of sildenafil in infants. The mortality rate at 36 weeks PMA was statistically negatively associated with each of the gestational ages at delivery and the maternity care provided during pregnancy. This is supported by the study by Hornik et al., where improvement in survival was demonstrated by the increase in gestational age and receiving of antenatal care [54]. The respiratory support provided by 36 weeks' PMA was statistically associated with each of the occurrences of intraventricular hemorrhage and necrotizing enterocolitis, gestational age, and with receiving antenatal care or postnatal steroids. The fraction of inspired oxygen was statistically related to the presence of retinopathy of prematurity and necrotizing enterocolitis, as well as with higher gestational age and receiving postnatal steroids.

In this study, antenatal care was provided to almost half of twin infants compared with singletons, triplets, and quadruplets who received more antenatal care. Our study showed that, despite earlier antenatal steroids and delivery by caesarean section, very low birth weight twins were at greater risk of death than singletons, triplets, or quadruplets, which was similar to the findings found in Bellizzi et al study [55]. Evidence suggests that twins, whose mothers received intensive prenatal care during pregnancy, have a lower infant mortality rate [56].

Many previous studies have shown a clear inverse correlation between plurality and gestational age. In a large population-based study by Alexander et al., the mean gestational age was 39 weeks in singletons, 35.8 weeks in twins, and 32.5 weeks in triplets [57]. Consequently, neonatal morbidity and mortality have been shown to be higher among twins and triplets [56, 58]. Similar to this study, studies such as that by Buekens and Wilcox showed that twins were at higher risk of mortality regardless of their weight [59].

There are several limitations which could explain the lack of sildenafil effect against placebo in this study. First, it is possible that a total daily dose of 2 mg/kg is too small for the study purpose. In the only other study of sildenafil for BPD prevention in preterm infants, by Konig et al., a dose of 3 mg/kg/day was given. We opted for a lesser dose due to the lack of any prior evidence to inform the safety of the very early use of sildenafil in the extremely preterm infants in this study, especially given a high variability in the serum concentration and gastrointestinal absorption of oral medications in infants [60]. Higher doses may possibly have a positive impact on the lung growth in the study population. Second, due to lack of safety evidence, the duration of therapy was one week. It is possible that this was not a sufficient duration to reach maximum sildenafil dosage, especially given the relatively small sildenafil dose administered. Third, the hypothesis that sildenafil may have a preventative effect against BPD is primarily based on animal studies, such as the rat model experiment discussed above, by Park et al [15]. Animals in such studies are well controlled, exposed to hyperoxia-induced BPD, which may not replicate the pathophysiology of infants, who are often receiving less mechanical ventilation in recent days. It is possible therefore that the impact of sildenafil is inherently lower in preterm infants compared with animals. The outcomes in this study may have been affected by these factors as Woynarowska et al and Jung et al showed that patients with late pneumonia and respiratory distress syndrome were significantly associated with an increased risk of BPD [61, 62]. Finally, despite the prospective nature of the study, it was conducted in a single center with small sample size. However, the sample size is larger than the sample size in the most RTCs in the literature on sildenafil in infants.

There are very few trials that have evaluated the off-label use of sildenafil in infants to date, and these have their limitations, which may be partly due to the difficulty in performing experiments in infants. Sildenafil seems to be a drug with limited evidence of efficacy, safety and, thus, appropriate dosing and indication to prevent BPD in infants. But given results so far and the uncertainties of oral administration in preterm infants, it is highly likely that sildenafil is not useful for the prevention of BPD in infants. A recent evaluation of the pharmacokinetics of sildenafil in premature infants demonstrated that sildenafil can be associated with stable concentrations that are consistent with previously reported ranges in older children [63]. However, this refers only to intravenous sildenafil. Other recently completed studies, such as the ClinicalTrials.gov Identifier: NCT02244528, further evaluated the pharmacokinetics and safety in the preterm infants, including oral sildenafil. While all of these studies are only for sildenafil in the treatment of BPD-associated PAH infants, they may address some of the knowledge gaps with sildenafil for BPD prevention. Until data from broader evaluations become available to guide the sildenafil use in infants, particularly regarding the appropriate dosing regimens and the intravenous use, it would be wise not

to recommend sildenafil for the prevention of BPD in the very preterm infant population.

5 Conclusion

Taking into consideration the study setting and limitations, and while was not associated with side effects, sildenafil did not demonstrate efficacy in prevention against BPD in very preterm infants. Future dosing and efficacy trials that target varying dosing regimens of sildenafil are needed to show any benefit for the off-label preparation in the prevention of BPD.

Compliance with Ethical Standards

Ethical approval The study was appropriately approved by the institutional human research ethics committee at the Medical Research Center of HMC (reference #11087/11). The study was conducted in full conformance with principles of the "Declaration of Helsinki", Good Clinical Practice and within the laws and regulations of Ministry of Public Health in Qatar. Prior to the randomization of an enrolled infant, written informed consent was obtained from parents.

Conflict of interest All authors declare that they have no conflict of interest.

References

1. Lemons JA, Bauer CR, Oh W, et al. Very low birth weight outcomes of the National Institute of Child health and human development neonatal research network, January 1995 through December 1996. NICHD Neonatal Research Network. Pediatrics. 2001;107:E.

2. Jensen EA, Schmidt B. Epidemiology of bronchopulmonary dysplasia. Birth Defects Res A Clin Mol Teratol. 2014;100:145–57.

3. Donn SM, Sinha SK, editors. Manual of neonatal respiratory care. New York: Springer; 2012.

4. Greenough A. Long-term pulmonary outcome in the preterm infant. Neonatology. 2008;93:324–7.

5. Bhatt-Mehta V, Donn SM. Sildenafil for pulmonary hypertension complicating bronchopulmonary dysplasia. Expert Rev Clin Pharmacol. 2014;7:393–5.

6. Collins JJP, Tibboel D, de Kleer IM, et al. The future of bronchopulmonary dysplasia: emerging pathophysiological concepts and potential new avenues of treatment. Front Med (Lausanne). 2017;4:61.

7. Malavolti AM, Bassler D, Arlettaz-Mieth R, et al. Bronchopulmonary dysplasia—impact of severity and timing of diagnosis on neurodevelopment of preterm infants: a retrospective cohort study. BMJ Paediatr Open. 2018;2:e000165.

8. Konig K, Barfield CP, Guy KJ, et al. The effect of sildenafil on evolving bronchopulmonary dysplasia in extremely preterm infants: a randomised controlled pilot study. J Matern Fetal Neonatal Med. 2014;27:439–44.

9. Papoff P, Cerasaro C, Caresta E, et al. Current strategies for treating infants with severe bronchopulmonary dysplasia. J Matern Fetal Neonatal Med. 2012;25:15–20.

10. Greenough A. Long term respiratory outcomes of very premature birth (< 32 weeks). Semin Fetal Neonatal Med. 2012;17:73–6.

11. Gough A, Spence D, Linden M, et al. General and respiratory health outcomes in adult survivors of bronchopulmonary dysplasia: a systematic review. Chest. 2012;141:1554–67.

12. Anderson PJ, Doyle LW. Neurodevelopmental outcome of bronchopulmonary dysplasia. Semin Perinatol. 2006;30:227–32.

13. Simonca L, Tulloh R. Sildenafil in infants and children. Children. 2017;4:60.

14. Ladha F, Bonnet S, Eaton F, et al. Sildenafil improves alveolar growth and pulmonary hypertension in hyperoxia-induced lung injury. Am J Respir Crit Care Med. 2005;172:750–6.

15. Park HS, Park JW, Kim HJ, et al. Sildenafil alleviates bronchopulmonary dysplasia in neonatal rats by activating the hypoxia-inducible factor signaling pathway. Am J Respir Cell Mol Biol. 2013;48:105–13.

16. Mourani PM, Sontag MK, Ivy DD, et al. Effects of long-term sildenafil treatment for pulmonary hypertension in infants with chronic lung disease. J Pediatr. 2009;154:379–84.

17. Caputo S, Furcolo G, Rabuano R, et al. Severe pulmonary arterial hypertension in a very premature baby with bronchopulmonary dysplasia: normalization with long-term sildenafil. J Cardiovasc Med (Hagerstown). 2010;11:704–6.

18. Hon KL, Cheung KL, Siu KL, et al. Oral sildenafil for treatment of severe pulmonary hypertension in an infant. Biol Neonate. 2005;88:109–12.

19. Nyp M, Sandritter T, Poppinga N, et al. Sildenafil citrate, bronchopulmonary dysplasia and disordered pulmonary gas exchange: any benefits? J Perinatol. 2012;32:64–9.

20. Trottier-Boucher M, Lapointe A, Malo J, et al. Treatment of pulmonary arterial hypertension using sildenafil in neonates with bronchopulmonary dysplasia. Paediatr Child Health. 2014;19:e64.

21. Baquero H, Soliz A, Neira F, et al. Oral sildenafil in infants with persistent pulmonary hypertension of the newborn: a pilot randomized blinded study. Pediatrics. 2006;117:1077–83.

22. Vargas-Origel A, Gomez-Rodriguez G, Aldana-Valenzuela C, et al. The use of sildenafil in persistent pulmonary hypertension of the newborn. Am J Perinatol. 2010;27:225–30.

23. Soliz A, Concha E, Romero F. Oral sildenafil treatment as therapy for persistent pulmonary hypertension of the newborn: a multicenter

randomized trial. Pediatric Academic Societies' Annual Meeting; 2009 May 2–5; Baltimore MD, United States; 2009.

24. Herrera TR, Concha GP, Holberto CJ, et al. Sildenafil oral como alternativa en el tratamiento de recién nacidos con hipertensión pulmonar persistente. Rev Mex Pediatr. 2006;73:159–63.

25. Barst RJ, Beghetti M, Pulido T, et al. STARTS-2: long-term survival with oral sildenafil monotherapy in treatment-naive pediatric pulmonary arterial hypertension. Circulation. 2014;129:1914–23.

26. FDA Drug Safety Communication: FDA clarifies warning about pediatric use of revatio (sildenafil) for pulmonary arterial hypertension. 2016. https://www.fda.gov/drugs/drugsafety/ucm390876. Accessed 10 Feb 2018.

27. Abman Steven H, Collaco Joseph M, Shepherd Edward G, et al. Interdisciplinary care of children with severe bronchopulmonary dysplasia. J Pediatr. 2017;181(12–28):e1.

28. Dhariwal AK, Bavdekar SB. Sildenafil in pediatric pulmonary arterial hypertension. J Postgrad Med. 2015;61:181–92.

29. Uslu S, Kumtepe S, Bulbul A, et al. A comparison of magnesium sulphate and sildenafil in the treatment of the newborns with persistent pulmonary hypertension: a randomized controlled trial. J Trop Pediatr. 2011;57:245–50.

30. Karatza AA, Bush A, Magee AG. Safety and efficacy of Sildenafil therapy in children with pulmonary hypertension. Int J Cardiol. 2005;100:267–73.

31. Karatza AA, Narang I, Rosenthal M, et al. Treatment of primary pulmonary hypertension with oral sildenafil. Respiration. 2004;71:192–4.

32. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med. 2001;163:1723–9.

33. Hellström A, Smith LEH, Dammann O. Retinopathy of prematurity. Lancet. 2013;382:1445–57.

34. Mancini MC, Barbosa NE, Banwart D, et al. Intraventricular hemorrhage in very low birth weight infants: associated risk factors and outcome in the neonatal period. Rev Hosp Clin Fac Med Sao Paulo. 1999;54:151–4.

35. Rezaie P, Dean A. Periventricular leukomalacia, inflammation and white matter lesions within the developing nervous system. Neuropathology. 2002;22:106–32.

36. Gregory KE, DeForge CE, Natale KM, et al. Necrotizing enterocolitis in the premature infant: neonatal nursing assessment, disease pathogenesis, and clinical presentation. Adv Neonatal Care. 2011;11:155–66.

37. Clyman RI. Ibuprofen and patent ductus arteriosus. N Engl J Med. 2000;343:728–30.

38. Stoll BJ, Hansen NI, Sánchez PJ, et al. Early onset neonatal sepsis: the burden of group B streptococcal and *E. coli* disease continues. Pediatrics. 2011;127:817–26.

39. Phase II Clinical Trial. https://www2.ccrb.cuhk.edu.hk/stat/Phase II.htm. Accessed 21 Dec 2018.

40. Khan I, Sarker S-J, Hackshaw A. Smaller sample sizes for phase II trials based on exact tests with actual error rates by trading-off their nominal levels of significance and power. Br J Cancer. 2012;107:1801–9.

41. Cooper TR, Berseth CL, Adams JM, et al. Actuarial survival in the premature infant less than 30 weeks' gestation. Pediatrics. 1998;101(6):975–8.

42. Boghossian NS, Geraci M, Edwards EM, et al. Sex differences in mortality and morbidity of infants born at less than 30 weeks' gestation. Pediatrics. 2018. https://doi.org/10.1542/peds.2018-2352.

43. Atz AM, Wessel DL. Sildenafil ameliorates effects of inhaled nitric oxide withdrawal. Anesthesiology. 1999;91:307–10.

44. Schindler T, Koller-Smith L, Lui K, et al. Causes of death in very preterm infants cared for in neonatal intensive care units: a population-based retrospective cohort study. BMC Pediatr. 2017;17:59.

45. Atalay D, Salihoğlu Ö, Can E, et al. Short-term outcomes of very low birth weight infants born at a tertiary care hospital, Istanbul, Turkey. Iran J Pediatr. 2013;23:205–11.

46. Backes CH, Reagan PB, Smith CV, et al. Sildenafil treatment of infants with bronchopulmonary dysplasia-associated pulmonary hypertension. Hosp Pediatr. 2016;6(1):27–33.

47. Steiner M, Salzer U, Baumgartner S, et al. Intravenous sildenafil i. v. as rescue treatment for refractory pulmonary hypertension in extremely preterm infants. Klin Padiatr. 2014;226:211–5.

48. Khorana M, Yookaseam T, Layangool T, et al. Outcome of oral sildenafil therapy on persistent pulmonary hypertension of the newborn at Queen Sirikit National Institute of Child Health. J Med Assoc Thai. 2011;94:S64–73.

49. Samada K, Shiraishi H, Aoyagi J, et al. Cerebral hemorrhage associated with sildenafil (revatio) in an infant. Pediatr Cardiol. 2009;30:998–9.

50. Morales A, Gingell C, Collins M, et al. Clinical safety of oral sildenafil citrate (VIAGRA) in the treatment of erectile dysfunction. Int J Impot Res. 1998;10:69–73.

51. Laties AM, Fraunfelder FT. Ocular safety of Viagra, (sildenafil citrate). Trans Am Ophthalmol Soc. 1999;97:115–28.

52. Jagle H, Jagle C, Serey L, et al. Visual short-term effects of Viagra: double-blind study in healthy young subjects. Am J Ophthalmol. 2004;137:842–9.

53. Samiee-Zafarghandy S, van den Anker JN, Laughon MM, et al. Sildenafil and retinopathy of prematurity risk in very low birth weight infants. J Perinatol. 2016;36:137–40.

54. Hornik CP, Sherwood AL, Cotten CM, et al. Daily mortality of infants born at less than 30 weeks' gestation. Early Hum Dev. 2016;96:27–30.

55. Bellizzi S, Sobel H, Betran AP, et al. Early neonatal mortality in twin pregnancy: findings from 60 low- and middle-income countries. J Glob Health. 2018;8:010404.

56. Kogan MD, Alexander GR, Kotelchuck M, et al. Trends in twin birth outcomes and prenatal care utilization in the United States, 1981–1997. JAMA. 2000;284:335–41.

57. Alexander GR, Kogen M, Martin J, et al. What are the fetal growth patterns of singletons, twins and triplets in the United States? Clin Obstet Gynecol. 1998;41:115–25.

58. Luke B, Keith L. The contribution of singletons, twins and triplets to low birth weight, infant mortality and handicap in the United States. J Reprod Med. 1992;37:661–5.

59. Buekens P, Wilcox A. Why do small twins have a lower mortality rate than small singletons? Am J Obstet Gynecol. 1993;168:937–41.

60. Samiee-Zafarghandy S, Smith PB, van den Anker JN. Safety of sildenafil in infants*. Pediatr Crit Care Med. 2014;15:362–8.

61. Woynarowska M, Rutkowska M, Szamotulska K. Risk factors, frequency and severity of bronchopulmonary dysplasia (BPD) diagnosed according to the new disease definition in preterm neonates. Med Wieku Rozwoj. 2008;12:933–41.

62. Jung YH, Park Y, Kim BI, et al. Length at birth z-score is inversely associated with an increased risk of bronchopulmonary dysplasia or death in preterm infants born before 32 gestational weeks: a nationwide cohort study. PLoS One. 2019;14:e0217739.

63. Thakkar N, Gonzalez D, Cohen-Wolkowiez M, et al. An opportunistic study evaluating pharmacokinetics of sildenafil for the treatment of pulmonary hypertension in infants. J Perinatol. 2016;36:744–7.

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