ANIMAL MODELS OF BRONCHOPULMONARY DYSPLASIA. III: THE PRETERM AND TERM RABBIT MODELS

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Abstract

Bronchopulmonary dysplasia (BPD) is an important lung developmental pathophysiology that affects many premature infants each year. Newborn animal models employing both premature and term animals have been used over the years to study various components of BPD. This review describes some of the neonatal rabbit studies that have contributed to the understanding of BPD, including those using term newborn hyperoxia exposure models, premature hyperoxia models, and a term newborn hyperoxia model with recovery in moderate hyperoxia, all designed to emulate aspects of BPD in human infants. Some investigators perturbed these models to include exposure to neonatal infection/inflammation or postnatal malnutrition. The similarities to lung injury in human premature infants include an acute inflammatory response with the production of cytokines, chemokines and growth factors that have been implicated in human disease, abnormal pulmonary function, disordered lung architecture and alveolar simplification, development of fibrosis, and abnormal vascular growth factor expression. Neonatal rabbit models have the drawback of limited access to reagents as well as the lack of readily available transgenic models, but, unlike smaller rodent models, are able to be manipulated easily, and are significantly less expensive than larger animal models.
Hyperoxia exposure has been used in a number of animal models to recapitulate the inflammation, fibrosis and developmental arrest seen during the development of human bronchopulmonary dysplasia (BPD) (13, 39, 113). Newborn rabbits have been used in hyperoxia research since the mid-20th century (90, 103). Rabbits have potential specific advantages as a model of newborn lung injury, including developmental similarities to the human newborn, the long-term viability of preterm animals, a size as newborns large enough to permit intensive instrumentation, and relatively low cost. This article will review the use of hyperoxia and other perturbations, such as malnutrition and chorioamnionitis, to reproduce features consistent with human BPD in the term and premature newborn rabbit (Table 1). Studies over the last quarter century have revealed similarities of the responses in these rabbit models to those of human newborns in areas including the acute inflammatory response, cytokine and growth factor production, pulmonary function, lung architecture and the development of fibrosis (5, 21, 25, 29). The relative advantages and disadvantages of rabbit models, as compared to other animals, will also be discussed.

Newborn Rabbit Models of Hyperoxia

Pulmonary oxygen toxicity has been recognized since the time of Lavoisier (68). By the 1960’s, investigators had found that neonatal animals of many species, including rabbits, were more resistant to oxygen toxicity than adult animals of the same species (90, 103). Ogawa and Saito reported in 1961 that premature rabbit kits were less resistant to hyperoxia exposure than term rabbit kits (90). Shanklin and colleagues noted that the severity of lung injury in a vagotomized newborn rabbit model increased with increasing
fractional inspired oxygen concentration (FiO₂), with lung injury in FiO₂ 0.60 midway between that of FiO₂ 0.21 and 1.0 (103). By the 1970’s, investigators interested in nutritional research had developed longer-term neonatal rabbit models that could be maintained for up to 10 days using artificial feeding formulas (6).

The early work in animal hyperoxia exposure suggested that hyperoxia exposure in the newborn rabbit might be a model for recapitulating the role of oxidant stress in the development of bronchopulmonary dysplasia (BPD) in human infants. In a comprehensive study of pulmonary oxygen toxicity reported in 1978, Frank and colleagues compared survival and lung injury between adult and neonatal animals of five species (rat, mouse, rabbit, guinea pig and hamster) (40). Neonatal guinea pigs and hamsters had no survival advantage over adults. However, nearly all neonatal rats, mice and rabbits survived 7 days of FiO₂ >0.95, while nearly all adult animals died before 7 days. Among rabbits, 50% of adults had died by 3.2 days of FiO₂ >0.95 and nearly all had died by 5 days, while 10/10 newborn rabbits survived a full 7 days. On histological examination, the lungs of the adult rabbits showed alveolar hemorrhage, edema of the interstitial, perivascular and alveolar spaces, and formation of “hyalinelike membranes.” Neonatal lungs, however, showed only occasional alveolar septal widening with mild interstitial edema and/or hypercellularity. The lung architecture among newborns was otherwise reportedly normal. Superoxide dismutase (SOD), catalase and glutathione peroxidase activities were upregulated in the hyperoxia-exposed newborn rabbits, but not their adult counterparts (40). Similar upregulation of antioxidant enzymes was also seen in the newborn rat and mouse (but not in the guinea pig or hamster), a phenomenon subsequently confirmed by others (15). Later investigations have shown that the relative
hyperoxia resistance displayed by newborn rabbits is diminished even if exposure begins
7 days of age, and is associated with a decreased ability to upregulate antioxidant
enzymes and decreased pulmonary eicosanoids as compared to the immediate newborn
(47, 48).

Other experiments found that physiological changes occur early during hyperoxia
exposure and described more significant histological changes than those reported by
Frank and colleagues. Wender and colleagues delivered rabbit kits by Caesarean section
at 1 day before term (31 days in the rabbit) and exposed the animals to air or FiO$\textsubscript{2}$ 1.0 for
up to 72 hours (122). Kits were fed an artificial formula once daily by gavage. The lungs
of hyperoxia-exposed animals displayed focal areas of atelectasis flanked by areas of
hyperexpansion. The investigators calculated that only 43% of the lung appeared normal
(i.e., without atelectasis or hyperexpansion), as opposed to 82% of the lung in air-
exposed animals. The lungs of hyperoxia-exposed animals showed evidence of lipid
peroxidation and upregulation of SOD, glutathione peroxidase and glutathione reductase.
The investigators reported that intramuscular injection of vitamin E reversed many of the
findings seen in hyperoxia.

Ward and Roberts, in a series of experiments, exposed newborn rabbits to FiO$\textsubscript{2}$
>0.95 for 48-96 hours (119). Kits were delivered by Caesarean section at term and were
gavage fed once daily using a commercial rabbit formula. The investigators found
modest but statistically significant disturbances of pressure-volume relationships and
maximum lung distensibility in the lungs of newborn rabbits exposed to FiO$\textsubscript{2}$ >0.95 for
48 hours, when these were compared to normoxia-exposed controls. Phospholipid
content in lung lavage was also decreased following 48 or 96 hours of hyperoxia, and
phosphatidylcholine release and turnover was decreased in lung slices from animals exposed to 48 hours of hyperoxia (118, 119). Morphometric analysis showed that the number of type I alveolar epithelial cells was decreased and alveolar hemorrhage was increased in the hyperoxia-exposed newborns (120). The administration of vitamin E subcutaneously during exposure prevented the changes in pressure-volume dynamics, phospholipid content and morphology (120). Similar experiments by the same investigative group, using rabbits exposed to FiO$_2$ >0.90 for 72 hours and maintained on subcutaneously administered fluids, showed no effect of hyperoxia on lipid peroxidation, but did show an increased mortality (40%) as compared to air-exposed controls (0%) (123).

Experiments performed by Horowitz and colleagues, using a similar 96-hour exposure to FiO$_2$ >0.95, found that mRNA expression for surfactant protein A (SP-A) and the tissue inhibitor of metalloproteinases (TIMP) was elevated in hyperoxia-exposed animals as opposed to air-exposed controls beginning at 24 hours, and metallothionein mRNA expression was elevated at 96 hours (50, 112). Polak and colleagues evaluated surfactant metabolism in lungs from newborn rabbits exposed to FiO$_2$ >0.95 for 72 hours and found decreased disaturated phosphatidylcholine synthesis (93).

Sherman and colleagues also used a 96-hour hyperoxia-exposure model with term newborn rabbits, but exposed animals to FiO$_2$ 0.21, 0.40, 0.80 or >0.95 (105). Animals treated with FiO$_2$ 0.21 and 0.40, but not those treated with 0.80 and >0.95, showed a steady increase in macrophage yield from bronchoalveolar lavage (BAL) over the course of exposure. The decreased macrophage numbers in hyperoxic animals were shown to be the result of 12-fold lower rates of macrophage replication.
The 96-hour hyperoxia exposure model has also been used to evaluate other potential therapeutic approaches to prevention of hyperoxic injury. Kertesz and colleagues exposed 7-day-old rabbits to FiO$_2$ >0.95 to test the effectiveness of an infused leukotriene inhibitor, but showed no difference between drug- and vehicle-exposed animals in measures of lung inflammation or edema (60).

Overall, the data suggest that newborn rabbits display a resistance to hyperoxic injury compared to adults, which wanes quickly following birth. Among newborn rabbits exposed to 48-96 hours of hyperoxia, multiple physiologic processes, including the surfactant system and control of inflammation and the extracellular matrix, are affected at a time before significant histological changes are always apparent (Figure, Table 1). The models also confirm the feasibility of collecting BAL, performing morphometric analyses, collecting pulmonary function data and testing interventions including infused agents in newborn rabbits.

**Term Newborn Rabbit Models with Extended Hyperoxia Exposure**

In an injury model that went beyond 96 hours of hyperoxia, Sherman and Condiotti exposed litters of newborn rabbits to either air or FiO$_2$ >0.95 for up to 7 days (Figure) (104). Mothers were placed into the exposure chamber for feeding for one 6-hour period daily. Mortality among newborns was 3% at 96 hours and 16% at 7 days of hyperoxia, compared to 1% mortality over 7 days of air exposure. The investigators found that by 96 hours, 1/3 of the hyperoxia-exposed animals were not growing and that the mean weights of hyperoxia-exposed animals were lower than those of air-exposed controls, consistent with other neonatal hyperoxia models. Histological evaluation
showed that hyperoxia-exposed animals had increased numbers of polymorphonuclear cells (PMN) in their airways at 96 hours and 7 days. Alveolar macrophages gathered by BAL from the animals had an increased ability to produce superoxide at 48 hours of hyperoxia, but this capacity fell by 96 hours. Animals exposed to hyperoxia to 96 hours or more also showed impaired killing of inhaled staphylococci. These data suggest a disordered inflammatory cell response early in hyperoxia exposure.

In a preliminary study, Dobkin and colleagues exposed newborn rabbits to FiO$_2$ 0.55 – 0.65 for up to 10 days. Compared to air-exposed animals, hyperoxia-exposed animals experienced an earlier (days 1-4) and higher peak of transforming growth factor (TGF) $\beta$ protein content in whole lung homogenate, which may be associated with stimulating the development of fibrosis (37).

Ahmed and colleagues reported administering a human extracellular SOD cDNA by nebulization to newborn rabbits exposed to air or FiO$_2$ 0.95 (with 5% CO$_2$) for 3 or 7 days (1). Lungs from hyperoxia-exposed rabbits had increased immunostaining for nuclear factor kappa B (NFkB) at 7 days, as compared to air-exposed control lungs. Transient transfection with SOD maintained the availability of nitric oxide and decreased NFkB activity in the hyperoxia lungs, indicating a possible protective effect against oxidative stress (1).

Overall, the results of longer hyperoxia exposures in newborn indicate that such exposures are feasible with acceptable mortality. The inflammatory response seen early in hyperoxia exposure continues to progress, and the effect of hyperoxia on weight gain becomes apparent (Table 1). Abnormalities in bacterial killing and TGF$\beta$, NFkB and nitric oxide production are also detected during these exposures. Exposure to moderate
hyperoxia (FiO\textsubscript{2} 0.55 – 0.65) as well as to higher FiO\textsubscript{2} also produces effects, at least on potential pro-fibrotic pathways. The experiments also demonstrate the feasibility of administering nebulized therapy in the neonatal rabbit model.

**Term Newborn Rabbit Model Involving Hyperoxia and Recovery**

In a series of experiments, D’Angio, Ryan and colleagues developed a model of acute severe hyperoxia, followed by recovery in moderate hyperoxia, to mimic the acute injury followed by recovery in the setting of continued oxidative stress that is experienced by some human infants developing BPD (Figure) (20, 26, 27, 72, 110, 121).

Pregnant rabbits delivered vaginally at term and the litter was placed in a separate exposure chamber. Litters were exposed as a whole to either humidified oxygen or humidified room air. Among hyperoxia-exposed litters, the fractional inspired oxygen concentration (FiO\textsubscript{2}) was maintained at >0.95 for 8-9 days and then reduced to 0.60 for a total exposure of up to 36 days. Mothers were placed in the exposure chamber once daily for 30 minutes to feed the litters, with the chamber returned to the exposure condition (air or hyperoxia) during feeding. Animals exposed beyond 22 days were weaned at 3-4 weeks of age.

*Physiological and histologic findings.* In initial experiments, litters were exposed to FiO\textsubscript{2} >0.95 until 50% of animals had died or had been killed electively to avoid further suffering (LD\textsubscript{50}). The time to LD\textsubscript{50} ranged from 7-11 days, with a median of 9 days, which was used to establish the timing of the switch to FiO\textsubscript{2} 0.60 (26). Rabbit kits exposed to hyperoxia gained weight poorly, with lower weights than controls by day 7 that persisted over 36 days of exposure. BAL protein was elevated in hyperoxia-exposed
animals at the LD<sub>50</sub> timepoint (7-11 days) and at 22 days (26). Hyperoxia-exposed animals developed mild alveolar edema by 4 days of exposure and significant alveolitis, with PMN and mononuclear cell infiltrates, septal edema and thickening, hyaline membranes and type II alveolar epithelial cell hyperplasia by 10 days (26, 27). Type II cell proliferation was increased at 8-12 days in hyperoxia animals (20). The alveolar architecture appeared simplified at 4 and 10 days of hyperoxia exposure (26, 27). By 14-22 days of total exposure (that is, 5-14 days of recovery in FiO<sub>2</sub> 0.60), hyperoxia-exposed animals showed resolving septal edema and persistent mild alveolitis, with a predominance of intraalveolar macrophages. Alveolar septal thickness and septal collagen content were elevated at 22 days (26). By 36 days of hyperoxia, patchy mild septal thickening persisted, but cellular infiltrates had largely resolved and overall septal thickness no longer differed from air-exposed animals (26).

**Chemokines and cytokines.** Proinflammatory cytokine mRNA expression for monocyte chemoattractant protein (MCP)-1 (CCL2) (localized to type II cells), interleukin (IL)-1β (localized to mononuclear cells), IL-8 (CXCL8) (localized to PMN), and growth-regulated protein (Gro) β (CXCL2) mRNA expression was elevated in hyperoxia-exposed animals, peaking between 6-10 days of exposure (27, 110). IL-8 protein in lung lavage fluid was elevated in a similar pattern. Tumor necrosis factor (TNF) α mRNA was not detected in lung tissue and TNF bioactivity could not be detected in lung lavage fluid (110). Cytokine expression decreased rapidly once FiO<sub>2</sub> was decreased to 0.60.

**Growth factors.** Keratinocyte growth factor (KGF) mRNA expression was noted to be elevated at 6 days in hyperoxia-exposed animals (20). TGFα protein in BAL was
increased at 6-8 days of hyperoxia, while the appearance of lower-molecular-weight, mature forms of TGFβ was delayed, rising above control levels at 22 and 36 days (114). BAL concentrations of parathyroid hormone-related protein (PTHrP), a putative regulator of type II cells, was increased during acute hyperoxia, fell at the LD50 time point and rebounded during recovery, and was inversely correlated with the number of type II cells expressing proliferating cell nuclear antigen (PCNA) (46). Vascular endothelial growth factor (VEGF)-1 mRNA abundance (expressed primarily by type II cells) decreased during exposure to FiO2 >0.95, reaching a nadir at 9 days, and returned to normal during recovery in FiO2 0.60 (72). VEGF-1 protein immunostaining followed a similar pattern, while VEGF-1 protein in lung lavage also decreased during FiO2 >0.95, but became elevated above room air levels during recovery in FiO2 0.60. The relative abundances of the splice variants of VEGF-1 were also altered by exposure to FiO2 >0.95, with a marked relative decrease of the 189-amino-acid variant, which is primarily bound to the extracellular matrix (121).

Surfactant and surfactant proteins. In keeping with the findings of others, surfactant function was diminished at the LD50 (26). Surfactant protein (SP)-A mRNA expression, was elevated in LD50 animals compared with controls and appeared to be localized in type II cells (26). SP-B and SP-C expression was unaffected. Overall, the acute injury/recovery model reproduced the findings of other investigators regarding the inflammation, architectural changes and surfactant system dysfunction accompanying acute hyperoxia (Table 1). The model revealed that the histological and physiological changes were closely associated with changes of proinflammatory cytokines and growth factors that are potential mediators of the
hyperoxia effect. However, the model did not result in apparent long term changes in alveolar size or structure. The changes seen during acute hyperoxia largely resolved in an atmosphere of moderate hyperoxia and the observed changes in growth factors such as VEGF-1 and PTHrP may facilitate the repair process. In addition to adding extensive characterization of hyperoxia-induced injury in the newborn rabbit, the injury/recovery model may provide a reasonable reproduction of the human experience of injury followed by recovery under continued oxidative stress.

**Premature Newborn Rabbit Models of Bronchopulmonary Dysplasia**

Early work in oxygen exposure showed that premature rabbits have increased sensitivity to hyperoxia, as compared to term newborn rabbits (90). Frank and Sosenko evaluated the development of the antioxidant systems during fetal life and found that in rabbits, as in several other species, the antioxidant system develops quickly in concert with the surfactant system during the last 10-15% of pregnancy, with lung antioxidant levels in the fetal rabbit rising 110-200% in the 3-5 days before the end of a typical 31 day gestation (42, 43). Lung structure matures from the early saccular to early-to-mid alveolar phase over the same period (39, 43, 67, 77). The same investigators exposed premature (delivered at 29 days of gestation) and term newborn rabbit kits to FiO₂ >0.90 for 48-72 hours (44). Animals were fed formula daily by gavage. Term rabbits responded to hyperoxia with elevations in activities of SOD, catalase, glutathione peroxidase and glucose-6-phosphate dehydrogenase, while premature rabbits did not show similar elevations. The premature rabbits displayed increases in BAL protein content and conjugated diene levels and more severe lung pathology, as compared to term
rabbits exposed to the same degree of hyperoxia. In subsequent experiments, the investigators found that subcutaneous administration of endotoxin at birth and 24 hours to 29-day-gestation rabbits exposed to FiO$_2$ for 48 hours improved SOD mRNA expression and activity, BAL protein content and survival (91%), as compared to hyperoxia alone (survival 76%) (107). Walther and colleagues confirmed a similar lack of upregulation of catalase and SOD in 28- or 29-day-gestation rabbit kits exposed to 24 hours of FiO$_2$ 1.0 and showed that administering surfactant liposomes containing CuZn-SOD and catalase increased lung SOD and catalase activity in both preterm and term rabbit kits (115). They also found that, despite the lack of increase in measured enzyme activity, both CuZn-SOD and catalase mRNA expression in 28-day-gestation rabbits was increased following 24 hours of hyperoxia exposure (116).

Using a similar model, Bany-Mohammed and colleagues exposed 29-day-gestation rabbit kits delivered by hysterotomy to either air or FiO$_2$ 1.0, providing nutrition by daily gavage feeding of formula (9). Animals received recombinant human erythropoietin or vehicle subcutaneously on days 0 and 2. Hyperoxia for 72-96 hours increased BAL protein, decreased the ability of BAL to prevent lipid peroxidation and caused alveolar thickening and alveolar proteinaceous exudate, as compared to air-exposed controls. Erythropoietin administration decreased plasma iron and decreased iron saturation of transferrin, and mitigated the BAL and histological findings. The same investigative group also tested the administration of intravenous transferrin in hyperoxic 29-day-gestation rabbits, and showed similar improvements in BAL protein and evidence of lipid peroxidation at 2 to 4 days (102). Inhaled nitric oxide (14 ppm) was also found to mitigate the negative effects of a brief, 20-hour period of FiO$_2$ 0.98 in 29-day-gestation
kits on BAL surfactant composition and activity, surfactant protein B content, lipid peroxidation and glutathione content (54).

Using a longer hyperoxia exposure of 11 days, Mascaretti and colleagues assessed lung histology and morphology following premature birth in rabbits (77). Twenty-eight-day-gestation preterm rabbits were delivered by Caesarean section and placed in either air or FiO$_2$ >95%. Animals were fed twice daily by gavage, using a rabbit formula, and treated with prophylactic vitamin K and antibiotics during exposure. Mortality was higher (89%) in hyperoxia-exposed animals than in air-exposed animals (69%). The lungs of hyperoxia-exposed animals showed a reduction in alveolar number and thickening of the interstitium with inflammatory cell infiltration. Morphometric analysis confirmed an increase in the mean linear intercept and a decrease in alveolar number. The number of collagen fibers was decreased and both collagen and elastic fibers were disorganized. Subsequent work by the same group compared 29-day gestation rabbits exposed to FiO$_2$ 0.80 for 11 days to 28-day gestation rabbits exposed to FiO$_2$ >0.95 for the same time (75). Both hyperoxia groups showed impaired alveolar development and septal thickening and, in contrast to earlier work, elevated proportions of collagen and elastic fibers, when compared to age-matched, air-exposed controls. Although the early survival was higher in the 29-day, FiO$_2$ 0.80 group than the 28-day FiO$_2$ >0.95 group, mortality by day 11 approached 90% in both groups, compared to 40-70% in the air-exposed groups.

Richter and colleagues have recently reported results of a 7-day hyperoxia exposure among preterm rabbits (95). Preterm kits were delivered at 28 days gestation by Caesarean section and term controls were allowed to deliver spontaneously at term.
Preterm animals were exposed to either air or FiO$_2$ >0.95 for 7 days. They were fed twice daily by gavage with a rabbit formula and treated with vitamin K and antibiotics. Term controls remained in air with their mothers for the duration of the exposure. Survival to 7 days was 83% among air-exposed preterm rabbits and 56% among hyperoxia-exposed preterm rabbits (and 84% among term controls). The investigators performed extensive pulmonary function testing using whole body plethysmography and forced oscillation. Hyperoxia-exposed animals had multiple lung function abnormalities, including decreased minute volume, decreased total lung capacity, decreased static compliance and increased tissue elasticity, when compared to air-exposed preterm rabbits (who were similar to term controls). In keeping with other models, histological and morphometric comparisons showed increased alveolar size and septal thickening with edema and inflammatory cell infiltration in the hyperoxia-exposed animals. Hyperoxia-exposed animals also had higher numbers of proliferating cells in the lung. Sirius Red staining showed increased collagen deposition in hyperoxia-exposed lungs.

Consistent, controlled morphometric measurements such as those described in the aforementioned studies (75, 95) are particularly important to provide reproducible, easily comparable measures, as differing models of differing lung diseases may require the use of differing morphometric techniques (83, 89). For instance, Mühlfeld and Ochs, in a recent review of quantitative microscopy of the lung, note that the appropriate stereological techniques to assess changes in blood vessels differ from those appropriate to assessing emphysematous changes; indeed, techniques that measure alveolar size may not be appropriate for assessing alveolar septa (83). The appropriate tissue preparation
and sectioning for each morphometric technique may differ, requiring separate fixation, processing and sectioning for various measures (89).

In summary, the lungs of premature rabbits have many similarities to those of human premature infants. Premature rabbit models of hyperoxia exposure show that preterm rabbits display diminished antioxidant response, as compared to term newborns, and develop lung pathology similar to that described in term rabbits. Although hyperoxia exposures as long as 11 days have been described, mortality appears unacceptably high with exposures beyond 7 days (Figure). Work to date in premature rabbit models of hyperoxia shows the feasibility of moderately prolonged hyperoxia exposure and their suitability for extensive physiological and morphometric testing and intratracheal and intravenous drug administration, although less mechanistic work has been done in premature rabbits than in the term models. The models tend to require supportive measures, such as gavage feeding and the administration Vitamin K and antibiotics. Overall, however, premature rabbit models of hyperoxia are practical and may be more relevant to human BPD than term newborn rabbit models.

**Other Newborn Rabbit Models**

Mataloun and colleagues have explored a model that combines the effects of malnutrition and hyperoxia in the preterm rabbit kit (78). Kits were delivered at 28 days gestation by Caesarean section and fed twice daily for 7 days by gavage with either a routine rabbit formula or a diet with 30% fewer calories than the routine diet. Malnutrition resulted in lower body weights, lower lung weights, lower lung water content, decreased alveolar numbers, decreased numbers of elastic fibers and decreased
lung collagen. Animals in each group were also exposed to either air or FiO$_2$ >0.95. Hyperoxia among control animals decreased alveolar number, elastic fibers and lung collagen, and produced septal thickening. Combined exposure to malnutrition and hyperoxia further reduced alveolar number, elastic fibers and lung collagen. These data are in agreement with earlier reports of malnutrition exacerbating hyperoxic lung injury in newborn rats (41). Spontaneously feeding rabbit models of hyperoxia may need to be evaluated for a nutritional deprivation component of their effect, as animals might feed less well as their lung function worsened.

Newborn rabbits have also been used to assess the effect of chorioamnionitis on pulmonary development. Gras-Le Guen and colleagues inoculated the uteruses of pregnant rabbits at gestational day 29 with *Escherichia coli*, treated the mothers with antibiotics beginning 8 hours after inoculation, and allowed kits born spontaneously 60-84 hours later to remain with their mothers for up to 15 days (45). Stillbirth and neonatal mortality were higher in *E. coli*-exposed pregnancies than in vehicle treated pregnancies. Compared to kits from vehicle-injected controls, surviving *E. coli*-exposed kits had lower body weights and absolute lung volumes by days 8-15 after birth. Alveolarization in the *E. coli*-exposed kits was impaired. Vehicle- and *E. coli*-exposed kits showed no differences on measures of inflammation or apoptosis. The findings in this model are reminiscent of the alveolar simplification that has been described in the “new” BPD among humans (55, 56).

Both term and preterm rabbits (delivered between 27 and 29 days of gestation) have been used in short-term (≤16 hours) experiments involving mechanical ventilation. These models have tested the effects of the administration of surfactant (24, 30, 64, 86),
of varying conventional mechanical ventilation and oxygen strategies (52) and of
conventional vs. high frequency oscillatory ventilation (88), as well as the effects of
conventional ventilation on SP-A and KGF (36). With animal weights ranging from
about 25 to 35 grams at 27 days gestation, the preterm model allowed ventilation using an
18 gauge catheter inserted into the trachea (30, 88).

The effect of 14 days in a heliox (21% oxygen, 79% helium) environment has
been tested in four-day old rabbit kits. No differences in weight, growth factors,
tissue/organ weights, blood chemistries or muscle enzyme activity were detected between
heliox- and air-exposed animals (106). This finding has relevance for human infants
treated with heliox to decrease gas viscosity in obstructive lung conditions.

Fetal rabbits have been used to study various aspects of lung development and conditions
such as lung growth following tracheal occlusion (34, 42, 61). Instrumented pregnant
rabbits have allowed study of the complex interactions between surfactant present in the
amniotic fluid and vernix caseosa in the subsequent stimulation and maturation of
intestinal enterocytes (85). Newborn rabbits have also been used to study other
conditions and disorders relevant to the human newborn, such as meconium aspiration,
methods of resuscitation, lung liquid clearance and surgically-created diaphragmatic
hernia, (22, 38, 49, 124, 125), but these models are not directly relevant to BPD.

Overall, newborn rabbit models including malnutrition and infection stress the
need to consider these factors in the pathogenesis of experimental BPD and point to
potential mechanisms for human disease. Newborn rabbit models have also been shown
to be versatile, allowing instrumentation and mechanical ventilation, as well as the study
of multiple other perturbations.
Other Relevant Rabbit Models of Lung Disease

It would be difficult in the course of a single review to detail the many uses to which the rabbit has been put to study the pathogenesis of lung disease, but a few examples bear mention. In a study of juvenile, rather than newborn, animals, 8-10 week old, tracheostomized, ventilated rabbits have been used as a model for perfluorochemical-enhanced delivery of recombinant human SOD in acute lung injury (14).

Adult rabbits have been used particularly extensively to model human asthma, as they are phylogenetically more closely related to humans than are rodents, have lungs that are more anatomically similar to humans, share qualities such as IgE mediation of anaphylaxis and relative capsaicin unresponsiveness with humans, and can model both early and late phase airway responses (58, 59). Recent work in a rabbit asthma model has elucidated the role of the proasthmatic cytokine IL-13 in producing impaired endogenous glucocorticoid activity via the upregulation of 11β-hydroxysteroid dehydrogenase, showing the value of well-developed rabbit models for dissecting the pathogenesis of human asthma (57).

Adult rabbits also have been used for the study of innate immunity in the lungs in response to mechanical ventilation and/or sepsis (76), an example of the many adult rabbit models of acute lung injury, including some of the earliest groundbreaking work on transfusion-associated lung injury (71). Rabbit models have contributed to air pollution research. In a recent manuscript, Miyata and colleagues described the ability of lovastatin to counter the systemic and bone marrow inflammatory responses to ambient particulates, showing a delay in the release of PMN from bone marrow pools, a reduction
in retention of PMN in the lung and a reduction in plasma IL-6 (82). In areas beyond the lung proper, rabbits have been used to model the pleural response to injury (66). In a recent study, Komissarov and colleagues described the role of α-macroglobulin as a “molecular cage” for urokinase used in fibrinolytic therapy following experimental pleural injury with tetracycline in rabbits (65).

Overall, the extensive use of the adult rabbit as a model for human disease and the increasingly mechanistic studies possible in the rabbit bode well for the extension of similar techniques into newborn rabbit models.

Rabbit Models in Context

Rabbits are born with lungs in the early to middle alveolar stage, similar to the lungs of newborn human infants, have mature surfactant and antioxidant systems, and have postnatal lung development similar to humans (39, 67) (Table 2). This is in contrast to mice and rats, which are born at term with lungs in the saccular stage, similar to premature human newborns, but which have mature surfactant and antioxidant systems and air exchange (39). Other animal models, such as the sheep and primate, are also born in the alveolar (rather than saccular) stage of development, but lack the small size and lower cost of rabbits and other small animal models (4, 23, 92, 126) (Table 2). Preterm rabbits at 28 or 29 days of gestation are in the early saccular phase of lung development and have immature surfactant and antioxidant systems, making them potentially valuable models of the premature human newborn, in that they possess both structural and functional similarities to the human (43, 77). Overall, further development
of such a premature model may offer significant advantages over both term rabbit models and term rodent (rat, mouse) models of BPD.

Rabbits, rats, mice and several other species, including sheep, pigs and baboons, all develop similar inflammation and alveolar simplification following hyperoxia exposure (13, 32, 35, 94, 113) (Table 1). Some potential mechanisms for these effects, such as the elaboration of proinflammatory cytokines and chemokines, appear to be relatively similar across species ranging from mouse to rabbit to baboon to human, lending support to the hypothesis that these are common, central mediators of the response to hyperoxia (23, 27, 98, 110). Similarly, elevations in TGFα and/or TGFβ have been reported in multiple animal models of newborn lung injury, including rabbits, rats and lambs (12, 18, 53). Protective effects of the exogenous administration or overexpression of various forms of SOD similar to those seen in the rabbit have been observed in a number of newborn animal models of lung injury, including mice, sheep and piglets (1, 2, 63, 81, 84, 91, 97, 115). Each these animal models may shed light on the protective effect reported in some studies of exogenous administration of SOD to human premature newborns (31, 33, 99, 100, 108). Other phenomena, however, such as VEGF expression and production, vary in direction and magnitude in response to hyperoxia across species from rodents to rabbits to humans (7, 8, 10, 11, 16, 28, 51, 69, 72-74, 80, 121). This led Buczynski and colleagues in a recent review to propose that interspecies differences in lung development, as well as timing and methods of VEGF measurement, might be at the root of such disparities (16).

Recent reviews of animal models in various species, including humans and some of the rabbit models here described, have extensively explored recent progress in areas
such as the mechanisms of acute lung injury in preterm fetuses and newborns (53), the mechanisms of derangement of late lung development and its relationship to BPD (70, 79), the use of large animal (sheep and baboon) models (3), the role of strain in the alveolus (96), the mechanism of hyperoxic injury in both animal models (16) and humans (101) and the role of stem cells in the pathogenesis of BPD (87). It is only through close evaluation of the similarities and differences among the various models and between the models and human disease that the most appropriate model for each aspect of the pathogenesis of BPD will become apparent.

**Advantages, Disadvantages and Conclusions**

Neonatal rabbit models of hyperoxia exposure for varying periods in both term and preterm rabbits appear to recapitulate many of the patterns seen in human neonatal lung disease and bronchopulmonary dysplasia (Table 1, Figure). These similarities include an acute inflammatory response with the production of cytokines, chemokines and growth factors that have been implicated in human disease, abnormal pulmonary function, disordered lung architecture and alveolar simplification, development of fibrosis, and abnormal vascular growth factor expression (5, 21, 25, 29). In a model involving recovery after acute hyperoxia, many of these abnormalities improve during recovery from the acute injury, despite the continuing moderate hyperoxia, the sort of conditions an infant developing the “new” BPD might encounter (26, 55, 56). Models involving malnutrition and infection, conditions also likely to be experienced by human premature infants, also show abnormalities (45, 78). Malnutrition alone causes alveolar simplification and collagen and elastin abnormalities, reminiscent of the “new” BPD;
hyperoxia exacerbates this process (78). Prenatal infection alone also produces alveolar simplification (45).

Specific advantages of rabbit models include their relatively brief course, compared to larger animals (Table 3). Rabbits reach adolescence by the close of exposure at 5-6 weeks (although, unlike the human, their alveoli are still developing rapidly at that stage), allowing early evaluation of near-adult animals following a neonatal perturbation (67). Rabbit models are also cost-effective compared to larger animal models, but large enough to permit perturbations, including nebulized treatments, mechanical ventilation and pulmonary function testing, and easy isolation of individual cells, even among newborn or premature animals (1, 30, 36, 52, 64, 86, 88, 95). Neonatal rabbits weigh about 50 grams, slightly above the size of an adult mouse, and even premature (27-29 day) rabbits have weights in the range of a mouse’s 30 grams. The once-daily feeding behavior of term rabbits allows mothers to be placed in the exposure chamber for brief periods once daily, avoiding the need for gavage feeding or cross-fostering and rotating mothers to prevent oxygen toxicity to the mothers. Preterm rabbits appear to grow well on twice daily, gavage feedings, also minimizing the need for interrupting exposure and handling the animals. Rabbits are relatively easy to breed in the local vivarium, and the cost of maintaining a colony is not prohibitive.

The developmental stage of rabbits at birth is both an asset and a liability to the use of rabbits to model BPD. As noted previously, when compared to species such as the mouse or rat, rabbits are more similar to humans at term, as both their alveolarization and surfactant and antioxidant systems have similar levels of maturity to the human. While this is an advantage for modeling the term human newborn, BPD overwhelmingly occurs
among premature infants, restricting the value of the term rabbit as a model. It is not clear, however, that term small rodent models, with discordant anatomic and functional maturation when compared to the human, serve the purpose better. Preterm rabbits in late gestation (28-29 weeks) are similar to premature human newborns in terms of maturity and appear capable both of surviving and of being instrumented, which make them perhaps better models for the human premature infant than either term rodents or term rabbits. The addition to the premature model of recovery in moderate hyperoxia may further improve the fidelity to the human experience.

Since rabbits are used less commonly than rats or mice for modeling BPD, no truly standard rabbit model has emerged. Described models vary by the type of exposure, length of exposure, gestational age of the animals, mode of delivery, type of feeding, and ancillary components such as humidity and the use of vitamins and antibiotics. Hyperoxia is the most common reported exposure, but investigators have used FiO\textsubscript{2} ranging from 0.55 to 1.0 for the acute injury portion of their models (37, 75, 122). Acute hyperoxia exposures have varied in length from short-term models under 24 hours to up to 11 days of exposure, with exposures from 7 to 11 days producing similar results (26, 52, 54, 77, 104, 112). Bacterial exposure and malnutrition as models have been explored infrequently, although the findings from those experiments suggest that investigators should be sensitive to the effects of nutrition and infection on their models and that these factors may be important in the human newborn (45, 78). Many rabbit models use gavaged formula, rather than suckled maternal milk, for feeding. Formulas may provide more consistent nutrition, but are unlikely to reflect fully the complex nutritional and immunological mix of fresh rabbit milk. Premature rabbit models may reflect human
premature newborns more accurately than term rabbit models, but survival has been low
in some investigators’ hands. A term newborn model of acute hyperoxia exposure and
recovery has been described, but, as noted, it may not faithfully reflect the developmental
state of the human premature newborn (26). Further, the term newborn recovery model
has not been critically evaluated for morphometric similarities to or differences from the
human infant recovering from BPD. Premature rabbit kits have been maintained briefly
on mechanical ventilation, showing evidence that it is possible to manipulate the model in
this manner (30, 36, 52, 64, 86, 88). However, there are no published longer-term
exposures to both hyperoxia and mechanical ventilation that would mimic more fully the
exposures faced by the human premature infant.

A notable disadvantage of newborn rabbit models of lung injury is the relative
paucity of reagents and transgenic models specific to the rabbit, particularly as the tools
available to study the mouse have proliferated during the early 21st century (58). siRNA
technology has been used in rabbits (117), rabbit-specific qPCR primers are now
commercially available and there are now companies devoted to producing antibodies
specific to rabbit proteins, but these resources remain rarer than those for humans or
mice. Transgenic rabbit models also remain rare (58).

An appropriate place for rabbit models of BPD among other models of neonatal
lung injury might be for the relatively rapid and inexpensive initial testing of hypotheses
that require instrumentation or other active intervention in preterm or term animals.
Promising hypotheses could then undergo further mechanistic testing in mouse models
and/or be moved into larger animal models as a step toward human interventions.


3. **Albertine KH.** Progress in understanding the pathogenesis of BPD using the baboon and sheep models. *Semin Perinatol* 37: 60-8, 2013.


44. Frank L, Sosenko IR. Failure of premature rabbits to increase antioxidant enzymes during hyperoxic exposure: increased susceptibility to pulmonary oxygen toxicity compared with term rabbits. *Pediatr Res* 29: 292-6, 1991.


### Pulmonary Findings in Rabbit Models of Bronchopulmonary Dysplasia

<table>
<thead>
<tr>
<th>Category</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hyperoxia Models (Term and Preterm)</strong></td>
<td></td>
</tr>
</tbody>
</table>
| Diminished Lung Function        | Decreased compliance (95, 119)  
|                                | Decreased total lung capacity (95, 119)  
|                                | Increased elasticity (95)                                                                                                                   |
| **Surfactant System Abnormalities** |                                                                                                                                            |
|                                | Decreased surfactant function (26, 54)  
|                                | Diminished surfactant phospholipid abundance and synthesis (54, 93, 118, 119)  
|                                | Increased SP-A expression (26, 50, 112)  
|                                | Increased SP-B in BAL (54)                                                                                                                   |
| **Altered Oxidant/Antioxidant Status** |                                                                                                                                            |
|                                | Increased SOD, catalase, glutathione peroxidase, glutathione reductase, G6PD activity (term newborns only) (40, 44, 54, 115, 116, 122)  
|                                | Increased lipid peroxidation (9, 44, 54, 102, 122)                                                                                           |
| **Lung Architectural Changes**   |                                                                                                                                            |
|                                | Atelectasis/overdistention (122)  
|                                | Hyaline membranes (9, 26)  
|                                | Septal thickening (9, 26, 40, 75, 77, 78, 95)  
|                                | Altered collagen and elastin deposition and networks (26, 75, 77, 78, 95)  
|                                | Alveolar simplification (26, 27, 75, 77, 78, 95)  
|                                | Decreased type I cell number (120)  
|                                | Type II cell hyperplasia and proliferation (19, 26)                                                                                          |
| **Disordered Inflammation**     |                                                                                                                                            |
|                                | Inflammatory infiltrates, primarily PMN and mononuclear cells (26, 27, 40, 77, 95, 104)  
|                                | Decreased alveolar macrophage proliferation (105)  
|                                | Diminished macrophage respiratory burst and bacterial killing (104)  
|                                | Alveolar and interstitial edema (26, 40, 95)  
|                                | Alveolar hemorrhage (120)  
|                                | Increased protein in BAL (9, 26, 44, 102)                                                                                                    |
| **Cytokines and Transcription and Growth Factors** |                                                                                                                                            |
|                                | Increased proinflammatory cytokine abundance – IL-1β (27), IL-8 (27), MCP-1 (27), Groβ (110)  
|                                | Altered growth factors – TGFα (114), TGFβ (37), VEGF (72, 121), PTHrP (46), KGF (20)  
|                                | Altered matrix factors – TIMP, metallothionein (50, 112)  
|                                | Increased NFκB activation (1)                                                                                                               |
| **Interventions That Mitigate Injury** |                                                                                                                                            |
|                                | Vitamin E (120, 122)  
<p>|                                | Exogenous or transiently expressed SOD (1, 115, 116)                                                                                         |</p>
<table>
<thead>
<tr>
<th>Endotoxin (107)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythropoetin (9)</td>
</tr>
<tr>
<td>Transferrin (102)</td>
</tr>
<tr>
<td>Nitric oxide (54)</td>
</tr>
</tbody>
</table>

**Other Models**
- Malnutrition (78)
  - Lower lung weight
  - Decreased alveolar number
  - Altered collagen and elastin networks
  - Findings worsened by concurrent hyperoxia
- Prenatal Bacterial Exposure (45)
  - Alveolar simplification

937
938
Table 2. Onset of alveolar stage of lung development in animals used in bronchopulmonary dysplasia models.

<table>
<thead>
<tr>
<th>Species</th>
<th>Approximate onset of alveolar stage</th>
<th>Days of gestation</th>
<th>Percent of completed gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse (92, 109, 126)</td>
<td>PD 1-5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Rat (17, 92, 109, 126)</td>
<td>PD 1-7</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Rabbit (62, 67, 92, 126)</td>
<td>28-30 days</td>
<td>90-97%</td>
<td></td>
</tr>
<tr>
<td>Sheep (4, 92, 126)</td>
<td>120 days</td>
<td>80%</td>
<td></td>
</tr>
<tr>
<td>Primate (baboon) (23, 92)</td>
<td>155-165 days</td>
<td>84-90%</td>
<td></td>
</tr>
<tr>
<td>Human (92, 126)</td>
<td>252 days</td>
<td>90%</td>
<td></td>
</tr>
</tbody>
</table>

PD = postnatal day
Table 3

<table>
<thead>
<tr>
<th>Table 3: Potential Advantages and Disadvantages of Rabbit Models of BPD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
</tr>
<tr>
<td>* Mimic many findings in human BPD</td>
</tr>
<tr>
<td>* Term models mimic lung maturity of term human newborns</td>
</tr>
<tr>
<td>* Preterm models mimic lung maturity of premature human newborns</td>
</tr>
<tr>
<td>* Relatively brief time from newborn to adulthood</td>
</tr>
<tr>
<td>* Once daily feeding behavior (term newborns)</td>
</tr>
<tr>
<td>* Established gavage feeding regimens (term and preterm newborns)</td>
</tr>
<tr>
<td>* Size of term and preterm newborns allows manipulation</td>
</tr>
<tr>
<td>* Ability to use mechanical ventilation (short term)</td>
</tr>
<tr>
<td>* Relatively low cost</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
</tr>
<tr>
<td>* Term models do not reflect preterm human anatomical or functional immaturity</td>
</tr>
<tr>
<td>* Lack of standardized model</td>
</tr>
<tr>
<td>* Fewer reagents and transgenic models</td>
</tr>
</tbody>
</table>
**FIGURE**

**Term Newborn Hyperoxia**

\[
\downarrow \downarrow \downarrow \downarrow
\]

**FiO₂ 0.90 – 1.0**

Birth 2 4 6 8 10

Days

**Premature Hyperoxia**

\[
\downarrow \downarrow \downarrow \downarrow
\]

**FiO₂ 0.80 – 1.0**

GD 2 4 6 8 10

27-29 Days

(Term = 31 days)

**Term Newborn Hyperoxia with Moderate Hyperoxia Recovery**

\[
\downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow
\]

**FiO₂ >0.95**

Birth 2 4 6 8 10

Days

**FiO₂ 0.60**

22

36
Figure. Attributes of common rabbit models of bronchopulmonary dysplasia. Term newborn models are delivered spontaneously or by Caesarean section at 30 or 31 days of a typical 31 day gestation (1, 26, 37, 40, 50, 93, 104, 105, 111, 118-120, 122, 123). Premature newborn models are delivered by Caesarean section at 27-29 days of gestation (GD) (9, 54, 75, 77, 78, 95, 102, 107, 115, 116). Animals either feed with mother (once daily) or are gavage fed (once to twice daily) with an artificial rabbit formula. All premature models are gavage fed. Arrows (↓) denote most common days of harvesting animals.