	Inventaris Wob-verzoek W17-07	wordt verstrekt				weigeringsgronden			
nr.	document NTS2016783	reeds openbaar	niet	geheel	deels	10.1.c	10.2.e	10.2.g	11.1
1	Aanvraagformulier				x		x	x	
2	Projectvoorstel			x					
3	Niet-technische samenvatting	x							
4	Bijlage beschrijving dierproeven 1			x					
5	Bijlage beschrijving dierproeven 2			x					
6	Referenties			x					
7	DEC-advies				x		x	x	
8	Ontvangstbevestiging				x		x	x	
9	Verzoek om aanvulling aan VH 23-12-16				x		x	x	
10	Verzoek om aanvulling CCD DEC 23-12-16				x		x	x	
11	Mail reactie VH 2-1-17				x		x	x	
12	bijlage reactie DEC op vragen 23-12			x					
13	bijlage herziene dierproeven 1			x					
14	bijlage herziene dierproeven 2			x					
15	mail reactie DEC 4_1_17				x		x	x	
16	verzoek nadere aanvulling aan VH 10-1-17				x		x	x	
17	Mail reactie VH 17-1-17				x		x	x	
18	bijlage reactie DEC vragen 5-1-17			x					
19	bijlage herziene dierproeven 1 definitief			x					
20	Advies CCD		x						x
21	Beschikking				x		x	x	

Centrale Commissie Dierproeven

20 DEC. 2016

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website www.centralecommissiedierproeven.nl. of in de toelichting op de website.
- Of bel met 0900-2800028 (10 ct/min).

1 Gegevens aanvrager

1.1 Heeft u een deelnemernummer van de NVWA?
Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.

☒ Ja > *Vul uw deelnemernummer in* 10700 / 783

☐ Nee > *U kunt geen aanvraag doen*

1.2 Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.

Naam instelling of organisatie	Universiteit Maastricht
Naam van de portefeuillehouder of diens gemachtigde	[redacted]
KvK-nummer	50169181

1.3 Vul de gegevens van het postadres in.
Alle correspondentie van de CCD gaat naar de portefeuillehouder of diens gemachtigde en de verantwoordelijke onderzoeker.

Straat en huisnummer	Minderbroedersberg 4-6
Postbus	616
Postcode en plaats	6200 MD Maastricht
IBAN	NL04 INGB 0679 5101 68
Tenaamstelling van het rekeningnummer	Universiteit Maastricht

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	[redacted] ☒ Dhr. ☐ Mw.
Functie	[redacted]
Afdeling	[redacted]
Telefoonnummer	[redacted]
E-mailadres	[redacted]

1.5 *(Optioneel)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	[redacted] ☒ Dhr. ☐ Mw.
Functie	[redacted]
Afdeling	[redacted]
Telefoonnummer	[redacted]
E-mailadres	[redacted]

1.6	*(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.	(Titel) Naam en voorletters	☐ Dhr. ☐ Mw.
		Functie	
		Afdeling	
		Telefoonnummer	
		E-mailadres	
1.7	Is er voor deze projectaanvraag een gemachtigde?	☐ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag*	
		☒ Nee	

2 Over uw aanvraag

2.1	Wat voor aanvraag doet u?	☒ Nieuwe aanvraag > Ga verder met vraag 3
		☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn
		Vul uw vergunde projectnummer in en ga verder met vraag 2.2
		☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn
		Vul uw vergunde projectnummer in en ga verder met vraag 2.3
2.2	Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?	☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier
		☐ Nee > Ga verder met vraag 3
2.3	Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?	☐ Nee > Ga verder met vraag 3
		☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	01 - 01 - 2017
		Einddatum	01 - 01 - 2022
3.2	Wat is de titel van het project?	Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis	
3.3	Wat is de titel van de niet-technische samenvatting?	Therapeutische maatregelen om de uitkomst van prematuren kinderen te verbeteren.	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	DEC-UM
		Postadres	Postbus 616, 6200 MD Maastricht
		E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 1187 Lege

☐ Wijziging € Lege

4.2 Op welke wijze wilt u dit bedrag aan de CCD voldoen.
Bij een eenmalige incasso geeft u toestemming aan de CCD om eenmalig het bij 4.1 genoemde bedrag af te schrijven van het bij 1.2 opgegeven rekeningnummer.

☐ Via een eenmalige incasso

☒ Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☒ Referentielijst

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD of per post naar:

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.7). De ondergetekende verklaart:

- dat het projectvoorstel is afgestemd met de Instantie voor Dierenwelzijn.
- dat de personen die verantwoordelijk zijn voor de opzet van het project en de dierproef, de personen die de dieren verzorgen en/of doden en de personen die de dierproeven verrichten voldoen aan de wettelijke eisen gesteld aan deskundigheid en bekwaamheid.
- dat de dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU, behalve in het voorkomende geval de in onderdeel F van de bijlage bij het bij de aanvraag gevoegde projectvoorstel gemotiveerde uitzonderingen.
- dat door het ondertekenen van dit formulier de verplichting wordt aangegaan de leges te betalen voor de behandeling van de aanvraag.
- dat het formulier volledig en naar waarheid is ingevuld.

Naam	[redacted]
Functie	[redacted]
Plaats	Maastricht
Datum	14-12-2016
Handtekening	[redacted]

Centrale Commissie Dierproeven

2

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10700
1.2	Provide the name of the licenced establishment.	Maastricht University
1.3	Provide the title of the project.	Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

- ☒ Basic research
- ☒ Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries
- ☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Preterm birth

Preterm birth, defined as birth before 37 weeks of gestation, is the leading cause of perinatal morbidity

and mortality in developed countries (1). Survival after preterm birth has sharply increased in the last decades (2). This positive trend can be largely contributed to reduction of early pulmonary complications, which has been established by widespread use of antepartum corticosteroids, postpartum surfactant administration and the development of improved ventilation strategies (2, 3). Unfortunately, preterm birth is still associated with mortality and long-term morbidity, despite the mentioned improvements in perinatal care. Given the magnitude of the problem of preterm birth, such a large scale health care challenge also forms a tremendous economic burden on society. The most important causes leading to preterm birth can be roughly divided in two major groups; intrauterine infection (chorioamnionitis) and fetal hypoxia-ischemia. In this project we will study the effects of chorioamnionitis.

Chorioamnionitis

Chorioamnionitis is characterized by microbial invasion of the amniotic cavity. The microorganisms responsible for this invasion, comprising *Ureaplasma urealyticum species,* most commonly originate from the lower reproductive tract (4). These microorganisms cause an inflammatory reaction of the fetal membranes (chorion and amnion), leading to release of inflammatory mediators into the amniotic fluid. As the fetus practices breathing and swallowing movements, the fetus is exposed to these micro-organisms and inflammatory mediators entering both the fetal digestive tract and the lungs, causing a fetal inflammatory response syndrome (FIRS), and subsequent injury to vital organs (e.g. lungs and brain). Moreover, the inflammatory cascade triggered by chorioamnionitis (e.g. release of prostaglandins and extracellular matrix degrading proteins) leads to (medically indicated) preterm delivery (5).

As outlined above, chorioamnionitis and/or preterm birth affect vital organ systems, most importantly the respiratory (lungs), gastrointestinal (gut) and central nervous system (brain):

Lungs

Respiratory distress syndrome

When infants are born preterm, their lungs are still immature, and not capable of production of surfactant. Surfactant lowers alveolar surface tensions. As a result of absence or an inadequate amount of pulmonary surfactant a premature baby often has difficulty expanding her lungs, thereby denying proper gas exchange, often referred to as respiratory distress syndrome (RDS).

Currently, RDS is prevented by surfactant replacement therapy in which tracheal administration of exogenous surfactant (of animal origin) lowers alveolar surface tension and improves pulmonary dynamic compliance. Surfactant replacement therapy has been the most significant advance in perinatal care to decrease neonatal mortality since the late 1980's.

Yet, these biological surfactant preparations are prone to in vivo inactivation as a result of plasma proteins leaking into the airways from areas of epithelial disruption and injury, mandating development and testing of new surfactant preparations that are more resistant to inactivation (6).

CHF 5633 is a fully synthetic surfactant containing two phospholipids and two peptides analogues of human surfactant proteins B and C, designed to be resistant to inactivation (6). Sato et al. demonstrated a superior oxygenation and lung compliance in ventilated preterm lambs treated with CHF 5633 compared to other, animal-derived surfactant preparations (7). Moreover, we previously reported CHF

5633 was more resistant to *in vivo* inactivation compared to animal-derived surfactant preparations and improved oxygenation and lung function of preterm lambs that were surfactant deficient due to their prematurity (6). The only clinically approved and used surfactant is a natural surfactant. The synthetic surfactant CHF5633 is not clinically approved. The experiments of natural surfactant represent therefore the status quo in clinical practice and serve as gold standard or benchmark for the translation of our results into clinical practice. The combination of steroids and surfactant is not recommended by the producer of surfactant.

Since (persisting antenatal) inflammation is clinically a major contributor to surfactant inactivation and RDS, we will assess an alternative approach to minimize inactivation by inflammation. We will test the suppression of the inflammatory response by administering glucocorticoids directly into the lung. We will assess the effectiveness of local administered glucocorticoids in animal-derived surfactants and in CHF5633.

Bronchopulmonary dysplasia

Antenatal exposure to inflammation accelerates lung maturation. However, by inducing accelerated early lung maturation, late lung development is impaired (8, 9). During the last phase of lung development which is called the alveolarization phase, functional alveoli are formed by secondary septation which subsequently increases the surface area needed for optimal gas exchange (10). Impairment of late lung development by intrauterine exposure to inflammation, will lead to a decreased number of alveoli and less surface area for gas exchange which eventually impairs the lung function of the newborn. This altered lung morphology can form the basis for bronchopulmonary dysplasia (BPD) (11). BPD is the most common chronic lung disease in preterm infants (12). Apart from intensive hospital care in early life, BPD infants also have an increased risk for recurrent respiratory complications such as wheezing and respiratory infections, and neurodevelopmental disabilities (13, 14). To date no effective treatment is available for BPD. Dysregulation of the pathways driving the alveolar growth by antenatal exposures could potentially result in disrupted lung morphology as seen in BPD patients.

The pathophysiological sequence leading to BPD is induced by lung immaturity combined with lung injury (15, 16). The latter is induced by inflammatory and airway remodeling processes, which are caused by **mechanical ventilation**, and/or **ante- or postnatal infections**. Especially, certain prenatal hits (namely chorioamnionitis) may prime the response of the immature lung, making it more vulnerable to postnatal hits (17, 18). This is of importance of the subsequent injuries or "hits" that the preterm lung may suffer. Mechanical ventilation is a risk factor per se for lung injury which may be aggravated by preceding injuries in a non-linear way. Two injuries are more than the addition but a potentiation of injury. Several sophisticated mechanical ventilation strategies have been clinically tested to reduce the incidence of BPD, which all failed to show improvement in clinical outcome.

Because inflammation significantly contributes to lung injury in BPD, POSTNATAL glucocorticoids have long been used as standard treatment of BPD, resulting in a reduced inflammatory response along with reduced lung damage in the preterm lungs (15, 16). But due to the risk of short- and long-term side effects, including impairment of neurological development, the routine use of SYSTEMIC POSTNATAL glucocorticoids has been drastically reduced in BPD therapy in the last years, and increased the demand for new therapeutic options for the treatment of BPD.

Still, glucocorticoids are very promising as a treatment option for BPD because of their anti-inflammatory properties and their effect on remodelling processes. At present glucocorticoids are under development which appear to be effective in treating different lung diseases like COPD and asthma. Studies in small animal models demonstrated a protective effect of glucocorticoids on BPD development through anti-inflammatory action and reversal of aberrant remodeling processes combined with prolongation of survival. However, a major challenge in a treatment using glucocorticoids is that the dose level required for therapeutic activity is about the threshold level for an induction of adverse effects. One option to reduce these systemic adverse effects could be the use of INTRATRACHEALLY administered glucocorticoids. Since BPD development is dependent on lung immaturity and lung injury caused by antenatal inflammation and postnatal ventilation, we aim to assess treatment of BPD in a preclinical animal model of BPD in which preterm ovine fetuses are exposed to antenatal inflammation (chorioamnionitis) followed by postnatal mechanical ventilation and/or glucocorticoid treatment which is further studied in locally administered and systemically administered approaches. Therefore, it is necessary to consider a trade-off between the desired effect on the lung of systemic glucocorticoids and the undesired effects on the brain.

Brain development

Chorioamnionitis and BPD have been repetitively associated with poor neurodevelopmental outcome, in particular with impaired learning skills and sensomotoric skills. The causes of the adverse outcomes are unclear, but are most likely the result of systemic inflammation which may be initiated in the lung.

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

The **main purpose** of this research project is to improve pulmonary outcomes by developing novel approaches. The synthetic surfactant is a complete new approach with biochemically designed analogues of natural proteins without the high risk of inactivation of the natural surfactant protein. This approach is to be considered innovative since it not only improves the known beneficial effects but also prevents the known adverse effects. No surfactant has ever been recommended in combination with steroids. We are therefore in an equipoise which surfactant is best suited in the combination with glucocorticoids. We assess both and continue with the one that is best suited.

AIM 1: Identifying the effect of glucocorticoids on surfactant activity in the presence of inflammation as a cause for surfactant inactivation (hereby identifying the best of the two tested surfactant carriers for endotracheal glucocorticoids) and in combination with a synthetic surfactant (CHF5633).

AIM 2: To assess the therapeutic potentials based on pharmacological and biological effects of

intratracheal glucocorticoids in the prevention of adverse effects of prematurity.

3.3 Relevance

What is the scientific and/or social relevance of the objectives described above?

General relevance:

Preterm birth is the leading cause of perinatal morbidity and mortality in developed countries. Although survival after preterm birth has increased in the last decades, still a large proportion of preterm infants suffer from long term morbidity and disability, which have a tremendous impact on patients and their families. Preterm birth: 13649 (7,7% or 77 per 1000 live and dead born children)

Of the 13649 preterm infants, 1148 infants died in the past 5 years due to preterm birth alone or complications of preterm birth (e.g. asphyxia, respiratory insufficiency)

Extreme preterm (born below 28 weeks gestational age) : 2637 (20% of preterms) of whom 1/3 dies; 1/3 survives with handicap and 1/3/ survives without morbidity.

In 25-40% of all preterm births are caused by intra-uterine inflammation (chorioamnionitis).

Lung:

Bronchopulmonary dysplasia occurs in approximately 20% of preterm infants and is an independent risk factor for lifelong pulmonary morbidity, but also impaired neurodevelopmental outcome. Because of the greater number of survivors of prematurity, but also because of the avoidance of systemic steroid therapy for BPD due to the known side effects on the brain, the incidence of BPD as complication of preterm birth rose over the past 20 years despite the therapeutic advances in neonatology.

We will conduct relevant experiments in preclinical/translational animal models in order to improve the outcome of this highly susceptible patient group by addressing different approaches. We are therefore aiming to improve **Surfactant replacement therapy.** Surfactant is a mixture of phospholipids and proteins that decreases surface tension in the alveoli, preventing its collapse and facilitating oxygen exchange. Surfactant replacement therapy is an **absolute necessity in neonatal care**. Without surfactant, survival of preterm neonates drastically decreases.

Despite surfactant replacement therapy, preterm infants develop long term complications such as **bronchopulmonary dysplasia** (BPD), which is an irreversible simplified lung structure resulting in reduced oxygen uptake and continuous oxygen demand. BPD is the result of injury of the immature lung caused by (1) mechanical ventilation and (2) ongoing inflammation.

Glucocortiocids are potent anti-inflammatory medications which may reduce inflammation and prevent BPD. However, glucocoriticoids also have an adverse effect on the brain - if they are given systemically. We want to give them intrapulmonary where we expect little adverse effects on the brain.

We want to study if prevention of BPD can be achieved by improving surfactant therapy. We will study different mechanisms:

1. Increasing resistance of surfactant to inactivation by adding glucocorticoids to surfactant will

result in maintenance of tissue oxygenation

2. the combination of a new surfactant with steroids will allow targeted therapy of the lung. Targeted therapy results in reduction of inflammation and modulates airway remodelling processes, while negative effects of glucocorticoids on the brain are avoided.

The only clinically approved and used surfactant is a natural surfactant. The synthetic surfactant CHF5633 is not clinically approved. The experiments of natural surfactant represent therefore the status quo in clinical practice and serve as gold standard or benchmark for the translation of our results into clinical practice. Natural and synthetic surfactants differ in ther composition and therefore exert different effects on the lung. Therefore, aim 1 is necessary to identify the best suitable surfactant for combination therapy with steroids. The combination of steroids and surfactant is not recommended by the producer of surfactant.

3.4 Research strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

We follow a research strategy in which we use a large mammalian animal model that reflects as good as possible the clinical situation. The synthetic surfactant has been tested for example in vitro. The data did not convince the EMA. The producer did animal experiments in young dogs which the EMA did not accept as appropriate animal model for the patient population. The EMA requested therefore in vivo experiments in a preclinical model which are preterm lambs.

The large animal model allows us to mimic the different components of disease mechanism and to test innovations and treatment in a preclinical model. The sheep model has the highest track record of changing clinical care in perinatal care in clinical medicine of all animal models. Our group has already successfully tested different surfactant preparations and different modes of surfactant administration. Further did we describe interaction between glucocorticoid therapy and chorioamnionitis in our sheep model.

In the current project we have formulated two individual experimental aims to develop and improve new therapeutic strategies for the treatment of perinatal insults in well-established ovine models of intra-uterine inflammation and mechanical ventilation.

Advantages of the sheep model

Ovine fetal development, in terms of lung alveolarization and white matter development, is comparable to human fetal development: both processes start prenatally and continue postnatally, whereas these processes start postnatally in rodents (figure 1).

Moreover, the size of the ovine fetus allows for chronic *in utero* instrumentation (hypoxia-ischemia model) and allows the use of ventilation equipment and ventilation strategies currently used in clinical practice.

Furthermore, the long gestational period (~147 days) allows for more precise timing of perinatal insults based on specific developmental processes for most organs.

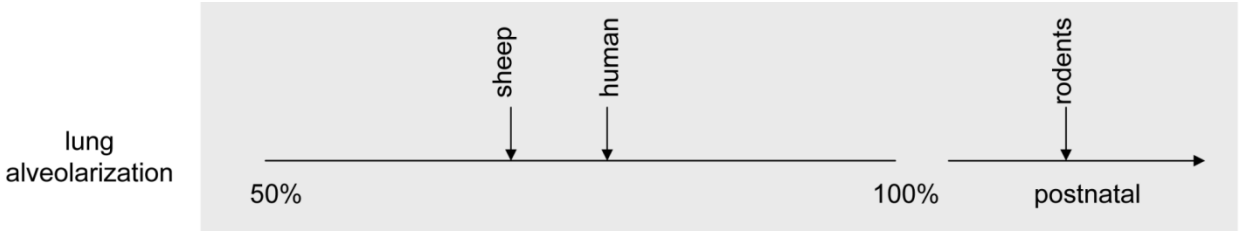


Figure 1 Lung development during gestation in humans, sheep and rodents.

We will focus on two aims. In aim 1, we will identify the effect of glucocorticoids on surfactant activity in the presence of inflammation as a cause for surfactant inactivation (hereby identifying the best of the two tested surfactant carriers for endotracheal glucocorticoids) and in combination with a synthetic surfactant (CHF5633). In aim 2, we assess the therapeutic potentials based on pharmacological and biological effects of intratracheal glucocorticoids in the prevention of adverse effects of prematurity.

The combination of steroids and surfactant is not recommended by the producer of surfactant. The only clinically approved and used surfactant is a natural surfactant. The synthetic surfactant CHF5633 is not clinically approved. The experiments of natural surfactant represent therefore the status quo in clinical practice and serve as gold standard or benchmark for the translation of our results into clinical practice.

1. **Intra-uterine inflammation:** What surfactant preparation plus glucocorticoids is the least susceptible to *in vivo* inactivation induced by antenatal inflammation (chorioamnionitis) and mechanical ventilation?

Persistent (antenatal) inflammation is a major contributor to inactivation of biological surfactant preparations and subsequent RDS in preterm infants and therefore the inactivation of new surfactant preparations cannot be tested in healthy animals. Synthetic surfactant has proven superiority in terms of resistance to inactivation when compared to biological preparations, due to altered peptide structures that cannot be destroyed by proteases. However, in vivo inactivation of synthetic surfactant in the presence and absence of glucocorticoids has not been tested in a clinical relevant model of chorioamnionitis-induced preterm birth.

2. **Ventilation-induced lung injury:** Is inhibition of inflammation and modulation of pulmonary developmental pathways by locally applied glucocorticoids effective in reducing bronchpulmonary dysplasia caused by chorioamnionitis and mechanical ventilation?

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

Intra-uterine inflammation: Time-mated Texel ewes receive an intra-amniotic injection of Ureaplasma Parvum (UP). UP is the most clinically relevant pathogen with respect to chorioamnionitis (35). Seven days after inoculation with UP the fetus is delivered preterm and subjected to mechanical ventilation and is subsequently treated with different surfactant preparations. The primary outcomes of this study are oxygenation (arterial oxygen partial pressure), repetitive dosing depending on oxygenation, and activity

of surfactant recovered from the animals after sacrifice.

The only clinically approved and used surfactant is a natural surfactant. The synthetic surfactant CHF5633 is not clinically approved. The experiments of natural surfactant represent therefore the status quo in clinical practice and serve as gold standrad or benchmark for the translation of our results into clinical practice. A previous version of the synthetic surfactant has been tested successfully in the absence of glucocortiocids.

Ventilation-induced lung injury

In order to model chronic lung disease, we combine intrauterine inflammation and postnatal mechanical ventilation, which are major contributors to BPD in preterms (15, 16). Time-mated Texel ewes receive an intra-amniotic injection of UP. Seven days after inoculation with UP the fetus is delivered preterm and subjected to long-term mechanical ventilation and subsequent treatment with glucocorticosteroids systemically or endotracheally.

Primary outcome parameter is survival, with BPD being a major cause of mortality in preterm infants.

Secondary outcome parameters include lung inflammation, lung compliance, lung development and lung injury. These parameters indicate how development of lung changes associated with BPD are affected by the therapeutic intervention.

The brains will be collected and scanned in a MRI analysis. Subsequent histologic analyses are based on the results of the scan.

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points.

Survival and adult health start *in utero* with a successful transition from *in utero* life to postnatal life. Our project comprises different complications in this transition. The coherence between the different components of our project lies in the fact that we focus on the biggest threats for impaired development in prematurity, namely infection (i.e. chorioamnionitis) and iatrogenic stressors (i.e. mechanical ventilation) which all result in inflammation of the airways of the newborn. The objective of this project is to develop therapeutic strategies to reduce the consequences of these threats and improve outcome in preterm infants.

The second point of coherence between the different components in this project is the effect of anti-inflammatory drugs which bear a high therapeutic potential, but also the potential of adverse effects. It has been tested in vitro. The data did not convince the EMA. The producer did animal experiments in young dogs which the EMA did not accept as appropirate animal model for the patient population. The EMA requested therefore in vivo experiments in a preclinical model.

The project includes:

AIM 1: Identifying a surfactant replacement therapy resistant to inactivation by inflammation/infection would mean a pivotal milestone in neonatal medicine solving a problem which neonatologists face on a daily basis. The end of the experiment for aim 1 will serve as a milestone (proof of principle) and as a go

or no go: if the combination of synthetic surfactant and local glucocorticosteroids does not prove to be superior over natural surfactants, we will not pursue further experimentation, this including experiments under aim 2 with this surfactant. The surfactant alone has already successfully been tested beforehand.

AIM 2: Reducing BPD using a synthetic anti-inflammatory drug would be a significant milestone in neonatal medicine creating the opportunity to reduce the pulmonary and neurodevelopmental sequelae of BPD in preterm infants. The end of the experiment for aim 1 will serve as a milestone (proof of principle) and as a go or no go: proof of principle that local glucocorticoids attenuates inflammation- and ventilation-induced lung injury will be a major milestone and will serve as the basis for further experimentation and future clinical trials. The group with systemic glucocorticoids serve as benchmark to validate the effectiveness of the locally applied glucocorticoids.

Go or no go's and milestones

The end of the experiment for aim 1 will serve as a milestone (proof of principle) and as a go or no go: if the combination of synthetic surfactant and glucocorticosteroids does not prove to be superior over natural surfactant plus glucocorticosteroids, we will not pursue further experimentation with this synthetic surfactant in combination with glucocorticoids as described in aim 2. Therefore, the experiments are intertwined.

3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	Intra-uterine inflammation
2	Ventilation-induced lung injury
3	
4	
5	
6	
7	
8	
9	
10	

Centrale Commissie Dierproeven

4

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10700
1.2	Provide the name of the licenced establishment.	Maastricht University
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 — Type of animal procedure: Intra-uterine inflammation

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

We induce intra-uterine inflammation with intra-amniotic injections of Ureaplasma Parvum (UP), since this micro-organism is the most commonly found micro-organism in human chorioamnionitis cases. Previous studies already validated this approach and the same amount of colony forming units will be administered in the current study. The presence of chorioamnionitis can only be confirmed after birth which will be done by histological examination of the fetal membranes and placenta. The fetuses are delivered preterm by C-section, sedated and mechanically ventilated for 48 hours during which they are treated with different surfactant preparations. Subsequently, animals receive ventilator support via CPAP for 5 days. We have formulated the following 6 experimental groups:

1. Control ventilation
2. UP + Control ventilation
3. UP + natural surfactant (Curosurf, clinical standard, porcine origin)
4. UP + CHF5633 (synthetic surfactant)
5. UP + natural surfactant (Curosurf) + glucocorticoid (Budesonide 0,25 mg/kg)
6. UP + CHF5633+ glucocorticoid (Budesonide 0,25 mg/kg)

Groups 1, 3 and 4 are necessary to assess the effects in the pattern of clinical practice. Group 1 will help to identify the inflammatory phenotype influencing treatment effect, group 3 is current clinical standard and group 4 will help to identify if the artificial surfactant itself has an effect on inflammation due to its different protein composition.

We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism. Based on a power calculation, and taking into account a loss of 25% in total of the animals due to complications of intra-uterine inflammation (10%), premature birth (5%) and humane endpoints in lambs (10%) we calculated a total number of animals per experimental groups will which be 12.

Within these experimental groups the following primary and outcome parameters will be assessed:

Primary outcomes:

- Oxygenation (arterial oxygen partial pressure) in the course of ventilation: oxygenation is the main clinical parameter indicative for adequate ventilation)
- Repetitive dosing: depending on oxygenation: due to inactivation of surfactant, oxygenation might fail and additional boluses of surfactant are needed.
- Activity of surfactant recovered from the animals after sacrifice

The animals get colostrum and breast milk from sheep during the mechanical ventilation according to a feeding protocol from the neonatal intensive care unit with gastric tube feeding.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Time-mated Texel ewes will receive an intra-amniotic injection of Ureaplasma Parvum (UP):

Under sedation UP is administered by ultrasound-guided intra-amniotic injection. 2 days before preterm delivery, the ewes will be given an intramuscular injection of dexamethasone, which enhances fetal lung maturation and allows the fetus to be ventilated postnatally. Moreover, maternal intramuscular dexamethasone administration mimics the clinical standard nowadays.

Maternal administration should not be confused with postnatal steroid treatment in the fetus for BPD: Prenatal treatment of the mother is beneficial for the child because lung maturation is induced and mortality is decreased.

Postnatal treatment of the preterm infant has also been shown to positively influence the infants' lung, and BPD is decreased. However, corticosteroids given systemically to the infant have serious side effects on the neurodevelopmental outcome. Therefore, postnatal therapy can only be administered after balancing the positive effects on the lung and the negative effects on the brain, leading frequently to a therapeutic dilemma.

Administration of corticosteroids for longer periods of time induces abortion in sheep. However, a single

injection of dexamethasone, as administered in our proposed experiments, will not increase the risk of abortion (Fehrholz et al., Am. J. Physiology 2015) provided that the fetus will be born 48 hours after injection.
Previous experiments with intra-uterine Ureaplasma Parvum infection and treatment with dexamethasone demonstrated that dexamethasone 2 days prior to delivery did not have any effects on the immune response towards Ureaplasma Parvum

Seven days after the inoculation with UP, the fetus will be born preterm by **C-section** (general anesthesia): while remaining on placental circulation umbilical vessel catheters are placed for administration of medication (i.e. anesthesia) and blood gas analysis and an endotracheal tube is placed for ventilatory support (modified EXIT procedure). The lamb is immediately placed in our lamb intensive care unit which resembles the clinical intensive care very closely. Before connection to mechanical ventilation the lambs receive **surfactant replacement therapy** (according to their allocation). The lamb remains mechanically ventilated for 24 hours while anesthesia is maintained. Surfactant replacement therapy is repeated throughout the experiment if necessary. After 24 h the animals will be switched to continuous positive pressure ventilation for additional 6 days under sedation.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

We have a preference for singleton pregnancies since this is more clinical relevant and twins can strongly affect each other's health (i.e. resulting in small for gestational age) however, to reduce the number of pregnant ewes we will use both singleton and twin pregnancies. Furthermore, we do not know in advance whether it will be a single or twin pregnancy and only selecting twin pregnancies will result in massive over-breeding. Group numbers were determined with the power-calculation according to Sachs, in which variance and expected therapeutic effects are based on previous experiments with surfactant in non-infectious sheep models.

We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism

In ventilated preterm sheep (non-infectious model, Seehase et al., 2012) CHF5633 has shown a comparable effect on oxygenation but superior resistance against inactivation. Surfactant re-dosing in a 48-hour period of ventilation is the critical outcome in this experiment.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Lambs are the most appropriate animals to address our hypothesis, for a number of reasons:
1. Development of the lungs in sheep has been sufficiently studied to allow informed experimental design and integration of findings with those from previous studies. The current surfactant replacement therapy was developed in preterm lambs.

2. Preterm lambs are large enough to use similar equipment for ventilation as in the neonatal intensive care unit, provide sufficient blood and tissue to allow enough biomolecular analyses, for thorough assessment of the biological systems being investigated, in individuals.

The total number of animals needed for the current study will by 6 groups * 12 animals = 72 animals. We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism. Based on a power calculation, and taking into account a loss of 25% in total of the animals due to complications of intra-uterine inflammation (10%), premature birth (5%) and humane endpoints in lambs (10%) we calculated a total number of animals per experimental groups will which be 12.

We cannot predict the number of twin pregnancies, since multiple factors (e.g. seasonal vs. out of season breeding and the age of the ewe) are of influence. Therefore, the number of ewes is based on singleton pregnancies. In the case of twin pregnancy, the total number of ewes used for the experiment will be reduced.

The gestational age at which the lambs are delivered (129-132d gestational age) is based on the developmental biology in which the sheep is very similar to a preterm infant (figure 1). In contrast to rodents, ovine lung development (alveolarization) starts prenatally and is prolonged postnatally. Moreover, the size allows for usage of similar equipment and ventilation strategies as is currently used in clinical practice. Animals will be time-mated to yield animals of the appropriate age. Two antenatal ultrasound scans will ensure correct gestational age and adequate in utero growth.

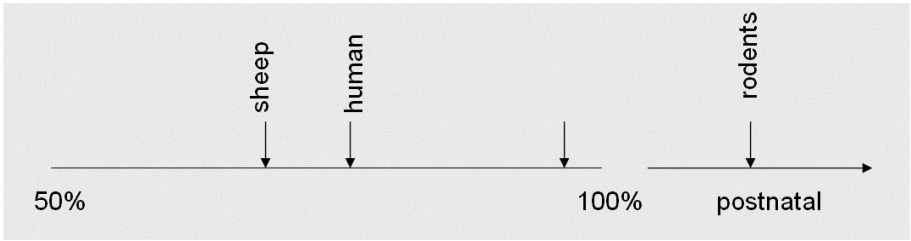


Figure 1 Time-points are shown when lung alveolarization, an important hallmark of lung maturation, occurs in different species. Lung maturation in sheep occurs prenatally, as in humans. Lung maturation in rodents takes place after birth.

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☐ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement: The use of preterm lambs has been suggested by the European Medical Agency (EMA) to pharmaceutical companies. We have done studies with synthetic surfactant (Seehase et al., 2012) with were mandated by the EMA in order to show in vivo the safety and feasibility of the new drug. It is unacceptable to test directly in human babies based on exclusively in vitro data by the standards of EMA and by good clinical practice since preterm babies is the most vulnerable patient population. In addition neonatology is full of examples of premature clinical trials with caused harm in the study population (e.g. phospholipid treatment for RDS).

Reduction: Due to large experience with sheep and maintenance of lambs on the lamb intensive care unit we are able to increase the number of animals that successfully reach the end of their experiments. Due to the expertise of our team, we have low numbers of drop-outs which decreased over the years with increasing experience, which reduces the numbers of animals in the experimental groups. Our experiments are conducted by highly experienced personnel according to well-established protocols. These methods are in favor of reproducibility and use of previously obtained historical data (also on CHF 5633 inactivation (2)) and prevent unnecessary use and loss of animals. Moreover, we do not differentiate between genders since our clinical population consists of both sexes.
Furthermore, we are able to incorporate twin pregnancies in our study. This results in a reduction of pregnant ewes that will be used. We rely on natural breeding. Therefore, we cannot control for singleton or twin pregnancies.

Refinement:
For this experiment in which different surfactant preparations are tested, the choice of animal model is based on the body size, use of similar equipment as in the neonatal intensive care and the developmental biology. In contrast to rodents, ovine and human the developmental biology are very similar. For example, the alveolar phase in lambs and humans starts before birth in a hypoxic environment, compared to rodents in which the alveolarization starts postnatally in normoxic conditions. Therefore, we have established an animal model of immature lung-development in preterm lambs. Findings in our animal model can be easily translated into clinical practice. Our staff and the staff of the animal care facility have been trained by a Texel sheep breeder to recognize symptoms of pending labor and give assistance to the sheep upon labor. Recognition of pending labor and assistance during labor reduces the risk of complications and improves animal well-being. Moreover, we aim to leave the sheep as much in their natural environment (group housing in a meadow) as we can with minimal human contact. Moreover, the fetus will receive analgesia and nutritional support (glucose) during mechanical ventilation.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects

on the environment.

Ewe

Before induction of intra-uterine inflammation the sheep are kept in their natural environment as long as possible with access to food and water *ad libitum*. After induction of intra-uterine inflammation the sheep are housed in groups with access to food and water *ad libitum*.

The sheep will be checked daily for discomfort due to intra-uterine inflammation. Discomfort due to intra-uterine inflammation is described in the humane endpoint section. Post-operative (after intra-amniotic injection) antibiotics, if necessary, will be administered, in order to prevent progression of (wound) infection.

The experiments will be performed by experienced animal technicians and researchers. This will speed up the progress of the experiment and therefore reduce animal suffering and fear (fast induction of anesthesia, fast surgery, and maximum results with minimal animal handling).

The majority of the animals have been exposed to Ureaplasma. Therefore, re-use is not possible. All ewes will be euthanized after the c-section.

Fetus

Previous experiments have demonstrated that fetal cortisol levels do not change in the course of chorioamnionitis (Jobe et al, Am J Respir Crit Care Med. 2000 Nov;162(5):1656-61). This suggests that the fetus will experience limited discomfort during intra-uterine inflammation in our experiments.

Chorioamnionitis is a multi-organ disease resulting in discomfort after birth, as they need to survive independently and start using their immature and inflammation affected organs. However, after C-section (birth) the fetus will stay on the Lamb Intensive Care Unit (LICU) and taken care of by highly trained personnel. During mechanical ventilation the fetus is constantly sedated (also receiving analgesia and parenteral feeding) with continuous monitoring and interpretation of vital parameters. Moreover, the environment in the LICU (temperature, light, sound) will be kept optimal in order to reduce discomfort.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

This experiment is new and is not a repetition. The previous in vitro work is used to reduced the number of animals by avoiding a dose finding study.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Ewe: All surgical procedures are performed under anesthesia (including analgetic block).

Lamb: whilst on placental circulation, the lamb is sedated through anesthesia of the ewe. Ex utero experiments are performed under sedation guided by depth of sedation.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Ewe:

Abortion might occur due to chorioamnionitis, but is very uncommon in sheep (8) (in retrospect over the past 10 years: 5 in 100). This is a major reason to use this species.

Fetus:

The fetus might experience pain, suffer from hemodynamic instability or respiratory complications (pneumothorax, hypoxia) or hypoglycemia.

Explain why these effects may emerge.

Due to their immaturity preterm fetuses are not always able to respond properly to environmental changes. The fetus might experience pain, suffer from hemodynamic instability or respiratory complications (pneumothorax, hypoxia).

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Ewe:

All invasive procedures are performed under aseptic conditions.

The ewe will will not recover from anesthesia.

Fetus:

The fetus will be sedated continuously after birth to minimise discomfort, also receiving analgesia. Environmental conditions (light, temperature, sounds) on the LICU are all in favour of the fetus.

Continuous monitoring of hemodynamic and respiratory enables us to treat or prevent the complications.

Intravenous administration of glucose will prevent hypoglycemia (regularly checked)

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Humane endpoints for ewes:

- Pending preterm labor
- Intra-uterine fetal death
- Sepsis/ infection not responding to treament
    - **Local (at the site of intra-amniotic injection):** redness, pain, and swelling with or without pus that cannot be treated with local antiseptics or antibiotics are considered a human endpoint.
    - **Systemic:** Fever, lethargy, excessive time lying down
- Untreatable pain: (a sheep that does not respond to analgesic treatment)

Humane endpoints for fetus:

- Untreatable pneumothorax
- Uncorrectable severe respiratory acidosis
- Sepsis Uncorrectable hypovolemia
- Multi-organ failure

Indicate the likely incidence.

Ewe: 5%

Fetus: 10%

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Ewe: Moderate

Lamb:Non-recovery. During the mechanical ventilation, the lambs are continuously anesthetized (sedation and analgesia), and will not regain conscience, until the end of the experiment (euthanasia). Therefore, we consider the classification of the experiment to be non-recovery.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

The ewe will be euthanized since we cannot reuse her for other purposes due to an intra-uterine infection an abdominal surgery.

The lamb will be euthanized since vital organs (i.e. lungs) have to be sampled for biochemical analysis.
Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?
☐ No > Describe the method of killing that will be used and provide justifications for this choice.
☒ Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

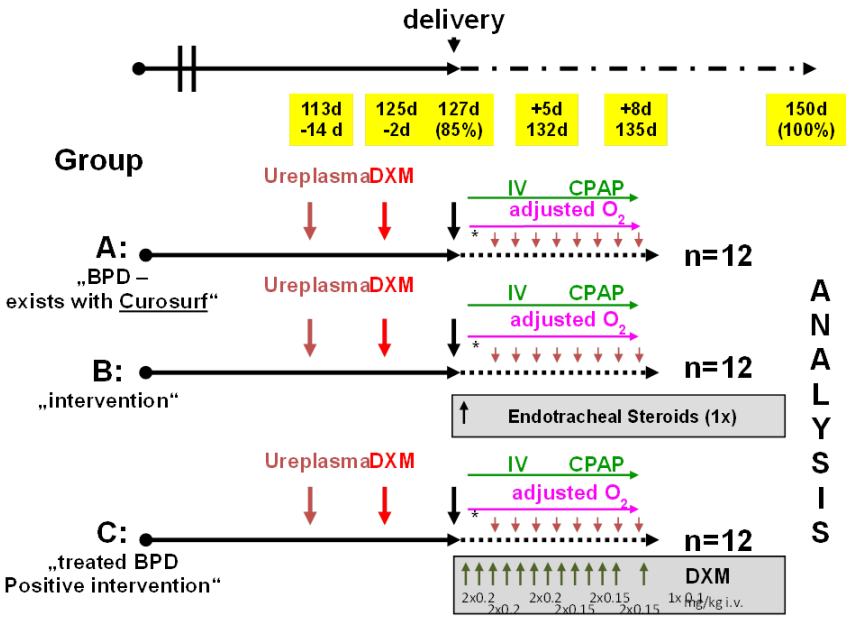
1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10700
1.2	Provide the name of the licenced establishment.	Maastricht University
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: Ventilation-induced lung injury

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

We induce intra-uterine inflammation with intra-amniotic injections of Ureaplasma Parvum, since this micro-organism is the most commonly found micro-organism in human chorioamnionitis cases. Previous studies already validated this approach and the same amount of colony forming units will be administered in the current study. The presence of chorioamnionitis can only be confirmed after birth which will be done by histological examination of the fetal membranes and placenta. The fetuses are delivered preterm by C-section (127d), sedated and mechanically ventilated for a maximum of 8 days in order to develop (histological) BPD [1]. During mechanical ventilation the lambs are treated with a glucocorticoid either by local instillation with surfactant or systemically. We compare endotracheal treatment (B) with intravenous treatment (C), a third group serves as control (A, see figure 1). A fourth group (D) will not be ventilated, but delivered on the same gestational age that autopsy is performed in the other three groups (127d+8d=135d). This group is used to identify the effects of chorioamnionitis with/without dexamethasone on lung inflammation, lung compliance, lung development and lung injury in comparison to mechanically ventilated animals (Figure 1). The following primary and secondary outcome parameters will assess feasibility, safety and efficacy of a topical administered glucocorticoids:



D: phenotype: lambs delivered at 135 d n=12

- D1 after DXM and ureaplasma; N=8
- D2 without any intervention; N=4

Figure 1 Experimental design: (Brown arrow: intra amniotic injection of UP, red arrow i.m. injection of dexamethason to mother; black arrow: delivery; small downward arrows: surfactant replacement therapy, * indicates that surfactant redosing does not follow a fixed scheme but is done dependent on oxygenation index; small upward arrows: corticosteroids to lamb.)

Primary outcome parameters: Survival. Both intrauterine inflammation and preterm birth are major risk factors for neonatal death due to complications that arise from underdeveloped organ systems. BPD is recognized as major factor in mortality in preterm infants. Treatment might improve survival compared to controls.

Secondary outcome parameters: Lung inflammation, lung compliance, lung development and lung injury, brain injury, brain development.

Lambs are the most appropriate animals to address our hypothesis, for a number of reasons:

1. Development of the lungs in sheep has been sufficiently studied to allow informed experimental design and integration of findings with those from previous studies. The current surfactant replacement therapy was developed in preterm lambs.
2. Preterm lambs are large enough to use similar equipment for ventilation as in the neonatal intensive care unit, provide sufficient blood and tissue to allow enough biomolecular analyses, for thorough assessment of the biological systems being investigated, in individuals.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

UP is administered by ultrasound-guided intra-amniotic injection. 2 days before preterm delivery, the ewes will be given an intramuscular injection of dexamethasone, which enhances fetal lung maturation and allows the fetus to be ventilated postnatally. Moreover, maternal intramuscular dexamethasone administration mimics the clinical standard nowadays.

Fourteen days after the inoculation with UP, the fetus will be born preterm by **C-section** (general anesthesia): while remaining on placental circulation umbilical vessel catheters are placed for administration of medication (i.e. anesthesia) and blood gas analysis and an endotracheal tube is placed for ventilatory support (modified EXIT procedure). The lamb is immediately placed in our lamb intensive care unit which resembles the clinical intensive care very closely. Before connection to mechanical ventilation the lambs receive surfactant replacement therapy which is repeated if necessary (based on oxygenation). The lambs remain under ventilatory support for 3 days while anesthesia is maintained and subsequently on respiratory support with CPAP (continuous positive airway pressure). Animals are maximum 8 days in the experiment. During these days, repetitive doses of glucocorticoids or sham treatment will be administered endotracheally or i.v. (Figure 1).

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism. Based on a power calculation, and taking into account a loss of 25% in total of the animals due to complications of intra-uterine inflammation (10%), premature birth (5%) and humane endpoints in lambs (10%) we calculated a total number of animals per experimental groups will which be **12**. The total number of animals needed for the current study will by 4 groups * 12 animals = 48 animals.

We cannot predict the number of twin pregnancies, since multiple factors (e.g. seasonal vs. out of season breeding and the age of the ewe) are of influence. Therefore, the number of ewes is based on singleton pregnancies. In the case of twin pregnancy, the total number of ewes used for the experiment will be reduced.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

The total number of animals needed for the current study will by 4 groups * 12 animals = 48 animals.

We will deliver 36 animals at **127d** (group A-C). Animals will receive receive ventilation and ventilator support for 8 days, therefore autopsy will also be performed on **day 135**. (figure 1). In group D, N=8 lambs are delivered at **135 d** after DXM and ureaplasma and N=4 without any intervention to assess the lung changes (lung inflammation, lung compliance, lung development and lung injury) due to chorioamnionitis and due to the immaturity at birth.

The other are intubated upon delivery.

Lambs are the most appropriate animals to address our hypothesis, for a number of reasons:

1. Development of the lungs in sheep has been sufficiently studied to allow informed experimental design and integration of findings with those from previous studies. The current surfactant replacement therapy was developed in preterm lambs.
2. Preterm lambs are large enough to use similar equipment for ventilation as in the neonatal intensive care unit, provide sufficient blood and tissue to allow enough biomolecular analyses, for thorough assessment of the biological systems being investigated, in individuals.

This experiment will be performed with a maximum of 48 pregnant sheep (Texel breed) and their respective singleton or twin fetuses (> 2/3 gestation, max. 48 fetuses) that are randomly allocated into 4 experimental groups consisting of 3 different glucocorticoid regimes, and one control group (cf. Appendix 1). If there are twin pregnancies less pregnant ewes will be needed. The gestational age at which the lambs in the ventilation groups are delivered (127d gestational age) is based on the developmental biology in which the sheep is very similar to a preterm infant (figure 2). In contrast to rodents, ovine lung development (alveolarization) starts prenatally and is prolonged postnatally. Moreover, the size allows for usage of similar equipment and ventilation strategies as is currently used in clinical practice. Animals will be time-mated to yield animals of the appropriate age. Two antenatal ultrasound scans will ensure correct gestational age and adequate in utero growth.

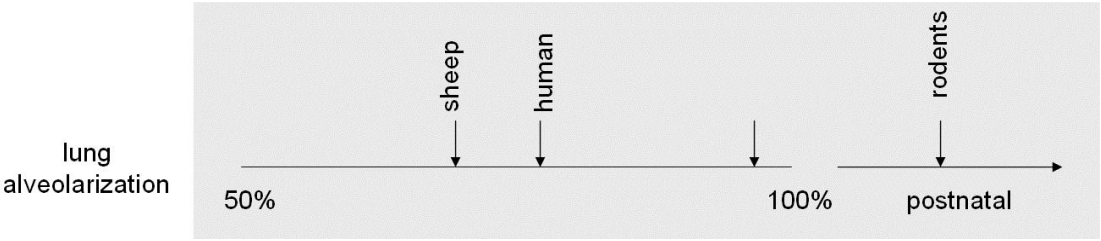


Figure 2 Time-points are shown when lung alveolarization, an important hallmark of lung maturation, occurs in different species. Lung maturation in sheep occurs prenatally, as in humans. Lung maturation in rodents takes place after birth.

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☒ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement: The use of preterm lambs has been suggested by the European Medical Agency (EMA)

to pharmaceutical companies. We have done studies with synthetic surfactant (Seehase et al., 2012) with were mandated by the EMA in order to show in vivo the safety and feasibility of the new drug. It is unacceptable to test directly in human babies based on exclusively in vitro data by the standards of EMA and by good clinical practice since preterm babies is the most vulnerable patient population. In addition neonatology is full of examples of premature clinical trials with caused harm in the study population (e.g. phospholipid treatment for RDS).

Reduction: The groups A and B of this appendix are identical to the corresponding groups of Appendix 1. Once the experiments of Appendix 1 have identified which surfactant is superior we intend to use the animals from Appendix 1 for the purposes of Appendix 2 in order to reduce the number of animals.
Further, due to large experience with sheep and maintenance of lambs on the lamb intensive care unit we are able to increase the number of animals that successfully reach the end of their experiments. Due to the expertise and experience of our team we have low numbers of drop-outs, which reduces the numbers of animals in the experimental groups. Our experiments are conducted by highly experienced personnel according to well-established protocols. These methods are in favor of reproducibility and use of previously obtained historical data and prevent unnecessary use and loss of animals. Moreover, we do not differentiate between genders since our clinical population consists of both sexes.
Furthermore, we are able to incorporate twin pregnancies in our study. This results in a reduction of pregnant ewes that will be used.
A variety of glucocorticoids have been tested in vitro and in vivo. In our current model we will use dosages defined on this pre-existing data.

Refinement: For this innovative technique of endotracheal administration of glucocorticoids to the preterm lung, the choice of animal model is based on the body size, use of similar equipment as in the neonatal intensive care and the developmental biology. In contrast to rodents, ovine and human the developmental biology are very similar (figure 2). For example, the alveolar phase in lambs and humans starts before birth in a hypoxic environment, compared to rodents in which the alveolarization starts postnatally in normoxic conditions. Therefore, we have established an animal model of immature lung-development in preterm lambs. Findings in our animal model can be easily translated into clinical practice. Our experiments will be conducted by a highly trained staff that can recognize and prevent discomfort. Moreover, we aim to leave the sheep as much in their natural environment (group housing in a meadow) as we can with minimal human contact.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Ewe

Before induction of intra-uterine inflammation and the C-section the sheep are kept in their natural environment as long as possible with access to food and water *ad libitum*. After induction of intra-uterine inflammation the sheep will be housed in groups with access to food and water ad libitum.
The sheep will be checked daily for discomfort due to intra-uterine inflammation. Discomfort due to intra-uterine inflammation is described in the human endpoint section. If necessary, analgesics are administered to treat pain.
The experiments will be performed by experienced animal technicians and researchers. This will speed up

the progress of the experiment and therefore reduce animal suffering and fear (fast induction of anesthesia, fast surgery, and maximum results with minimal animal handling).The UP exposure does not result in pain, distress or sepsis.

Fetus

During intrauterine inflammation fetuses will not experience distress as demonstrated by no changes of fetal cortisol levels during chorioamnionitis [7].

Chorioamnionitis is a multi-organ disease resulting in discomfort after birth, as they need to survive independently and start using their immature and inflammation affected organs. However, after C-section (birth) the fetus will stay on the Lamb Intensive Care Unit (LICU) and taken care of by highly trained personnel. During mechanical ventilation the fetus is constantly sedated (also receiving analgesia and parenteral feeding) with continuous monitoring and interpretation of vital parameters. Moreover, the environment in the LICU (temperature, light, sound) will be kept optimal in order to reduce discomfort.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

This experiment is new and is not a repetition. The previous in vitro work is used to reduced the number of animals by avoiding a dose finding study.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Ewe: anesthesia during operation, local analgesia of surgical wounds.

Lamb: whilst on placental circulation, the lamb is sedated through anesthesia of the ewe. Ex utero experiments are performed under sedation.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Ewe:

Abortion might occur due to chorioamnionitis, but is very uncommon in sheep [8]. This is a major reason to use this species.

Fetus:

The fetus will be sedated continuously after birth to minimise discomfort, also receiving analgesia.

Environmental conditions (light, temperature, sounds) on the LICU are all in favour of the fetus.

Explain why these effects may emerge.

Due to their immaturity preterm fetuses are not always able to respond properly to environmental changes.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Ewe:

All invasive procedures are performed under aseptic conditions.

The ewe will not recover from anesthesia.

The majority of the animals have been exposed to Ureaplasma. Therefore, re-use is not possible. All ewes will be euthanized after the c-section.

Fetus:

Environmental conditions (light, temperature, sounds) on the LICU are all in favour of the fetus. Pain will be prevented by continuous anesthesia. Continuous monitoring of hemodynamic and respiratory enables us to treat or prevent the complications. Intravenous administration of glucose will prevent hypoglycemia (regularly checked)

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Humane endpoints for ewes:

- Pending preterm labor
- Abortion caused by intra-uterine fetal death
- Sepsis/ infection not responding to treatment
    - **Local (at site of injection):** redness, pain, and swelling with or without pus that cannot be

treated with local antiseptics or antibiotics are considered a human endpoint.

- **Systemic:** Fever, lethargy, excessive time lying down

- Untreatable pain: (a sheep that does not respond to analgesic treatment)
  - **Assessment of pain:**
    - Lack of appetite
    - Grinding of teeth
    - Reluctance to stand/ excessive time lying down
    - Lethargy/depression: an unresponsive sheep with hung head and dull eyes can indicate pain, illness or discomfort

Humane endpoints for lambs:

- Untreatable pneumothorax (absence of breath sounds)
- Uncorrectable severe respiratory acidosis(based on blood gas analysis)
- Sepsis (elevation of body temperature, elevation heart rate, blood gas analysis)
- Uncorrectable hypovolemia (blood pressure, heart rate, blood gas analysis)

Multi-organ failure (blood-pressure, heart rate, blood gas analysis)

Indicate the likely incidence.

Ewe: 5%

Fetus: 10%

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Ewe: moderate

Lamb:Non-recovery. During the mechanical ventilation, the lambs are continuously sedated and will not regain conscience, until the end of the experiment (euthanasia).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

The fetus will be euthanized at the end of the experiment since examination of organ tissues is crucial to determine the effects of our treatment(s).

The ewes will be euthanized since we cannot reuse her for other purposes due to an intra-uterine infection an abdominal surgery.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes

Proposal

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Appendix 1

[1] Seehase M, Collins JJ, Kuypers E, Jellema RK, Ophelders DR, Ospina OL, et al. New surfactant with SP-B and C analogs gives survival benefit after inactivation in preterm lambs. PLoS One. 2012;7:e47631.

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Appendix 2

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7

DEC-advies PV 2016-012/[redacted]

Preambule;
De DEC-UM verzoekt U eventuele aanvullende vragen rechtstreeks aan de aanvrager te stellen met een afschrift aan de DEC-UM.

A. Algemene gegevens over de procedure

1. **Aanvraagnummer:** 10700

2. **Titel van het project:** *Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis*.

3. **Titel van de NTS:** *Therapeutische maatregelen om de uitkomst van premature kinderen te verbeteren.*

4. **Type aanvraag:**
   X nieuwe aanvraag projectvergunning

5. **Contactgegevens DEC:**
   - naam DEC; *DEC-UM*
   - telefoonnummer contactpersoon; [redacted]
   - e-mailadres contactpersoon; [redacted]

6. **Adviestraject:** (data dd-mm-jjjj):
   X ontvangen door DEC-UM; 03-11-2016
   ☐ aanvraag compleet
   X in vergadering besproken; 11-11-2016
   ☐ anderszins behandeld
   ☐ termijnonderbreking(en) van / tot
   ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   ☐ aanpassing aanvraag
   ☐ advies aan CCD

7. **Afstemming IvD:**
   - De aanvrager heeft het projectvoorstel afgestemd met de IvD *dd. 03-11-2016*.

8. **Eventueel horen van aanvrager**: **N.V.T.**

9. **Correspondentie met de aanvrager:**
   - Datum 16-11-2016
   -

Gestelde vragen en antwoorden:
3 Algemene projectbeschrijving
3.1 Achtergrond
Vragen:
1. **De DEC-UM waardeert de prima geschetste achtergrond van dit PV. De DEC-UM vraagt wel nog om een paragraaf om de bijwerkingen op neurologische ontwikkelingen aan te geven.**

Antwoord:
Chorioamnionitis and BPD have been repetitively associated with poor neurodevelopmental outcome, in particular with impaired learning skills and sensomotoric skills. The causes of the adverse outcomes are unclear, but are most likely the result of systemic inflammation which may be initiated in the lung.

2. **Het is niet duidelijk waarom nog moeite gedaan dient te worden om dier-gerelateerde surfactant in combinatie met glucocorticoids te onderzoeken als het synthetische surfactant CHF5633 superieure effecten vertoont?**
Antwoord:
The only clinically approved and used surfactant is a natural surfactant. The synthetic surfactant CHF5633 is not clinically approved. The experiments of natural surfactant represent therefore the status quo in clinical practice and serve as gold standard or benchmark for the translation of our results into clinical practice. The combination of steroids and surfactant is not recommended by the producer of surfactant**.**

3.2 Doel
Vragen:
1. **De DEC-UM vraagt zich af of de twee te testen doelstellingen geclassificeerd moeten worden als "novel" of eerder als "verbetering" van bestaande therapieën. In de achtergrond wordt ook geen kader geschetst voor nieuwe behandeling strategieën.**
Antwoord:
The main purpose of this research project is to improve pulmonary outcomes by developing novel approaches. The synthetic surfactant is a complete new approach with biochemically designed analogues of natural proteins without the high risk of inactivation of the natural surfactant protein. This approach is to be considered innovative since it not only improves the known beneficial effects but also prevents the known adverse effects.

2. **De DEC-UM vraagt zich af hoe in aim 1 de bijwerkingen voor neurologische ontwikkeling worden voorkomen aangezien dit een van de problemen is bij deze behandeling.**
Antwoord:
The kinetics of glucocorticoid adverse events are increased in the presence of systemic inflammation which is induced by mechanical ventilation. The various surfactant preparations have different effects in the presence of inflammation which allows the comparison even in the "back ground noise" of adverse effects on neurodevelopment**.**

3. **Aim 1 met dier-gerelateerde surfactant lijkt de DEC-UM weinig zinvol als er een superieur synthetisch surfactant bestaat.**
Antwoord:
The only clinically approved and used surfactant is a natural surfactant. The synthetic surfactant CHF5633 is not clinically approved. The experiments of natural surfactant represent therefore the status quo in clinical practice and serve as gold standard or benchmark for the translation of our results into clinical practice. The combination of steroids and surfactant is not recommended by the producer of surfactant.

4. **Wordt het therapeutisch potentieel enkel uitgezocht in combinatie met CHF5633? Zie ook relevantie kopje (… combination of the new surfactant with steroids…).**

Antwoord:
No, we clarified this part. We are in an equipoise which surfactant is best suited in the combination with glucocorticoids. We assess both and continue with the one that is best suited.

3.3. Belang

Vragen:

1. **De DEC-UM vraagt zich ook hier af waarom glucocorticoids worden gebruikt om inactivatie van surfactant te voorkomen maar niet wordt aangegeven wat wordt gedaan om de bijwerkingen op bijvoorbeeld neurologische ontwikkeling te voorkomen.**
   Dit wordt pas in aim 2 aangehaald.
   Antwoord:
   Glucocorticoids are potent anti-inflammatory medications which may reduce inflammation and prevent BPD. However, glucocorticoids also have an adverse effect on the brain - if they are given systemically. We want to give them intrapulmonary where we expect little adverse effects on the brain.

2. **De DEC-UM vraagt de onderzoekers het verschil tussen aims 1 en 2 beter aan te geven, omdat het nu lijkt dat aim 1 overbodig is als er in aim 2 gezocht wordt naar nieuwe surfactant behandeling in combinatie met glucocorticoids en hierin ook wordt getracht de negatieve bijwerkingen te reduceren.**
   Antwoord:
   Correct, however the only clinically approved and used surfactant is a natural surfactant. The synthetic surfactant CHF5633 is not clinically approved. The experiments of natural surfactant represent therefore the status quo in clinical practice and serve as gold standard or benchmark for the translation of our results into clinical practice. Natural and synthetic surfactants differ in their composition and therefore exert different effects on the lung. Therefore, aim 1 is necessary to identify the best suitable surfactant for combination therapy with steroids. The combination of steroids and surfactant is not recommended by the producer of surfactant.

3. **Voor welk gebied gelden de gepresenteerde cijfers, o.a. 13649 vroeg-geborenen, voor Nederland?**
   Antwoord:
   Yes.

3.4 Onderzoeksstrategie

3.4.1

Vragen:

1. **De DEC-UM vraagt zich af of de experimentele opzet onder aim 1 niet uitgevoerd kan worden *in vitro* en of –en waarom – het noodzakelijk is dit in een pre-klinisch model te testen.**
   Antwoord:
   The synthetic surfactant has been tested in vitro. The data did not convince the EMA. The producer did animal experiments in young dogs which the EMA did not accept as appropriate animal model for the patient population. The EMA requested therefore in vivo experiments in a preclinical model which are preterm lambs.

2. **Het is duidelijk om de noodzaak van *in vivo* inactivation van synthetische surfactant te testen in aan/afwezigheid van glycocorticoids. Waarom dient dier-gerelateerde surfactant nog meegenomen te worden?**
   Antwoord:
   The only clinically approved and used surfactant is a natural surfactant. The synthetic surfactant CHF5633 is not clinically approved. The experiments of natural surfactant represent therefore the status quo in clinical practice and serve as gold standard or benchmark for the translation of our results into clinical practice. The combination of steroids and surfactant is not recommended by the producer of surfactant.

3. **De DEC-UM vraagt zich onder aim 1 af welke surfactant al succesvol is getest? Is dit de synthetische variant vergeleken met de natuurlijke? En wordt dit in de kliniek al toegepast?**
   Antwoord:
   The only clinically approved and used surfactant is a natural surfactant. The synthetic surfactant CHF5633 is not clinically approved. The experiments of natural surfactant represent therefore the status quo in clinical practice and serve as gold standard or benchmark for the translation of our results into clinical practice. A previous version of the synthetic surfactant has been tested successfully in the absence of glucocortiocids.

3.4.2
Vragen:
1. **De DEC-UM vraagt zich af of er geen hersen/neurologisch onderzoek wordt verricht?**
   Antwoord:
   The brains will be collected and scanned in a MRI analysis. Subsequent histologic analyses are based on the results of the scan.

2. **Waarin verschillen long-term ventilation (ventilation-induced lung injury) met ventilation (intra-uterine inflammation) qua tijdsduur aangezien ventilation in beide gebeurt?**
   Antwoord:
   This is our mistake: in appendix 1 we have 1 day of mechanical ventilation and 6 days of CPAP. In appendix 2 mechanical ventilation is increased to 3 days in order to reflect the clinical situation. The overall time of ventilation/animal experimentation remains the same in appendices 1 and 2.

3.4.3
Vragen:
1. **De DEC-UM vraagt zich onder aim 1 af welke surfactant al succesvol is getest? Is dit de synthetische variant vergeleken met de natuurlijke?**
   Antwoord:
   A previsous version of the synthetic surfactant has been tested successfully in the absence of glucocorticoids.

2. **De DEC-UM vraagt zich af of de combinatie van synthetisch surfactant en glucocorticoid behandeling niet *in vitro* getest kan worden. Verder vraagt de DEC-UM zich af of niet alles onder aim 2 uitgevoerd kan worden?**

Antwoord:
It has been tested in vitro. The data did not convince the EMA. The producer did animal experiments in young dogs which the EMA did not accept as appropriate animal model for the patient population. The EMA requested therefore in vivo experiments in a preclinical model.

3.4.4
Appendix 1
Vragen:
A. Experimentele aanpak en primaire uitkomstparameters.

1. **Het doel van deze aim is het testen of synthetisch surfactant in combinatie met glucocorticoid, stabiliteit vertoond t.o.v. natuurlijk surfactant. Is het daarom noodzakelijk om groepen 1, 3, en 4 uit te voeren? Dit is geen onderdeel van deze vraagstelling.**
   Antwoord:
   Groups 1, 3 and 4 are necessary to assess the effects in the pattern of clinical practice. Group 1 will help to identify the inflammatory phenotype influencing treatment effect, group 3 is current clinical standard and group 4 will help to identify if the artificial surfactant itself has an effect on inflammation due to its different protein composition.

2. **Zijn groep 3 en 5 noodzakelijk indien men al weet dat er een superieur synthetisch surfactant bestaat?**
   Antwoord:
   Yes. The combination of steroids and surfactant is not recommended by the producer of surfactant.

3. **Worden de lammetjes gevoed tijdens 6 dagen anesthesie? Zo niet, hoe beïnvloedt dit de outcome? Hoe wordt outcome beïnvloed door 6 dagen anesthesie?**
   Antwoord:
   The animals get colostrum and breast milk from sheep during the mechanical ventilation according to a feeding protocol from the neonatal intensive care unit with gastric tube feeding.

4. **Wat gebeurt er met de moeders na de keizersnede?**
   Antwoord:
   The majority of the animals have been exposed to Ureaplasma. Therefore, re-use is not possible. All ewes will be euthanized after the c-section.

5. **Voor het berekenen van het aantal proefdieren nodig per groep, kan dit exact gebeuren (op basis van vorige experimenten) met een uitvalsberekening? Het aantal dieren in B gaat reeds uit van 12 per groep.**
   Antwoord:
   We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism. Based on a power calculation, and taking into account a loss of 25% in total of the animals due to complications of intra-uterine inflammation (10%), premature birth (5%) and humane endpoints in lambs (10%) we calculated a total number of animals per experimental groups will which be 12.

6. **De DEC-UM vraagt zich onder aim 1 af welke surfactant al succesvol is getest? Is dit de synthetische variant vergeleken met de natuurlijke? En wordt dit in de kliniek al toegepast?**
   Antwoord:
   The only clinically approved and used surfactant is a natural surfactant. The synthetic surfactant CHF5633 is not clinically approved. The experiments of natural surfactant represent therefore the status quo in clinical practice and serve as gold standard or benchmark for the translation of our results into clinical practice. A previous version of the synthetic surfactant has been tested successfully in the absence of glucocorticoids.

B. De dieren.

7. **Is er iets bekend over uitval? Gaarne toelichten.**
   Antwoord:
   We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism. Based on a power calculation, and taking into account a loss of 25% in total of the animals due to complications of intra-uterine inflammation (10%), premature birth (5%) and humane endpoints in lambs (10%) we calculated a total number of animals per experimental groups will which be 12.

D.
Vervanging, vermindering en verfijning.

8. **De DEC-UM vraagt zich af of hier geen *in vitro* experiment op zijn plaats is.**
   Antwoord:
   It has been tested in vitro. The data did not convince the EMA. The producer did animal experiments in young dogs which the EMA did not accept as appropriate animal model for the patient population. The EMA requested therefore in vivo experiments in a preclinical model.

9. **In appendix 1 wordt superieure surfactant bepaald, maar overal wordt vermeld dat het superieure surfactant de synthetische is. Hoe leidt dit tot vermindering van het aantal dieren als beide opnieuw getest worden?**
   Antwoord:
   It has been tested in vitro. The data did not convince the EMA. The producer did animal experiments in young dogs which the EMA did not accept as appropriate animal model for the patient population. The EMA requested therefore in vivo experiments in a preclinical model.

E. Herhaling.

10. **Niet beantwoord?**
    Antwoord:
    This experiment is new and is not a repetition. The previous in vitro work is used to reduced the number of animals by avoiding a dose finding study.

I. Overige aantasting van het welzijn en maatregelen.

11. **De DEC-UM merkt op dat hier voor de eerste keer aangegeven wordt dat het schaap niet uit de narcose bijkomt.**
    Antwoord:
    The majority of the animals have been exposed to Ureaplasma. Therefore, re-use is not possible. All ewes will be euthanized after the c-section.

See above
Appendix 2
Vragen:
A. Experimentele aanpak en primaire uitkomstparameters.

1. **De DEC-UM vraagt zich af of er geen neurologische analyses/ hersen analyses uitgevoerd worden om de nadelige effecten van glucocorticoid behandeling te testen**.
   Antwoord:
   We added these analyses.

D.
Vervanging, vermindering en verfijning.

2. **Kan men voor groep D meer pijn verwachten ten gevolge van de UP behandeling en het langer moeten leven met infectie, die dus steeds sterker wordt?**
   Antwoord:
   No. Ureaplasma infections have not been associated with sepsis, pain and distress**.**

E. Herhaling.

3. **Niet ingevuld?**
   Antwoord:
   This experiment is new and is not a repetition. The previous in vitro work is used to reduced the number of animals by avoiding a dose finding study.

- Datum antwoord 24-11-2016
- Verstrekte antwoorden: ***Zie hierboven****.*
- De antwoorden hebben geleid tot aanpassing van de aanvraag.

10. **Eventuele adviezen door experts:** (niet lid van de DEC-UM) ***N.V.T*.**

B. Beoordeling (adviesvraag en behandeling)

1. Is het project vergunningplichtig (dierproeven in de zin der wet)? Indien van toepassing, licht toe waarom het project niet vergunningplichtig is en of daar discussie over geweest is. ***JA***
   Indien niet vergunningplichtig, ga verder met onderdeel E. Advies.

2. De aanvraag betreft een ***nieuwe*** aanvraag.

3. Is de DEC competent om hierover te adviseren? ***Ja***

4. Geef aan of DEC-leden, met het oog op onafhankelijkheid en onpartijdigheid, zijn uitgesloten van de behandeling van de aanvraag en het opstellen van het advies. Indien van toepassing, licht toe waarom. ***N.V.T.***

C. Beoordeling (inhoud)

1. Beoordeel of de aanvraag toetsbaar is en voldoende samenhang heeft.

   Deze aanvraag heeft een concrete doelstelling en kan getypeerd worden als een project. Het is helder welke handelingen en ongerief individuele dieren zullen ondergaan.

De DEC-UM vertrouwt erop dat de aanvrager gedurende het project op zorgvuldige wijze besluiten zal nemen over de voortgang van het project en er niet onnodig dieren gebruikt zullen worden. Gezien bovenstaande is de DEC-UM van mening dat de aanvraag toetsbaar is en voldoende samenhang heeft.

2. Geef aan of er aspecten in deze aanvraag zijn die niet in overeenstemming zijn met wet- en regelgeving anders dan de Wod? Denk hierbij aan bijvoorbeeld de Flora en fauna wet en Wet dieren. Indien van toepassing, leg uit om welke aspecten het gaat en waarom hier sprake van is.

   N.v.t., daar dit buiten de taakstelling van de DEC valt overeenkomstig artikel 18a.2.b van de Wod.

3. Beoordeel of de in de projectaanvraag aangekruiste doelcategorie(ën) aansluit(en) bij de hoofddoelstelling. Nevendoelstellingen van beperkt belang hoeven niet te worden aangekruist in het projectvoorstel.

   Het projectvoorstel heeft inderdaad kenmerken van zowel fundamenteel als translationeel onderzoek.

   Belangen en waarden

4. Benoem zowel het directe doel als het uiteindelijke doel en geef aan of er een reële relatie is tussen beide doelstellingen.

   Zie antwoord op vraag C5.

5. Benoem de belanghebbenden in het project en beschrijf voor elk van de belanghebbenden welke morele waarden in het geding zijn of bevorderd worden.

   De belangrijkste belanghebbenden in dit fundamenteel en toegepast wetenschappelijke project, dat gericht is op het verbeteren van de behandeling van vroeg-geborenen door nieuwe strategieën te testen voor de verbetering van hun pulmonaire functie, zijn de proefdieren, de onderzoekers, de doelgroep, d.w.z. de pasgeborenen en hun naasten, en ook de medische wetenschap en de samenleving als geheel.
   Waarden die voor de proefdieren in het geding zijn: De integriteit van de dieren zal worden aangetast door de experimentele handelingen en het leven met de gevolgen daarvan gedurende de proeven en de opoffering aan het eind daarvan. Ze zullen ongerief ondervinden dat voor de ooien wordt geclassificeerd als matig en voor de lammeren als non-recovery.
   Waarden die voor de onderzoekers bevorderd worden: De onderzoekers zullen medisch-wetenschappelijke kennis verkrijgen en delen met de wetenschappelijke gemeenschap. De onderzoekers vergaren kennis over mogelijke methoden om longproblemen bij vroeg geborenen te verhelpen. Uiteindelijk kan deze kennis leiden tot verbeterde therapeutische mogelijkheden in de neonatologie.
   Waarden die voor de patiënten c.q. de vroeg geborenen bevorderd worden: Verbeterde therapie en vergrote levensverwachting. Daardoor kan de kwaliteit van leven verbeterd worden van deze patiënten en hun naasten.
   Groei van medische kennis op een gebied waar daaraan behoefte is, wordt eveneens bevorderd door het onderhavige onderzoek. Vroeggeboorte is een voorname oorzaak van perinatale ziektelast en sterfte. Daarom heeft dit onderzoek ook belang voor de samenleving als geheel.

6. Geef aan of er sprake kan zijn van substantiële milieueffecten. Zo ja, benoem deze, leg uit waarom daar sprake van kan zijn en of geef aan of deze effecten afgedekt worden door specifieke wetgeving.

   N.v.t., daar dit buiten de taakstelling van de DEC valt overeenkomstig artikel 18a.2.b van de Wod.

   Proefopzet en haalbaarheid

7. Beoordeel of de kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven voldoende gewaarborgd zijn. Licht uw antwoord toe.

   Voor zover de DEC-UM kan beoordelen zijn de kennis en kunde van de onderzoeksgroep adequaat gezien de wetenschappelijke output, de verworven interne- en externe financiering alsmede de aandacht voor de drie V's.

8. Beoordeel of het project goed is opgezet, de voorgestelde experimentele opzet en uitkomstparameters logisch en helder aansluiten bij de aangegeven doelstellingen en of de gekozen strategie en experimentele aanpak kan leiden tot het behalen van de doelstelling binnen het kader van het project. Licht uw antwoord toe.

   De DEC-UM is er van overtuigd dat het projectvoorstel aansluit bij recente wetenschappelijke inzichten en geen hiaten bevat die de bruikbaarheid van de resultaten in de weg zullen staan. De voorgestelde experimentele opzet en uitkomstparameters zijn logisch en helder gekozen en sluiten aan bij de aangegeven doelstellingen en de gekozen strategie en experimentele aanpak kunnen naar de mening van de DEC-UM leiden tot het behalen van de doelstelling in het kader van het project.

   Welzijn dieren

9. Geef aan of er sprake is van één of meerdere bijzondere categorieën van dieren, omstandigheden of behandeling van de dieren. Beoordeel of de keuze hiervoor voldoende wetenschappelijk is onderbouwd en de aanvrager voldoet aan de in de Wod voor de desbetreffende categorie genoemde beperkende voorwaarden. Licht uw antwoord toe. ***N.V.T.***

10. Geef aan of de dieren gehuisvest en verzorgd worden op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van de richtlijn. Indien niet aan deze minimale eisen kan worden voldaan omdat het om wetenschappelijke redenen noodzakelijk is hiervan af te wijken, beoordeel of dit in voldoende mate is onderbouwd. Licht toe waarom wel/niet.

    De DEC-UM heeft zich ervan verzekerd dat zulks het geval is.

11. Beoordeel of het ongerief als gevolg van de dierproeven realistisch is ingeschat en geclassificeerd, waarbij uitgegaan wordt van de kans op angst, pijn, stress en/of ziekte bij individuele dieren.

    De DEC-UM vertrouwt erop dat de aanvrager al het mogelijke zal doen om het eventuele ongerief voor de proefdieren te identificeren, te verminderen en waar mogelijk te voorkomen.

12. Geef aan op welke wijze de integriteit van de dieren wordt aangetast.

De integriteit van de dieren zal worden aangetast door: De experimentele handelingen en het leven met de gevolgen daarvan gedurende de proeven. De dieren worden aan het eind van de proef opgeofferd.

13. Beoordeel of de criteria voor humane eindpunten goed zijn gedefinieerd en of goed is ingeschat welk percentage dieren naar verwachting een humaan eindpunt zal bereiken. Licht uw antwoord toe.

    Naar de mening van de DEC-UM zijn de humane eindpunten zorgvuldig beschreven en is de inschatting van het percentage dieren dat naar verwachting een humaan eindpunt zal bereiken eveneens zorgvuldig beschreven in de projectaanvraag.

14. Beoordeel of de aanvrager voldoende aannemelijk heeft gemaakt dat er geen geschikte vervangingsalternatieven zijn? Onderbouw uw antwoord.

    De DEC-UM is van mening dat de doelstellingen van de proef niet behaald kunnen worden, anders dan met de aangevraagde dieren, daar geschikte vervangingsalternatieven ontbreken, zoals beschreven in onderhavig projectvoorstel.

15. Beoordeel of het aantal te gebruiken dieren realistisch is ingeschat en of er een heldere strategie is om ervoor te zorgen dat tijdens het project met zo min mogelijk dieren wordt gewerkt waarmee een betrouwbaar resultaat kan worden verkregen. Onderbouw uw antwoord.

    Naar de mening van de DEC-UM is het aantal te gebruiken dieren realistisch ingeschat en wel zodanig dat niet meer dan nodig, maar ook niet minder dan nodig dieren worden gebruikt voor het behalen van een betrouwbaar wetenschappelijke resultaat zulks mede gebaseerd op statistische analyse middels een poweranalyse.

16. Beoordeel of het project in overeenstemming is met de vereiste van verfijning van dierproeven en het project zodanig is opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd? Licht uw antwoord toe.

    De DEC-UM heeft zich ervan verzekerd dat de aanvrager al het mogelijke zal doen om het eventuele ongerief voor de proefdieren te identificeren, te verminderen en waar mogelijk te voorkomen. Hierbij heeft de DEC-UM onder andere de pijnbestrijding en huisvesting in haar beoordeling betrokken.

17. Beoordeel, indien het wettelijk vereist onderzoek betreft, of voldoende aannemelijk is gemaakt dat er geen duplicatie plaats zal vinden en of de aanvrager beschikt over voldoende expertise en informatie om tijdens de uitvoering van het project te voorkomen dat onnodige duplicatie plaatsvindt. Onderbouw uw antwoord.

    Voor zover de DEC-UM kan beoordelen zijn de kennis en kunde van de onderzoeksgroep adequaat en mede gezien het daartoe strekkende antwoord van de aanvrager in de projectaanvraag heeft de DEC-UM reden aan te nemen dat onnodige duplicatie achterwege blijft.

    Dieren in voorraad gedood en bestemming dieren na afloop proef

18. Geef aan of dieren van beide geslachten in gelijke mate ingezet zullen worden. Indien alleen dieren van één geslacht gebruikt worden, beoordeel of de aanvrager dat in voldoende mate wetenschappelijk heeft onderbouwd? Geef ook aan welke maatregelen verder zijn getroffen om bij fok of aankoop van dieren het aantal in voorraad gedood te beperken.

In onderhavige projectaanvraag worden wel dieren van een eenvormig geslacht gebruikt. Uit de projectaanvraag blijkt dat de onderzoeker zich bewust is van het nadeel van het gebruiken van dieren van een eenvormig geslacht zulks in relatie tot de vermindering van proefdieren in voorraad gedood en de onderzoeker heeft in de projectaanvraag naar de mening van de DEC-UM dit voldoende onderbouwd.

Alhoewel de DEC-UM vermindering van proefdieren in voorraad gedood toejuicht is zij overigens van mening dat dit aspect met name met de centrale dienst proefdieren en de aanvrager kortgesloten dient te worden daar de DEC niet betrokken is bij de fok en aankoop van proefdieren.

19. Geef aan of dieren gedood worden in kader van het project (tijdens of na afloop van de dierproef). Indien dieren gedood worden, geef aan of en waarom dit noodzakelijk is voor het behalen van de doelstellingen van het project. Indien dieren gedood worden, geef aan of er een voor de diersoort passende dodingsmethode gebruikt wordt die vermeld staat in bijlage IV van de richtlijn. Zo niet, beoordeel of dit in voldoende mate is onderbouwd. Licht dit toe. Indien van toepassing, geeft ook aan of er door de aanvrager ontheffing is aangevraagd.

    Naar de mening van de DEC-UM is dit genoegzaam beschreven in de projectaanvraag door de aanvrager.

20. Indien dieren worden gedood, is adoptie of hergebruik overwogen? Licht toe waarom dit wel/niet mogelijk is.

    Adoptie is ten aanzien van onderhavige aanvraag niet opportuun.

    NTS

21. Is de niet-technische samenvatting een evenwichtige weergave van het project en begrijpelijk geformuleerd?

    Naar de mening van de DEC-UM is zulks het geval.

D. Ethische afweging

1. Benoem de centrale morele vraag.
Rechtvaardigt het verkrijgen van kennis over nieuwe strategieën ter verbetering van de pulmonaire functie van vroeg geborenen, de opoffering en het matige ongerief dat de dieren wordt aangedaan in het voorliggende project "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis?".

2.
Waarden die voor de proefdieren in het geding zijn: *matig nadeel.*
Waarden die voor onderzoekers bevorderd worden: *substantieel voordeel.*
Waarden die voor de doelgroep bevorderd worden: *substantieel voordeel.*
Algemeen: *relevante groei van medische kennis.*

De DEC-UM is van mening dat de belangen van de samenleving in het algemeen en de patiëntjes en hun naasten in het bijzonder, binnen het project "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis" zwaarder wegen dan de belangen/waarden van de proefdieren. Voor de betrokken proefdieren leiden deze proeven tot de dood na, voor de ooien, matig ongerief. Het ongerief voor de lammeren wordt gekwalificeerd als non-recovery. De dieren worden door de experimenten in hun welzijn geschaad. De integriteit van de dieren zal worden aangetast door de experimentele handelingen en het leven met de gevolgen daarvan gedurende de proeven en de opoffering aan het eind daarvan.

Indien de doelstellingen bereikt worden, zal dit project echter leiden tot kennis over nieuwe methoden om de longfunctie van vroeg geborenen te verbeteren. In de neonatologische kliniek is op dit punt behoefte aan meer kennis en effectievere therapie. Hierdoor kan de levensverwachting, de ziektelast en uiteindelijk ook de kwaliteit van leven verbeterd worden van deze patiëntjes en hun naasten.
Vroeggeboorte is een belangrijke oorzaak van perinatale morbiditeit en sterfte. Daarom heeft dit onderzoek ook belang voor de samenleving als geheel. Vandaar dat de DEC-UM het onderhavige onderzoek van substantieel belang acht.
Het is aannemelijk dat de doelstelling behaald zal worden. De onderzoekers zullen zoveel mogelijk trachten het lijden van de dieren te beperken d.m.v. pijnbestrijding, waardoor het werkelijke ongerief van de dieren beperkt blijft in relatie tot het te behalen voordeel.

3.
De DEC-UM beantwoordt de centrale morele vraag: Rechtvaardigt het verkrijgen van kennis over nieuwe strategieën ter verbetering van de pulmonaire functie van vroeg geborenen, de opoffering en het matige ongerief dat de dieren wordt aangedaan in het voorliggende project "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis?" bevestigend.
Hoewel de DEC-UM de intrinsieke waarde van het dier onderschrijft en oog heeft voor het te ondergane ongerief van de proefdieren, weegt het substantiële belang van dit project naar haar mening zwaarder.
De DEC-UM is van mening dat de voorgestelde experimentele opzet en de uitkomstparameters logisch en helder aansluiten bij de aangegeven doelstellingen en dat de gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. De onderzoekers beschikken over de benodigde kennis en technische expertise. Er is geen sprake van duplicatie.
In de gekozen strategie wordt op bevredigende wijze tegemoet gekomen aan de vereisten van vervanging, vermindering en verfijning. De DEC-UM is er van overtuigd dat de aanvrager voldoende maatregelen treft om zowel het ongerief van de dieren als het aantal benodigde dieren tot een minimum te beperken. Er zijn voldoende go/no go momenten voorzien om onnodige dierproeven te vermijden. De DEC-UM is ervan overtuigd dat er geen alternatieven zijn, waardoor deze dierproef met minder ongerief of met minder, dan wel zonder levende dieren zou kunnen worden uitgevoerd.
Op grond van deze overwegingen beschouwt de DEC-UM de voorgestelde dierproeven in het projectvoorstel "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis metabolism" als ethisch gerechtvaardigd. Derhalve voorziet de DEC-UM het projectvoorstel "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis" van een positief advies.

E. Advies

1. Advies aan de CCD
   X De DEC-UM adviseert de vergunning te verlenen.

2. Het uitgebrachte advies is ***unaniem*** tot stand gekomen.

3. Omschrijf de knelpunten/dilemma's die naar voren zijn gekomen tijdens het beoordelen van de aanvraag en het opstellen van het advies zowel binnen als buiten de context van het project.

   N.v.t. Daarenboven is de DEC-UM niet gewoon projectaanvragen buiten de context c.q. haar verantwoordelijkheid en competentie te beoordelen.

Centrale Commissie Dierproeven

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> Retouradres Postbus 20401 2500 EK Den Haag

Universiteit Maastricht
[redacted]
Postbus 616
6200 MD MAASTRICHT

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD107002016783

Bijlagen
2

Datum 15 december 2016
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [redacted]

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 15 december 2016. Het gaat om uw project "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD107002016783. Gebruik dit nummer wanneer u contact met de CCD opneemt.

Wacht met de uitvoering van uw project
Als wij nog informatie van u nodig hebben dan ontvangt u daarover bericht. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn bijgeschreven op de rekening van de CCD. U ontvangt binnen veertig werkdagen een beslissing op uw aanvraag. Als wij nog informatie van u nodig hebben, wordt deze termijn opgeschort. In geval van een complexe aanvraag kan deze termijn met maximaal vijftien werkdagen verlengd worden. U krijgt bericht als de beslisperiode van uw aanvraag vanwege complexiteit wordt verlengd. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Factuur
Bijgaand treft u de factuur aan voor de betaling van de leges. Wij verzoeken u de leges zo spoedig mogelijk te voldoen, zodat we uw aanvraag in behandeling kunnen nemen. Is uw betaling niet binnen dertig dagen ontvangen, dan kan uw aanvraag buiten behandeling worden gesteld. Dit betekent dat uw aanvraag niet beoordeeld wordt en u uw project niet mag starten.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
15 december 2016
Aanvraagnummer:
AVD107002016783

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Datum:
15 december 2016
Aanvraagnummer:
AVD107002016783

Gegevens aanvrager

Uw gegevens	
Deelnemersnummer NVWA:	10700
Naam instelling of organisatie:	Universiteit Maastricht
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	50169181
Straat en huisnummer:	Minderbroedersberg 4-6
Postbus:	616
Postcode en plaats:	6200 MD MAASTRICHT
IBAN:	NL04 INGB 0679 5101 68
Tenaamstelling van het rekeningnummer:	Universiteit Maastricht

Gegevens verantwoordelijke onderzoeker	
Naam:	[redacted]
Functie:	[redacted]
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Datum:
15 december 2016
Aanvraagnummer:
AVD107002016783

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	[redacted]
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Over uw aanvraag

Wat voor aanvraag doet u?	[x] Nieuwe aanvraag [ ] Wijziging op een (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn [ ] Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Over uw project

Geplande startdatum:	1 januari 2017
Geplande einddatum:	1 januari 2022
Titel project:	Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis
Titel niet-technische samenvatting:	Therapeutische maatregelen om de uitkomst van prematuren kinderen te verbeteren.
Naam DEC:	DEC-UM
Postadres DEC:	Postbus 616, 6200 MD Maastricht
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 1.187,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

Verplichte bijlagen:	[x] Projectvoorstel [x] Beschrijving Dierproeven [x] Niet-technische samenvatting
Overige bijlagen:	[x] DEC-advies

Datum:
15 december 2016
Aanvraagnummer:
AVD107002016783

Ondertekening

Naam:	[redacted]
Functie:	[redacted]
Plaats:	Maastricht
Datum:	14 december 2016

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Universiteit Maastricht
[redacted]
Postbus 616
6200 MD MAASTRICHT

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD107002016783

Bijlagen
2

Datum 15 december 2016
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 15 december 2016
Vervaldatum: 14 januari 2017
Factuurnummer: 16700783

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD107002016783	€ 1.187,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL29INGB 070.500.1512 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 93144, 2509 AC te 's Gravenhage.

Centrale Commissie Dierproeven

9

> Retouradres Postbus 20401 2500 EK Den Haag

Universiteit Maastricht
[redacted]
Postbus 616
6200 MD MAASTRICHT

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD107002016783

Datum 23 december 2016
Betreft aanvraag projectvergunning Dierproeven

Geachte [redacted],

Op 15 december 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis" met aanvraagnummer AVD107002016783. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In deze brief leest u wat wij nog nodig hebben en wanneer u een beslissing kunt verwachten.

Welke informatie nog nodig
Wij hebben de volgende informatie van u nodig om uw aanvraag verder te kunnen beoordelen:

Onduidelijkheden
1) In uw aanvraag motiveert u de noodzaak om dit onderzoek uit te voeren door te melden dat de EMA naar het testen van de synthetische surfactant in lammetjes heeft gevraagd. Indien zo, zou dit project niet onder 'wettelijk vereist' onderzoek moeten vallen? Is het mogelijk om een officiële verzoek van de EMA naar ons toe te sturen? Indien niet, dan verzoeken we u om de reden dit al eerder uitgevoerd onderzoek in schapen te herhalen nader te onderbouwen.

2) In de aanvraag geeft u aan dat het ongerief van de lammetjes in de categorie terminaal valt, omdat de dieren vanaf de keizersnede onder verdoving worden gehouden. Daarnaast schrijft u onder punt I dat de foetussen pijn zouden kunnen ervaren, aan hemodynamische instabiliteit, respiratorische complicaties of hypoglycaemie zouden kunnen leiden. Bedoelt u dat dit gebeurt alleen als de dieren niet onder verdoving zijn of kunnen de lammetjes ook onder verdoving pijn ervaren?

3) In uw NTS geeft u aan 120 ooien en 120 lammetjes voor dit onderzoek te willen gebruiken, maar in de bijlages dierproeven zijn deze aantallen niet duidelijk aangegeven. In bijlage 1 is alleen het aantal lammetjes benoemd. We verzoeken u het aantal benodigde dieren duidelijker in de bijlages dierproeven te melden.

Leges
De leges die u verschuldigd bent zijn nog niet door ons ontvangen of de betaling is nog niet verwerkt. Uw aanvraag is niet compleet als de leges niet zijn ontvangen.
Zonder deze aanvullende informatie kan de beslissing nadelig voor u uitvallen omdat de gegevens onvolledig of onduidelijk zijn.

Opsturen binnen veertien dagen
Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. U kunt dit aanleveren via NetFTP. Stuurt u het per post op, gebruik dan het formulier dat u bij deze brief krijgt.

Wanneer een beslissing
De behandeling van uw aanvraag wordt opgeschort tot het moment dat wij de aanvullende informatie hebben ontvangen. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn ontvangen. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Centrale Commissie Dierproeven

Melding bijlagen

U wilt één of meerdere bijlagen naar ons versturen? Voeg altijd deze Melding Bijlagen toe.
Wij weten dan welke documenten van u zijn en hoeveel documenten u opstuurt.
Meer informatie vindt u op www.centralecommissiedierproeven.nl
Of bel met ons: 0900 28 000 28 (10 ct/min).

1 Uw Gegevens

Naam instelling: Universiteit Maastricht

Adres: .....

Postcode en plaats: .....

Aanvraagnummer: AVD107002016783

2 Bijlagen
Welke bijlagen stuurt u mee?
Vink de bijlagen aan of vul de naam of omschrijving in.

O Projectvoorstel

O Beschrijving Dierproeven

O Niet-technische samenvatting

O Melding Machtiging

O Aanvraagformulier

O .....

O .....

O .....

Datum:
23 december 2016
Aanvraagnummer:
AVD107002016783

3 Ondertekening

Naam: .....

Datum: ..... - ..... - .....

Handtekening: .....

Onderteken het formulier en stuur het met alle bijlagen op naar:
Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag

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[redacted]

Van: Info-zbo
Verzonden: vrijdag 23 december 2016 15:36
Aan: [redacted]
Onderwerp: aanvullende informatie aanvraag AVD107002016783

Geachte DEC–UM,

Enige tijd geleden heeft u advies uitgebracht op een projectaanvraag met titel "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis" met aanvraagnummer AVD107002016783.

De volgende vragen zijn aan de aanvrager voorgelegd:

1) In uw aanvraag motiveert u de noodzaak om dit onderzoek uit te voeren door te melden dat de EMA naar het testen van de synthetische surfactant in lammetjes heeft gevraagd. Indien zo, zou dit project niet onder 'wettelijk vereist' onderzoek moeten vallen? Is het mogelijk om een officiële verzoek van de EMA naar ons toe te sturen? Indien niet, dan verzoeken we u om de reden dit al eerder uitgevoerd onderzoek in schapen te herhalen nader te onderbouwen.

2) In de aanvraag geeft u aan dat het ongerief van de lammetjes in de categorie terminaal valt, omdat de dieren vanaf de keizersnede onder verdoving worden gehouden. Daarnaast schrijft u onder punt I dat de foetussen pijn zouden kunnen ervaren, aan hemodynamische instabiliteit, respiratorische complicaties of hypoglycaemie zouden kunnen leiden. Bedoelt u dat dit gebeurt alleen als de dieren niet onder verdoving zijn of kunnen de lammetjes ook onder verdoving pijn ervaren?

3) In uw NTS geeft u aan 120 ooien en 120 lammetjes voor dit onderzoek te willen gebruiken, maar in de bijlages dierproeven zijn deze aantallen niet duidelijk aangegeven. In bijlage 1 is alleen het aantal lammetjes benoemd. We verzoeken u het aantal benodigde dieren duidelijker in de bijlages dierproeven te melden.

We zouden graag de mening van de DEC willen horen over de categorie van dit project. Heeft de DEC een mogelijk wettelijk vereist karakter van dit project overwogen?

Het is niet duidelijk aangegeven in het advies of de DEC met de inschatting van het ongerief die in de aanvraag is ingevuld het eens is. Valt het ongerief van de lammetjes onder terminaal of onder een andere categorie?

We ontvangen graag uw reactie uiterlijk donderdag 5 januari 2017, om deze mee te kunnen nemen in de eerstvolgende CCD vergadering.

Alvast hartelijk dank.
Fijne feestdagen en Gelukkig Nieuwjaar!

Met vriendelijke groet,

[redacted]
Uitvoeringsexpert

Centrale Commissie Dierproeven www.centralecommissiedierproeven.nl
Postbus 20401 | 2500 EK | Den Haag
T: 0900 2800028
E: info@zbo-ccd.nl

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[redacted]

Van: [redacted]
Verzonden: maandag 2 januari 2017 12:30
Aan: Info-zbo; [redacted]
Onderwerp: Re: aanvullende informatie aanvraag AVD107002016783
Bijlagen: Project DEC CCD.docx; PV 2016-012 Appendix 1 - description animal procedure - Intra-uterine inflammation_revised.docx; PV 2016-012 Appendix 2 - description animal procedure - Ventilation-induced lung injury_revised.docx

Project "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis" met aanvraagnummer AVD107002016783

Reply to questions from 23.12.2016

1) In uw aanvraag motiveert u de noodzaak om dit onderzoek uit te voeren door te melden dat de EMA naar het testen van de synthetische surfactant in lammetjes heeft gevraagd. Indien zo, zou dit project niet onder 'wettelijk vereist' onderzoek moeten vallen? Is het mogelijk om een officiële verzoek van de EMA naar ons toe te sturen? Indien niet, dan verzoeken we u om de reden dit al eerder uitgevoerd onderzoek in schapen te herhalen nader te onderbouwen.

The EMA has requested the producer of the surfactant to test the synthetic surfactant who has contracted us to do so. The legislation of "wettelijk vereist onderzoek" is therefore not applicable.

The synthetic surfactant is a drug under development and not yet licensed. Batch to batch variability still exists. The company has requested - based on EMAs suggestions - to include the control group with the current synthetic surfactant batch to have a control group in order to reproduce the effect of the drug in the current experiment and not from a historic control respectively batch.

2) In de aanvraag geeft u aan dat het ongerief van de lammetjes in de categorie terminaal valt, omdat de dieren vanaf de keizersnede onder verdoving worden gehouden. Daarnaast schrijft u onder punt I dat de foetussen pijn zouden kunnen ervaren, aan hemodynamische instabiliteit, respiratorische complicaties of hypoglycaemie zouden kunnen leiden. Bedoelt u dat dit gebeurt alleen als de dieren niet onder verdoving zijn of kunnen de lammetjes ook onder verdoving pijn ervaren?

Please accept our apologies for the imprecision. We meant that animals WITHOUT sedation can suffer pain but NOT if they are sedated.

3) In uw NTS geeft u aan 120 ooien en 120 lammetjes voor dit onderzoek te willen gebruiken, maar in de bijlages dierproeven zijn deze aantallen niet duidelijk aangegeven. In bijlage 1 is alleen het aantal lammetjes benoemd. We verzoeken u het aantal benodigde dieren duidelijker in de bijlages dierproeven te melden.

We have clarified the appendices. We need 120 animals.

Appendix 1: per group N=12 animals/lambs, 6x 12= 72 animals/lambs
Appendix 2: As shown in figure 1: four groups of N=12 animals/lambs per group
4 x 12 = 48 animals/lambs for appendix 2

Total: 72 + 48 animals = 120 animals/lambs = 120 ewes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10700
1.2	Provide the name of the licenced establishment.	Maastricht University
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 — Type of animal procedure: Intra-uterine inflammation

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

We induce intra-uterine inflammation with intra-amniotic injections of Ureaplasma Parvum (UP), since this micro-organism is the most commonly found micro-organism in human chorioamnionitis cases. Previous studies already validated this approach and the same amount of colony forming units will be administered in the current study. The presence of chorioamnionitis can only be confirmed after birth which will be done by histological examination of the fetal membranes and placenta. The fetuses are delivered preterm by C-section, sedated and mechanically ventilated for 48 hours during which they are treated with different surfactant preparations. Subsequently, animals receive ventilator support via CPAP for 5 days. We have formulated the following 6 experimental groups:

1. Control ventilation N=12
2. UP + Control ventilation N=12
3. UP + natural surfactant (Curosurf, clinical standard, porcine origin) N=12
4. UP + CHF5633 (synthetic surfactant) N=12
5. UP + natural surfactant (Curosurf) + glucocorticoid (Budesonide 0,25 mg/kg) N=12
6. UP + CHF5633+ glucocorticoid (Budesonide 0,25 mg/kg) N=12

Groups 1, 3 and 4 are necessary to assess the effects in the pattern of clinical practice. Group 1 will help to identify the inflammatory phenotype influencing treatment effect, group 3 is current clinical standard and group 4 will help to identify if the artificial surfactant itself has an effect on inflammation due to its different protein composition.

We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism. Based on a power calculation, and taking into account a loss of 25% in total of the animals due to complications of intra-uterine inflammation (10%), premature birth (5%) and humane endpoints in lambs (10%) we calculated a total number of animals per experimental groups will which be 12.

Within these experimental groups the following primary and outcome parameters will be assessed:

Primary outcomes:

- Oxygenation (arterial oxygen partial pressure) in the course of ventilation: oxygenation is the main clinical parameter indicative for adequate ventilation)
- Repetitive dosing: depending on oxygenation: due to inactivation of surfactant, oxygenation might fail and additional boluses of surfactant are needed.
- Activity of surfactant recovered from the animals after sacrifice

The animals get colostrum and breast milk from sheep during the mechanical ventilation according to a feeding protocol from the neonatal intensive care unit with gastric tube feeding.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Time-mated Texel ewes will receive an intra-amniotic injection of Ureaplasma Parvum (UP):

Under sedation UP is administered by ultrasound-guided intra-amniotic injection. 2 days before preterm delivery, the ewes will be given an intramuscular injection of dexamethasone, which enhances fetal lung maturation and allows the fetus to be ventilated postnatally. Moreover, maternal intramuscular dexamethasone administration mimics the clinical standard nowadays.

Maternal administration should not be confused with postnatal steroid treatment in the fetus for BPD: Prenatal treatment of the mother is beneficial for the child because lung maturation is induced and mortality is decreased.

Postnatal treatment of the preterm infant has also been shown to positively influence the infants' lung, and BPD is decreased. However, corticosteroids given systemically to the infant have serious side effects on the neurodevelopmental outcome. Therefore, postnatal therapy can only be administered after balancing the positive effects on the lung and the negative effects on the brain, leading frequently to a therapeutic dilemma.

Administration of corticosteroids for longer periods of time induces abortion in sheep. However, a single

injection of dexamethasone, as administered in our proposed experiments, will not increase the risk of abortion (Fehrholz et al., Am. J. Physiology 2015) provided that the fetus will be born 48 hours after injection.
Previous experiments with intra-uterine Ureaplasma Parvum infection and treatment with dexamethasone demonstrated that dexamethasone 2 days prior to delivery did not have any effects on the immune response towards Ureaplasma Parvum

Seven days after the inoculation with UP, the fetus will be born preterm by **C-section** (general anesthesia): while remaining on placental circulation umbilical vessel catheters are placed for administration of medication (i.e. anesthesia) and blood gas analysis and an endotracheal tube is placed for ventilatory support (modified EXIT procedure). The lamb is immediately placed in our lamb intensive care unit which resembles the clinical intensive care very closely. Before connection to mechanical ventilation the lambs receive **surfactant replacement therapy** (according to their allocation). The lamb remains mechanically ventilated for 24 hours while anesthesia is maintained. Surfactant replacement therapy is repeated throughout the experiment if necessary. After 24 h the animals will be switched to continuous positive pressure ventilation for additional 6 days under sedation.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

We have a preference for singleton pregnancies since this is more clinical relevant and twins can strongly affect each other's health (i.e. resulting in small for gestational age) however, to reduce the number of pregnant ewes we will use both singleton and twin pregnancies. Furthermore, we do not know in advance whether it will be a single or twin pregnancy and only selecting twin pregnancies will result in massive over-breeding. Group numbers were determined with the power-calculation according to Sachs, in which variance and expected therapeutic effects are based on previous experiments with surfactant in non-infectious sheep models.

We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism

In ventilated preterm sheep (non-infectious model, Seehase et al., 2012) CHF5633 has shown a comparable effect on oxygenation but superior resistance against inactivation. Surfactant re-dosing in a 48-hour period of ventilation is the critical outcome in this experiment.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Lambs are the most appropriate animals to address our hypothesis, for a number of reasons:
1. Development of the lungs in sheep has been sufficiently studied to allow informed experimental design and integration of findings with those from previous studies. The current surfactant replacement therapy was developed in preterm lambs.

2. Preterm lambs are large enough to use similar equipment for ventilation as in the neonatal intensive care unit, provide sufficient blood and tissue to allow enough biomolecular analyses, for thorough assessment of the biological systems being investigated, in individuals.

The total number of animals needed for the current study will by 6 groups * 12 animals = 72 animals. We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism. Based on a power calculation, and taking into account a loss of 25% in total of the animals due to complications of intra-uterine inflammation (10%), premature birth (5%) and humane endpoints in lambs (10%) we calculated a total number of animals per experimental groups will which be 12.

We cannot predict the number of twin pregnancies, since multiple factors (e.g. seasonal vs. out of season breeding and the age of the ewe) are of influence. Therefore, the number of ewes is based on singleton pregnancies. In the case of twin pregnancy, the total number of ewes used for the experiment will be reduced.

The gestational age at which the lambs are delivered (129-132d gestational age) is based on the developmental biology in which the sheep is very similar to a preterm infant (figure 1). In contrast to rodents, ovine lung development (alveolarization) starts prenatally and is prolonged postnatally. Moreover, the size allows for usage of similar equipment and ventilation strategies as is currently used in clinical practice. Animals will be time-mated to yield animals of the appropriate age. Two antenatal ultrasound scans will ensure correct gestational age and adequate in utero growth.

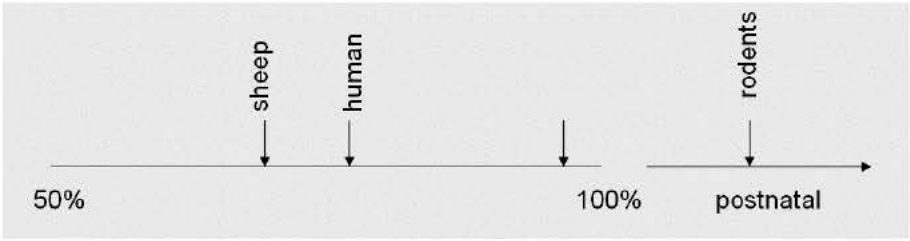


Figure 1 Time-points are shown when lung alveolarization, an important hallmark of lung maturation, occurs in different species. Lung maturation in sheep occurs prenatally, as in humans. Lung maturation in rodents takes place after birth.

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☐ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement: The use of preterm lambs has been suggested by the European Medical Agency (EMA) to pharmaceutical companies. We have done studies with synthetic surfactant (Seehase et al., 2012) with were mandated by the EMA in order to show in vivo the safety and feasibility of the new drug. It is unacceptable to test directly in human babies based on exclusively in vitro data by the standards of EMA and by good clinical practice since preterm babies is the most vulnerable patient population. In addition neonatology is full of examples of premature clinical trials with caused harm in the study population (e.g. phospholipid treatment for RDS).

Reduction: Due to large experience with sheep and maintenance of lambs on the lamb intensive care unit we are able to increase the number of animals that successfully reach the end of their experiments. Due to the expertise of our team, we have low numbers of drop-outs which decreased over the years with increasing experience, which reduces the numbers of animals in the experimental groups. Our experiments are conducted by highly experienced personnel according to well-established protocols. These methods are in favor of reproducibility and use of previously obtained historical data (also on CHF 5633 inactivation (2)) and prevent unnecessary use and loss of animals. Moreover, we do not differentiate between genders since our clinical population consists of both sexes.
Furthermore, we are able to incorporate twin pregnancies in our study. This results in a reduction of pregnant ewes that will be used. We rely on natural breeding. Therefore, we cannot control for singleton or twin pregnancies.

Refinement:
For this experiment in which different surfactant preparations are tested, the choice of animal model is based on the body size, use of similar equipment as in the neonatal intensive care and the developmental biology. In contrast to rodents, ovine and human the developmental biology are very similar. For example, the alveolar phase in lambs and humans starts before birth in a hypoxic environment, compared to rodents in which the alveolarization starts postnatally in normoxic conditions. Therefore, we have established an animal model of immature lung-development in preterm lambs. Findings in our animal model can be easily translated into clinical practice. Our staff and the staff of the animal care facility have been trained by a Texel sheep breeder to recognize symptoms of pending labor and give assistance to the sheep upon labor. Recognition of pending labor and assistance during labor reduces the risk of complications and improves animal well-being. Moreover, we aim to leave the sheep as much in their natural environment (group housing in a meadow) as we can with minimal human contact. Moreover, the fetus will receive analgesia and nutritional support (glucose) during mechanical ventilation.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects

on the environment.

Ewe

Before induction of intra-uterine inflammation the sheep are kept in their natural environment as long as possible with access to food and water *ad libitum*. After induction of intra-uterine inflammation the sheep are housed in groups with access to food and water *ad libitum*.

The sheep will be checked daily for discomfort due to intra-uterine inflammation. Discomfort due to intra-uterine inflammation is described in the humane endpoint section. Post-operative (after intra-amniotic injection) antibiotics, if necessary, will be administered, in order to prevent progression of (wound) infection.

The experiments will be performed by experienced animal technicians and researchers. This will speed up the progress of the experiment and therefore reduce animal suffering and fear (fast induction of anesthesia, fast surgery, and maximum results with minimal animal handling).

The majority of the animals have been exposed to Ureaplasma. Therefore, re-use is not possible. All ewes will be euthanized after the c-section.

Fetus

Previous experiments have demonstrated that fetal cortisol levels do not change in the course of chorioamnionitis (Jobe et al, Am J Respir Crit Care Med. 2000 Nov;162(5):1656-61). This suggests that the fetus will experience limited discomfort during intra-uterine inflammation in our experiments.

Chorioamnionitis is a multi-organ disease resulting in discomfort after birth, as they need to survive independently and start using their immature and inflammation affected organs. However, after C-section (birth) the fetus will stay on the Lamb Intensive Care Unit (LICU) and taken care of by highly trained personnel. During mechanical ventilation the fetus is constantly sedated (also receiving analgesia and parenteral feeding) with continuous monitoring and interpretation of vital parameters. Moreover, the environment in the LICU (temperature, light, sound) will be kept optimal in order to reduce discomfort.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

This experiment is new and is not a repetition. The previous in vitro work is used to reduced the number of animals by avoiding a dose finding study.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Ewe: All surgical procedures are performed under anesthesia (including analgetic block).

Lamb: whilst on placental circulation, the lamb is sedated through anesthesia of the ewe. Ex utero experiments are performed under sedation guided by depth of sedation.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Ewe:

Abortion might occur due to chorioamnionitis, but is very uncommon in sheep (8) (in retrospect over the past 10 years: 5 in 100). This is a major reason to use this species.

Fetus:

The fetus might experience pain, suffer from hemodynamic instability or respiratory complications (pneumothorax, hypoxia) or hypoglycemia.

Explain why these effects may emerge.

Due to their immaturity preterm fetuses are not always able to respond properly to environmental changes. The fetus might experience pain, suffer from hemodynamic instability or respiratory complications (pneumothorax, hypoxia).

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Ewe:

All invasive procedures are performed under aseptic conditions.

The ewe will will not recover from anesthesia.

Fetus:

The fetus will be sedated continuously after birth to minimise discomfort, also receiving analgesia. Environmental conditions (light, temperature, sounds) on the LICU are all in favour of the fetus.

Continuous monitoring of hemodynamic and respiratory enables us to treat or prevent the complications.

Intravenous administration of glucose will prevent hypoglycemia (regularly checked)

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Humane endpoints for ewes:

- Pending preterm labor
- Intra-uterine fetal death
- Sepsis/ infection not responding to treament
  - **Local (at the site of intra-amniotic injection):** redness, pain, and swelling with or without pus that cannot be treated with local antiseptics or antibiotics are considered a human endpoint.
  - **Systemic:** Fever, lethargy, excessive time lying down
- Untreatable pain: (a sheep that does not respond to analgesic treatment)

Humane endpoints for fetus:

- Untreatable pneumothorax
- Uncorrectable severe respiratory acidosis
- Sepsis Uncorrectable hypovolemia
- Multi-organ failure

Indicate the likely incidence.

Ewe: 5%

Fetus: 10%

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Ewe: Moderate

Lamb:Non-recovery. During the mechanical ventilation, the lambs are continuously anesthetized (sedation and analgesia), and will not regain conscience, until the end of the experiment (euthanasia). Therefore, we consider the classification of the experiment to be non-recovery.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

The ewe will be euthanized since we cannot reuse her for other purposes due to an intra-uterine infection an abdominal surgery.

The lamb will be euthanized since vital organs (i.e. lungs) have to be sampled for biochemical analysis.
Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?
☐ No > Describe the method of killing that will be used and provide justifications for this choice.
☒ Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

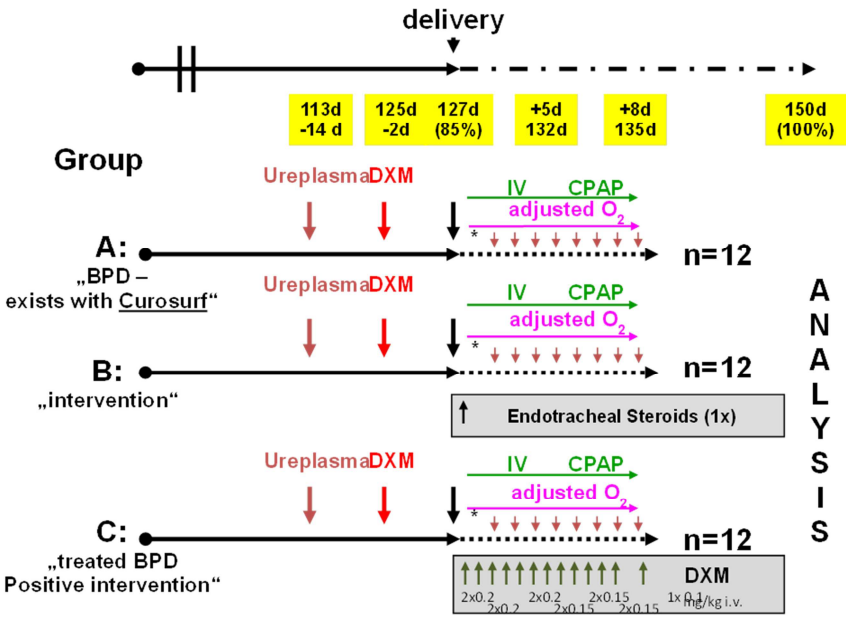
1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10700
1.2	Provide the name of the licenced establishment.	Maastricht University
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: Ventilation-induced lung injury

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

We induce intra-uterine inflammation with intra-amniotic injections of Ureaplasma Parvum, since this micro-organism is the most commonly found micro-organism in human chorioamnionitis cases. Previous studies already validated this approach and the same amount of colony forming units will be administered in the current study. The presence of chorioamnionitis can only be confirmed after birth which will be done by histological examination of the fetal membranes and placenta. The fetuses are delivered preterm by C-section (127d), sedated and mechanically ventilated for a maximum of 8 days in order to develop (histological) BPD [1]. During mechanical ventilation the lambs are treated with a glucocorticoid either by local instillation with surfactant or systemically. We compare endotracheal treatment (B) with intravenous treatment (C), a third group serves as control (A, see figure 1). A fourth group (D) will not be ventilated, but delivered on the same gestational age that autopsy is performed in the other three groups (127d+8d=135d). This group is used to identify the effects of chorioamnionitis with/without dexamethasone on lung inflammation, lung compliance, lung development and lung injury in comparison to mechanically ventilated animals (Figure 1). The following primary and secondary outcome parameters will assess feasibility, safety and efficacy of a topical administered glucocorticoids:



D: phenotype: lambs delivered at 135 d n=12

- D1 after DXM and ureaplasma; N=8
- D2 without any intervention; N=4

Figure 1 Experimental design: (Brown arrow: intra amniotic injection of UP, red arrow i.m. injection of dexamethason to mother; black arrow: delivery; small downward arrows: surfactant replacement therapy, * indicates that surfactant redosing does not follow a fixed scheme but is done dependent on oxygenation index; small upward arrows: corticosteroids to lamb.)

Primary outcome parameters: Survival. Both intrauterine inflammation and preterm birth are major risk factors for neonatal death due to complications that arise from underdeveloped organ systems. BPD is recognized as major factor in mortality in preterm infants. Treatment might improve survival compared to controls.

Secondary outcome parameters: Lung inflammation, lung compliance, lung development and lung injury, brain injury, brain development.

Lambs are the most appropriate animals to address our hypothesis, for a number of reasons:

1. Development of the lungs in sheep has been sufficiently studied to allow informed experimental design and integration of findings with those from previous studies. The current surfactant replacement therapy was developed in preterm lambs.
2. Preterm lambs are large enough to use similar equipment for ventilation as in the neonatal intensive care unit, provide sufficient blood and tissue to allow enough biomolecular analyses, for thorough assessment of the biological systems being investigated, in individuals.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

UP is administered by ultrasound-guided intra-amniotic injection. 2 days before preterm delivery, the ewes will be given an intramuscular injection of dexamethasone, which enhances fetal lung maturation and allows the fetus to be ventilated postnatally. Moreover, maternal intramuscular dexamethasone administration mimics the clinical standard nowadays.

Fourteen days after the inoculation with UP, the fetus will be born preterm by **C-section** (general anesthesia): while remaining on placental circulation umbilical vessel catheters are placed for administration of medication (i.e. anesthesia) and blood gas analysis and an endotracheal tube is placed for ventilatory support (modified EXIT procedure). The lamb is immediately placed in our lamb intensive care unit which resembles the clinical intensive care very closely. Before connection to mechanical ventilation the lambs receive surfactant replacement therapy which is repeated if necessary (based on oxygenation). The lambs remain under ventilatory support for 3 days while anesthesia is maintained and subsequently on respiratory support with CPAP (continuous positive airway pressure). Animals are maximum 8 days in the experiment. During these days, repetitive doses of glucocorticoids or sham treatment will be administered endotracheally or i.v. (Figure 1).

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism. Based on a power calculation, and taking into account a loss of 25% in total of the animals due to complications of intra-uterine inflammation (10%), premature birth (5%) and humane endpoints in lambs (10%) we calculated a total number of animals per experimental groups will which be **12**. The total number of animals needed for the current study will by 4 groups * 12 animals = 48 animals.

We cannot predict the number of twin pregnancies, since multiple factors (e.g. seasonal vs. out of season breeding and the age of the ewe) are of influence. Therefore, the number of ewes is based on singleton pregnancies. In the case of twin pregnancy, the total number of ewes used for the experiment will be reduced.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

The total number of animals needed for the current study will by 4 groups * 12 animals = 48 animals.

We will deliver 36 animals at **127d** (group A-C). Animals will receive receive ventilation and ventilator support for 8 days, therefore autopsy will also be performed on **day 135**. (figure 1). In group D, N=8 lambs are delivered at **135 d** after DXM and ureaplasma and N=4 without any intervention to assess the lung changes (lung inflammation, lung compliance, lung development and lung injury) due to chorioamnionitis and due to the immaturity at birth.

The other are intubated upon delivery.

Lambs are the most appropriate animals to address our hypothesis, for a number of reasons:

1. Development of the lungs in sheep has been sufficiently studied to allow informed experimental design and integration of findings with those from previous studies. The current surfactant replacement therapy was developed in preterm lambs.
2. Preterm lambs are large enough to use similar equipment for ventilation as in the neonatal intensive care unit, provide sufficient blood and tissue to allow enough biomolecular analyses, for thorough assessment of the biological systems being investigated, in individuals.

This experiment will be performed with a maximum of 48 pregnant sheep (Texel breed) and their respective singleton or twin fetuses (> 2/3 gestation, max. 48 fetuses) that are randomly allocated into 4 experimental groups consisting of 3 different glucocorticoid regimes, and one control group (cf. Appendix 1). If there are twin pregnancies less pregnant ewes will be needed. The gestational age at which the lambs in the ventilation groups are delivered (127d gestational age) is based on the developmental biology in which the sheep is very similar to a preterm infant (figure 2). In contrast to rodents, ovine lung development (alveolarization) starts prenatally and is prolonged postnatally. Moreover, the size allows for usage of similar equipment and ventilation strategies as is currently used in clinical practice. Animals will be time-mated to yield animals of the appropriate age. Two antenatal ultrasound scans will ensure correct gestational age and adequate in utero growth.

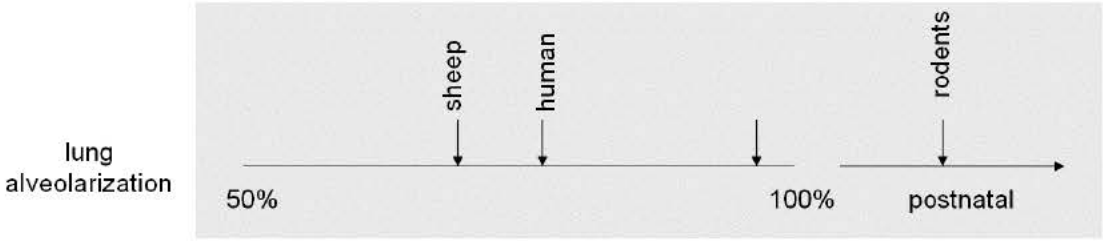


Figure 2 Time-points are shown when lung alveolarization, an important hallmark of lung maturation, occurs in different species. Lung maturation in sheep occurs prenatally, as in humans. Lung maturation in rodents takes place after birth.

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☒ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement: The use of preterm lambs has been suggested by the European Medical Agency (EMA)

to pharmaceutical companies. We have done studies with synthetic surfactant (Seehase et al., 2012) with were mandated by the EMA in order to show in vivo the safety and feasibility of the new drug. It is unacceptable to test directly in human babies based on exclusively in vitro data by the standards of EMA and by good clinical practice since preterm babies is the most vulnerable patient population. In addition neonatology is full of examples of premature clinical trials with caused harm in the study population (e.g. phospholipid treatment for RDS).

Reduction: The groups A and B of this appendix are identical to the corresponding groups of Appendix 1. Once the experiments of Appendix 1 have identified which surfactant is superior we intend to use the animals from Appendix 1 for the purposes of Appendix 2 in order to reduce the number of animals.
Further, due to large experience with sheep and maintenance of lambs on the lamb intensive care unit we are able to increase the number of animals that successfully reach the end of their experiments. Due to the expertise and experience of our team we have low numbers of drop-outs, which reduces the numbers of animals in the experimental groups. Our experiments are conducted by highly experienced personnel according to well-established protocols. These methods are in favor of reproducibility and use of previously obtained historical data and prevent unnecessary use and loss of animals. Moreover, we do not differentiate between genders since our clinical population consists of both sexes.
Furthermore, we are able to incorporate twin pregnancies in our study. This results in a reduction of pregnant ewes that will be used.
A variety of glucocorticoids have been tested in vitro and in vivo. In our current model we will use dosages defined on this pre-existing data.

Refinement: For this innovative technique of endotracheal administration of glucocorticoids to the preterm lung, the choice of animal model is based on the body size, use of similar equipment as in the neonatal intensive care and the developmental biology. In contrast to rodents, ovine and human the developmental biology are very similar (figure 2). For example, the alveolar phase in lambs and humans starts before birth in a hypoxic environment, compared to rodents in which the alveolarization starts postnatally in normoxic conditions. Therefore, we have established an animal model of immature lung-development in preterm lambs. Findings in our animal model can be easily translated into clinical practice. Our experiments will be conducted by a highly trained staff that can recognize and prevent discomfort. Moreover, we aim to leave the sheep as much in their natural environment (group housing in a meadow) as we can with minimal human contact.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Ewe

Before induction of intra-uterine inflammation and the C-section the sheep are kept in their natural environment as long as possible with access to food and water *ad libitum*. After induction of intra-uterine inflammation the sheep will be housed in groups with access to food and water ad libitum.
The sheep will be checked daily for discomfort due to intra-uterine inflammation. Discomfort due to intra-uterine inflammation is described in the human endpoint section. If necessary, analgesics are administered to treat pain.
The experiments will be performed by experienced animal technicians and researchers. This will speed up

the progress of the experiment and therefore reduce animal suffering and fear (fast induction of anesthesia, fast surgery, and maximum results with minimal animal handling).The UP exposure does not result in pain, distress or sepsis.

Fetus

During intrauterine inflammation fetuses will not experience distress as demonstrated by no changes of fetal cortisol levels during chorioamnionitis [7].

Chorioamnionitis is a multi-organ disease resulting in discomfort after birth, as they need to survive independently and start using their immature and inflammation affected organs. However, after C-section (birth) the fetus will stay on the Lamb Intensive Care Unit (LICU) and taken care of by highly trained personnel. During mechanical ventilation the fetus is constantly sedated (also receiving analgesia and parenteral feeding) with continuous monitoring and interpretation of vital parameters. Moreover, the environment in the LICU (temperature, light, sound) will be kept optimal in order to reduce discomfort.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

This experiment is new and is not a repetition. The previous in vitro work is used to reduced the number of animals by avoiding a dose finding study.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Ewe: anesthesia during operation, local analgesia of surgical wounds.

Lamb: whilst on placental circulation, the lamb is sedated through anesthesia of the ewe. Ex utero experiments are performed under sedation.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Ewe:

Abortion might occur due to chorioamnionitis, but is very uncommon in sheep [8]. This is a major reason to use this species.

Fetus:

The fetus will be sedated continuously after birth to minimise discomfort, also receiving analgesia. Environmental conditions (light, temperature, sounds) on the LICU are all in favour of the fetus.

Explain why these effects may emerge.

Due to their immaturity preterm fetuses are not always able to respond properly to environmental changes.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Ewe:

All invasive procedures are performed under aseptic conditions.

The ewe will not recover from anesthesia.

The majority of the animals have been exposed to Ureaplasma. Therefore, re-use is not possible. All ewes will be euthanized after the c-section.

Fetus:

Environmental conditions (light, temperature, sounds) on the LICU are all in favour of the fetus. Pain will be prevented by continuous anesthesia. Continuous monitoring of hemodynamic and respiratory enables us to treat or prevent the complications. Intravenous administration of glucose will prevent hypoglycemia (regularly checked)

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Humane endpoints for ewes:

- Pending preterm labor
- Abortion caused by intra-uterine fetal death
- Sepsis/ infection not responding to treatment
    - **Local (at site of injection):** redness, pain, and swelling with or without pus that cannot be

treated with local antiseptics or antibiotics are considered a human endpoint.

- **Systemic:** Fever, lethargy, excessive time lying down

- Untreatable pain: (a sheep that does not respond to analgesic treatment)
  - **Assessment of pain:**
    - Lack of appetite
    - Grinding of teeth
    - Reluctance to stand/ excessive time lying down
    - Lethargy/depression: an unresponsive sheep with hung head and dull eyes can indicate pain, illness or discomfort

Humane endpoints for lambs:

- Untreatable pneumothorax (absence of breath sounds)
- Uncorrectable severe respiratory acidosis(based on blood gas analysis)
- Sepsis (elevation of body temperature, elevation heart rate, blood gas analysis)
- Uncorrectable hypovolemia (blood pressure, heart rate, blood gas analysis)

Multi-organ failure (blood-pressure, heart rate, blood gas analysis)

Indicate the likely incidence.

Ewe: 5%

Fetus: 10%

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Ewe: moderate

Lamb:Non-recovery. During the mechanical ventilation, the lambs are continuously sedated and will not regain conscience, until the end of the experiment (euthanasia).

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

The fetus will be euthanized at the end of the experiment since examination of organ tissues is crucial to determine the effects of our treatment(s).

The ewes will be euthanized since we cannot reuse her for other purposes due to an intra-uterine infection an abdominal surgery.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes

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Van: Dec Secretariaat [redacted]
Verzonden: woensdag 4 januari 2017 9:52
Aan: 'Info-zbo'
Onderwerp: RE: aanvullende informatie aanvraag AVD107002016783

Categorieën: Dossier: [redacted]

Geachte commissie,
De DEC-UM dankt U voor Uw vragen en opmerkingen.
Hierbij de reactie van de DEC-UM:
Het PV bestaat uit twee strategieën, namelijk: 1) surfactant + glucocorticoiden --> geeft minder inactivatie van surfactant. 2) Glucocorticoiden postnataal intratracheaal vs systemisch ---> is een minder hoge dosis nodig? = hoopvolle behandeling tegen BPD.

Het stuk over wettelijk vereiste experimenten is volgens de DEC-UM correct, echter zijn de aangevraagde experimenten gericht op een nieuwe behandelstrategie (surfactant + intratracheale glucocorticoiden) en niet op het testen van (de veiligheid van) een potentieel nieuw middel. Zowel het (natuurlijke) surfactant als de corticosteroïden zijn al in gebruik in de kliniek. Het testen van de veiligheid van deze middelen is ook niet de focus van dit onderzoek. De focus is de mogelijke vermindering van negatieve effecten van (systemisch toegediende) corticosteroïden door deze in plaats van systemisch, direct in de longen toe te dienen. Wat het precieze effect daarvan is, is onbekend, en dus is translationeel onderzoek nodig, om te onderzoeken of het inderdaad een goede interventie zou zijn bij prematuren.

Wat betreft het ongerief: de lammetjes zijn eerst onder invloed van maternale anesthesie, en worden gedurende het hele verdere experiment onder sedatie met analgesie gehouden en worden nooit wakker. De DEC-UM is van mening dat dit een terminaal experiment, zoals de onderzoekers hebben aangegeven.

Met vriendelijke groet,

[redacted]
[redacted]
[redacted]**/DEC-UM**

[redacted]
[redacted]
www.maastrichtuniversity.nl/dec
Postbus 616, box 48, 6200 MD Maastricht
[redacted]

From: Info-zbo [mailto:info@zbo-ccd.nl]
Sent: vrijdag 23 december 2016 15:36
To: Dec Secretariaat [redacted]
Subject: aanvullende informatie aanvraag AVD107002016783
Geachte DEC–UM,
Enige tijd geleden heeft u advies uitgebracht op een projectaanvraag met titel "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis" met aanvraagnummer AVD107002016783.
De volgende vragen zijn aan de aanvrager voorgelegd:
1) In uw aanvraag motiveert u de noodzaak om dit onderzoek uit te voeren door te melden dat de EMA naar het testen van de synthetische surfactant in lammetjes heeft gevraagd. Indien zo, zou dit project niet onder 'wettelijk vereist' onderzoek moeten vallen? Is het mogelijk om een officiële verzoek van de EMA naar ons toe te sturen? Indien niet, dan verzoeken we u om de reden dit al eerder uitgevoerd onderzoek in schapen te herhalen nader te onderbouwen.
2) In de aanvraag geeft u aan dat het ongerief van de lammetjes in de categorie terminaal valt, omdat de dieren vanaf de keizersnede onder verdoving worden gehouden. Daarnaast schrijft u onder punt I dat de foetussen pijn zouden kunnen ervaren, aan hemodynamische instabiliteit, respiratorische complicaties of hypoglycaemie zouden kunnen leiden. Bedoelt u dat dit gebeurt alleen als de dieren niet onder verdoving zijn of kunnen de lammetjes ook onder verdoving pijn ervaren?
3) In uw NTS geeft u aan 120 ooien en 120 lammetjes voor dit onderzoek te willen gebruiken, maar in de bijlages dierproeven zijn deze aantallen niet duidelijk aangegeven. In bijlage 1 is alleen het aantal lammetjes benoemd. We verzoeken u het aantal benodigde dieren duidelijker in de bijlages dierproeven te melden.

We zouden graag de mening van de DEC willen horen over de categorie van dit project. Heeft de DEC een mogelijk wettelijk vereist karakter van dit project overwogen?
Het is niet duidelijk aangegeven in het advies of de DEC met de inschatting van het ongerief die in de aanvraag is ingevuld het eens is. Valt het ongerief van de lammetjes onder terminaal of onder een andere categorie?
We ontvangen graag uw reactie uiterlijk donderdag 5 januari 2017, om deze mee te kunnen nemen in de eerstvolgende CCD vergadering.
Alvast hartelijk dank.
Fijne feestdagen en Gelukkig Nieuwjaar!
Met vriendelijke groet,

[redacted]

Uitvoeringsexpert
Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**
.....
Postbus 20401 | 2500 EK | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl

Centrale Commissie Dierproeven

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> Retouradres Postbus 20401 2500 EK Den Haag

Universiteit Maastricht
t.a.v. [redacted]
Minderbroedersberg 4-6
Postbus 616
6200MD Maastricht

Centrale Commissie Dierproeven

Postbus 20401
2500 EK Den Haag
www.centralecommissiedierproeven.nl

T 0900-28 000 28 (10 ct /min)

info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD107002016783

Uw referentie

Bijlagen

Datum 10 januari 2017
Betreft Aanvulling Aanvraag projectvergunning dierproeven

Geachte [redacted]

Wij hebben een aanvraag voor een projectvergunning dierproeven van u in behandeling. Het gaat om het project 'Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis' met aanvraagnummer AVD107002016783.

De CCD heeft uw aanvraag besproken, maar om een besluit te kunnen nemen heeft zij nog aanvullende informatie nodig. In deze brief leest u wat de CCD nog nodig heeft en wanneer u een beslissing kunt verwachten.

Welke informatie nog nodig
Wij hebben de volgende informatie van u nodig om uw aanvraag verder te kunnen beoordelen:

1) In uw aanvraag beschrijft u onderzoek te willen doen naar de efficiëntie van een synthetische surfactant in vergelijking met een natuurlijke (klinisch geaccepteerde) surfactant. Daarnaast meldt u dat alleen de natuurlijke surfactant geaccepteerd is voor gebruik in patiënten.
Het blijkt toch dat in de Verenigde Staten al fase 2 klinische trials met de synthetische surfactant plaatsvinden. Dit betekent dat het gebruik van de synthetische surfactant in mens al toegestaan is, ongeacht de resultaten uit het in deze aanvraag beschreven project.
Kunt u nader onderbouwen wat de toegevoegde waarde van dit experiment is gezien de al lopende klinische trials? Waarom is het noodzakelijk om wel de dierproeven uit te voeren?

2) In bijlage dierproeven 1 (Intra-uterine inflammation) schrijft u onder punt A op pagina 1: '*The fetuses are delivered preterm by C-section, sedated and mechanically ventilated for 48 hours during which they are treated with different surfactant preparations. Subsequently, animals receive ventilator support via CPAP for 5 days.*' en op pagina 3: '*The lamb remains mechanically ventilated for 24 hours while anesthesia is maintained. Surfactant replacement therapy is repeated throughout the experiment if necessary. After 24 h the animals will be switched to continuous positive pressure ventilation for additional 6 days under*

sedation.' Het feit dat een keer het protocol wordt beschreven als 48h mecanische ventilatie + 5 dagen CPAP en een keer 24h + 6 dagen is verwarrend. Is er een verschil tussen de twee protocollen? Zo ja, graag dat uitleggen. Zo nee, graag uw aanvraag aanpassen met het correcte protocol.

Opsturen binnen veertien dagen
Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. U kunt dit aanleveren via NetFTP. Gebruik hierbij het formulier dat u bij deze brief krijgt indien u uw antwoord per post verstuurt.

Leges
De leges die u verschuldigd bent zijn nog niet door ons ontvangen of de betaling is nog niet verwerkt. Uw aanvraag is niet compleet als de leges niet zijn ontvangen.

Wanneer een beslissing
De behandeling van uw aanvraag wordt opgeschort tot het moment dat uw aanvraag compleet is. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn ontvangen. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project. De CCD zal uw aanvraag in de eerstkomende vergadering opnieuw bespreken.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Centrale Commissie Dierproeven

Melding

Bijlagen via de post

- U wilt één of meerdere bijlagen naar ons versturen? Voeg *altijd* deze Melding Bijlagen toe. Wij weten dan welke documenten van u zijn en hoeveel documenten u opstuurt.
- Meer informatie vindt u op www.centralecommissiedierproeven.nl
- Of bel met ons: 0900 28 000 28 (10 ct/min).

1 Uw gegevens

1.1 Vul de gegevens in.

Naam aanvrager			
Postcode		Huisnummer	

1.2 Bij welke aanvraag hoort de bijlage?
Het aanvraagnummer staat in de brief of de ontvangstbevestiging.

Aanvraagnummer	

2 Bijlagen

2.1 Welke bijlagen stuurt u mee?
Vul de naam of omschrijving van de bijlage in.

☐	
☐	
☐	

3 Ondertekening

3.1 Onderteken het formulier en stuur het met alle bijlagen op naar:

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Naam	
Datum	- - 20
Handtekening	

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[redacted]

Van: [redacted]
Verzonden: dinsdag 17 januari 2017 10:25
Aan: Info-zbo; [redacted])
Onderwerp: Re: aanvullende informatie aanvraag AVD107002016783
Bijlagen: Project DEC CCD 5-1-2017.docx; PV 2016-012 Appendix 1 - description animal procedure - Intra-uterine inflammation_revised_48h mechanical.docx

Dear [redacted]

Please find our additional information in the enclosure and the revised appendix.

Please do not hesitate to contact us for further information.

Best regards

[redacted]

[redacted]
Maastricht University Medical Center

From: Info-zbo
Sent: Tuesday, January 10, 2017 1:25 PM
To: [redacted]
Cc: [redacted]
Subject: aanvullende informatie aanvraag AVD107002016783

Geachte [redacted]
Wij hebben een aanvraag voor een projectvergunning dierproeven van u in behandeling. Het gaat om het project 'Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis' met aanvraagnummer AVD107002016783.
De CCD heeft uw aanvraag besproken, maar om een besluit te kunnen nemen heeft zij nog aanvullende informatie nodig. In de bijgaande brief leest u wat de CCD nog nodig heeft en wanneer u een beslissing kunt verwachten.
Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op.
De behandeling van uw aanvraag wordt opgeschort tot het moment dat uw aanvraag compleet is. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn ontvangen. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project. De CCD zal uw aanvraag in de eerstkomende vergadering opnieuw bespreken.
Met vriendelijke groet,

[redacted]
Uitvoeringsexpert
Centrale Commissie Dierproeven www.centralecommissiedierproeven.nl
.....
Postbus 20401 | 2500 EK | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl

Project "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis" met aanvraagnummer AVD107002016783

Reply to questions from 5.1.2017

1) In uw aanvraag beschrijft u onderzoek te willen doen naar de efficiëntie van een synthetische surfactant in vergelijking met een natuurlijke (klinisch geaccepteerde) surfactant. Daarnaast meldt u dat alleen de natuurlijke surfactant geaccepteerd is voor gebruik in patiënten.
Het blijkt toch dat in de Verenigde Staten al fase 2 klinische trials met de synthetische surfactant plaatsvinden. Dit betekent dat het gebruik van de synthetische surfactant in mens al toegestaan is, ongeacht de resultaten uit het in deze aanvraag beschreven project.
Kunt u nader onderbouwen wat de toegevoegde waarde van dit experiment is gezien de al lopende klinische trials? Waarom is het noodzakelijk om wel de dierproeven uit te voeren?

The only licensed surfactant is a natural surfactant. The synthetic surfactant is in study but not approved/licensed. We want to study the effects in chorioamnionitis which is known to inactivate the exogenous surfactant [J Pediatr. 2010 Jan;156(1):10-15.e1]. In the clinical trial that you mention, chorioamnionitis is not included. Chorioamnionitis is a clinical situation in humans which has a high clinical variability since it is not known when the infection of the chorio/amnion starts and which bacteria cause it. The animal model of sheep offers a standardisation since it known which bacteria causes the infection from which time point onwards. Therefore, the experiments are necessary to be performed in sheep since they cannot be performed in human perterm babies.

2) In bijlage dierproeven 1 (Intra-uterine inflammation) schrijft u onder punt A op pagina 1: '*The fetuses are delivered preterm by C-section, sedated and mechanically ventilated for 48 hours during which they are treated with different surfactant preparations. Subsequently, animals receive ventilator support via CPAP for 5 days.*' en op pagina 3: '*The lamb remains mechanically ventilated for 24 hours while anesthesia is maintained. Surfactant replacement therapy is repeated throughout the experiment if necessary. After 24 h the animals will be switched to continuous positive pressure ventilation for additional 6 days under sedation.*' Het feit dat een keer het protocol wordt beschreven als 48h mechanische ventilatie + 5 dagen CPAP en een keer 24h + 6 dagen is verwarrend. Is er een verschil tussen de twee protocollen? Zo ja, graag dat uitleggen. Zo nee, graag uw aanvraag aanpassen met het correcte protocol.

The description in the appendix is incorrect. It must read 48h of mechanical ventilation plus 5 days of CPAP throughout the experiments. We apologize for the mistake.

The document has been amended accordingly.

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10700	
1.2	Provide the name of the licenced establishment.	Maastricht University	
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number	Type of animal procedure
		1	Intra-uterine inflammation

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

We induce intra-uterine inflammation with intra-amniotic injections of Ureaplasma Parvum (UP), since this micro-organism is the most commonly found micro-organism in human chorioamnionitis cases. Previous studies already validated this approach and the same amount of colony forming units will be administered in the current study. The presence of chorioamnionitis can only be confirmed after birth which will be done by histological examination of the fetal membranes and placenta. The fetuses are delivered preterm by C-section, sedated and mechanically ventilated for 48 hours during which they are treated with different surfactant preparations. Subsequently, animals receive ventilator support via CPAP for 5 days. We have formulated the following 6 experimental groups:

1. Control ventilation N=12
2. UP + Control ventilation N=12
3. UP + natural surfactant (Curosurf, clinical standard, porcine origin) N=12
4. UP + CHF5633 (synthetic surfactant) N=12
5. UP + natural surfactant (Curosurf) + glucocorticoid (Budesonide 0,25 mg/kg) N=12
6. UP + CHF5633+ glucocorticoid (Budesonide 0,25 mg/kg) N=12

Groups 1, 3 and 4 are necessary to assess the effects in the pattern of clinical practice. Group 1 will help to identify the inflammatory phenotype influencing treatment effect, group 3 is current clinical standard and group 4 will help to identify if the artificial surfactant itself has an effect on inflammation due to its different protein composition.

We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism. Based on a power calculation, and taking into account a loss of 25% in total of the animals due to complications of intra-uterine inflammation (10%), premature birth (5%) and humane endpoints in lambs (10%) we calculated a total number of animals per experimental groups will which be 12.

Within these experimental groups the following primary and outcome parameters will be assessed:

Primary outcomes:

- Oxygenation (arterial oxygen partial pressure) in the course of ventilation: oxygenation is the main clinical parameter indicative for adequate ventilation)
- Repetitive dosing: depending on oxygenation: due to inactivation of surfactant, oxygenation might fail and additional boluses of surfactant are needed.
- Activity of surfactant recovered from the animals after sacrifice

The animals get colostrum and breast milk from sheep during the mechanical ventilation according to a feeding protocol from the neonatal intensive care unit with gastric tube feeding.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Time-mated Texel ewes will receive an intra-amniotic injection of Ureaplasma Parvum (UP):

Under sedation UP is administered by ultrasound-guided intra-amniotic injection. 2 days before preterm delivery, the ewes will be given an intramuscular injection of dexamethasone, which enhances fetal lung maturation and allows the fetus to be ventilated postnatally. Moreover, maternal intramuscular dexamethasone administration mimics the clinical standard nowadays.

Maternal administration should not be confused with postnatal steroid treatment in the fetus for BPD: Prenatal treatment of the mother is beneficial for the child because lung maturation is induced and mortality is decreased.

Postnatal treatment of the preterm infant has also been shown to positively influence the infants' lung, and BPD is decreased. However, corticosteroids given systemically to the infant have serious side effects on the neurodevelopmental outcome. Therefore, postnatal therapy can only be administered after balancing the positive effects on the lung and the negative effects on the brain, leading frequently to a therapeutic dilemma.

Administration of corticosteroids for longer periods of time induces abortion in sheep. However, a single

injection of dexamethasone, as administered in our proposed experiments, will not increase the risk of abortion (Fehrholz et al., Am. J. Physiology 2015) provided that the fetus will be born 48 hours after injection.
Previous experiments with intra-uterine Ureaplasma Parvum infection and treatment with dexamethasone demonstrated that dexamethasone 2 days prior to delivery did not have any effects on the immune response towards Ureaplasma Parvum

Seven days after the inoculation with UP, the fetus will be born preterm by **C-section** (general anesthesia): while remaining on placental circulation umbilical vessel catheters are placed for administration of medication (i.e. anesthesia) and blood gas analysis and an endotracheal tube is placed for ventilatory support (modified EXIT procedure). The lamb is immediately placed in our lamb intensive care unit which resembles the clinical intensive care very closely. Before connection to mechanical ventilation the lambs receive **surfactant replacement therapy** (according to their allocation). The lamb remains mechanically ventilated for 48 hours while anesthesia is maintained. Surfactant replacement therapy is repeated throughout the experiment if necessary. After 48 h the animals will be switched to continuous positive pressure ventilation for additional 5 days under sedation.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

We have a preference for singleton pregnancies since this is more clinical relevant and twins can strongly affect each other's health (i.e. resulting in small for gestational age) however, to reduce the number of pregnant ewes we will use both singleton and twin pregnancies. Furthermore, we do not know in advance whether it will be a single or twin pregnancy and only selecting twin pregnancies will result in massive over-breeding. Group numbers were determined with the power-calculation according to Sachs, in which variance and expected therapeutic effects are based on previous experiments with surfactant in non-infectious sheep models.

We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism

In ventilated preterm sheep (non-infectious model, Seehase et al., 2012) CHF5633 has shown a comparable effect on oxygenation but superior resistance against inactivation. Surfactant re-dosing in a 48-hour period of ventilation is the critical outcome in this experiment.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Lambs are the most appropriate animals to address our hypothesis, for a number of reasons:
1. Development of the lungs in sheep has been sufficiently studied to allow informed experimental design and integration of findings with those from previous studies. The current surfactant replacement therapy was developed in preterm lambs.

2. Preterm lambs are large enough to use similar equipment for ventilation as in the neonatal intensive care unit, provide sufficient blood and tissue to allow enough biomolecular analyses, for thorough assessment of the biological systems being investigated, in individuals.

The total number of animals needed for the current study will by 6 groups * 12 animals = 72 animals. We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism. Based on a power calculation, and taking into account a loss of 25% in total of the animals due to complications of intra-uterine inflammation (10%), premature birth (5%) and humane endpoints in lambs (10%) we calculated a total number of animals per experimental groups will which be 12.

We cannot predict the number of twin pregnancies, since multiple factors (e.g. seasonal vs. out of season breeding and the age of the ewe) are of influence. Therefore, the number of ewes is based on singleton pregnancies. In the case of twin pregnancy, the total number of ewes used for the experiment will be reduced.

The gestational age at which the lambs are delivered (129-132d gestational age) is based on the developmental biology in which the sheep is very similar to a preterm infant (figure 1). In contrast to rodents, ovine lung development (alveolarization) starts prenatally and is prolonged postnatally. Moreover, the size allows for usage of similar equipment and ventilation strategies as is currently used in clinical practice. Animals will be time-mated to yield animals of the appropriate age. Two antenatal ultrasound scans will ensure correct gestational age and adequate in utero growth.

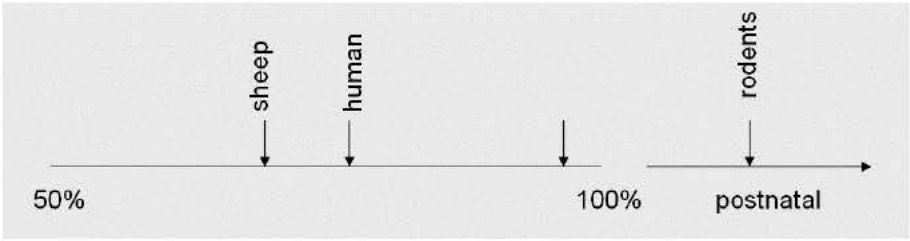

Figure 1 Time-points are shown when lung alveolarization, an important hallmark of lung maturation, occurs in different species. Lung maturation in sheep occurs prenatally, as in humans. Lung maturation in rodents takes place after birth.

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☐ No

☐ Yes> Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement: The use of preterm lambs has been suggested by the European Medical Agency (EMA) to pharmaceutical companies. We have done studies with synthetic surfactant (Seehase et al., 2012) with were mandated by the EMA in order to show in vivo the safety and feasibility of the new drug. It is unacceptable to test directly in human babies based on exclusively in vitro data by the standards of EMA and by good clinical practice since preterm babies is the most vulnerable patient population. In addition neonatology is full of examples of premature clinical trials with caused harm in the study population (e.g. phospholipid treatment for RDS).

Reduction: Due to large experience with sheep and maintenance of lambs on the lamb intensive care unit we are able to increase the number of animals that successfully reach the end of their experiments. Due to the expertise of our team, we have low numbers of drop-outs which decreased over the years with increasing experience, which reduces the numbers of animals in the experimental groups. Our experiments are conducted by highly experienced personnel according to well-established protocols. These methods are in favor of reproducibility and use of previously obtained historical data (also on CHF 5633 inactivation (2)) and prevent unnecessary use and loss of animals. Moreover, we do not differentiate between genders since our clinical population consists of both sexes.
Furthermore, we are able to incorporate twin pregnancies in our study. This results in a reduction of pregnant ewes that will be used. We rely on natural breeding. Therefore, we cannot control for singleton or twin pregnancies.

Refinement:
For this experiment in which different surfactant preparations are tested, the choice of animal model is based on the body size, use of similar equipment as in the neonatal intensive care and the developmental biology. In contrast to rodents, ovine and human the developmental biology are very similar. For example, the alveolar phase in lambs and humans starts before birth in a hypoxic environment, compared to rodents in which the alveolarization starts postnatally in normoxic conditions. Therefore, we have established an animal model of immature lung-development in preterm lambs. Findings in our animal model can be easily translated into clinical practice. Our staff and the staff of the animal care facility have been trained by a Texel sheep breeder to recognize symptoms of pending labor and give assistance to the sheep upon labor. Recognition of pending labor and assistance during labor reduces the risk of complications and improves animal well-being. Moreover, we aim to leave the sheep as much in their natural environment (group housing in a meadow) as we can with minimal human contact. Moreover, the fetus will receive analgesia and nutritional support (glucose) during mechanical ventilation.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects

on the environment.

Ewe

Before induction of intra-uterine inflammation the sheep are kept in their natural environment as long as possible with access to food and water *ad libitum*. After induction of intra-uterine inflammation the sheep are housed in groups with access to food and water *ad libitum*.

The sheep will be checked daily for discomfort due to intra-uterine inflammation. Discomfort due to intra-uterine inflammation is described in the humane endpoint section. Post-operative (after intra-amniotic injection) antibiotics, if necessary, will be administered, in order to prevent progression of (wound) infection.

The experiments will be performed by experienced animal technicians and researchers. This will speed up the progress of the experiment and therefore reduce animal suffering and fear (fast induction of anesthesia, fast surgery, and maximum results with minimal animal handling).

The majority of the animals have been exposed to Ureaplasma. Therefore, re-use is not possible. All ewes will be euthanized after the c-section.

Fetus

Previous experiments have demonstrated that fetal cortisol levels do not change in the course of chorioamnionitis (Jobe et al, Am J Respir Crit Care Med. 2000 Nov;162(5):1656-61). This suggests that the fetus will experience limited discomfort during intra-uterine inflammation in our experiments.

Chorioamnionitis is a multi-organ disease resulting in discomfort after birth, as they need to survive independently and start using their immature and inflammation affected organs. However, after C-section (birth) the fetus will stay on the Lamb Intensive Care Unit (LICU) and taken care of by highly trained personnel. During mechanical ventilation the fetus is constantly sedated (also receiving analgesia and parenteral feeding) with continuous monitoring and interpretation of vital parameters. Moreover, the environment in the LICU (temperature, light, sound) will be kept optimal in order to reduce discomfort.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

This experiment is new and is not a repetition. The previous in vitro work is used to reduced the number of animals by avoiding a dose finding study.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Ewe: All surgical procedures are performed under anesthesia (including analgetic block).

Lamb: whilst on placental circulation, the lamb is sedated through anesthesia of the ewe. Ex utero experiments are performed under sedation guided by depth of sedation.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals' welfare may be expected?

Ewe:

Abortion might occur due to chorioamnionitis, but is very uncommon in sheep (8) (in retrospect over the past 10 years: 5 in 100). This is a major reason to use this species.

Fetus:

The fetus might experience pain, suffer from hemodynamic instability or respiratory complications (pneumothorax, hypoxia) or hypoglycemia.

Explain why these effects may emerge.

Due to their immaturity preterm fetuses are not always able to respond properly to environmental changes. The fetus might experience pain, suffer from hemodynamic instability or respiratory complications (pneumothorax, hypoxia).

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Ewe:

All invasive procedures are performed under aseptic conditions.

The ewe will will not recover from anesthesia.

Fetus:

The fetus will be sedated continuously after birth to minimise discomfort, also receiving analgesia. Environmental conditions (light, temperature, sounds) on the LICU are all in favour of the fetus.

Continuous monitoring of hemodynamic and respiratory enables us to treat or prevent the complications.

Intravenous administration of glucose will prevent hypoglycemia (regularly checked)

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Humane endpoints for ewes:

- Pending preterm labor
- Intra-uterine fetal death
- Sepsis/ infection not responding to treament
  - **Local (at the site of intra-amniotic injection):** redness, pain, and swelling with or without pus that cannot be treated with local antiseptics or antibiotics are considered a human endpoint.
  - **Systemic:** Fever, lethargy, excessive time lying down
- Untreatable pain: (a sheep that does not respond to analgesic treatment)

Humane endpoints for fetus:

- Untreatable pneumothorax
- Uncorrectable severe respiratory acidosis
- Sepsis Uncorrectable hypovolemia
- Multi-organ failure

Indicate the likely incidence.

Ewe: 5%

Fetus: 10%

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

Ewe: Moderate

Lamb:Non-recovery. During the mechanical ventilation, the lambs are continuously anesthetized (sedation and analgesia), and will not regain conscience, until the end of the experiment (euthanasia). Therefore, we consider the classification of the experiment to be non-recovery.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

The ewe will be euthanized since we cannot reuse her for other purposes due to an intra-uterine infection an abdominal surgery.

The lamb will be euthanized since vital organs (i.e. lungs) have to be sampled for biochemical analysis.
Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?
☐ No > Describe the method of killing that will be used and provide justifications for this choice.
☒ Yes

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Universiteit Maastricht
[redacted]
Postbus 616
6200 MD MAASTRICHT

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD107002016783
Bijlagen
1

Datum 1 februari 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 15 december 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis" met aanvraagnummer AVD107002016783. Wij hebben uw aanvraag beoordeeld.

Op 2 en 17 januari 2017 heeft u uw aanvraag aangevuld. U heeft de vragen van de CCD met betrekking tot de toegevoegde waarde van uw project, over de ongeriefclassificatie en over het aantal dieren.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning.
Met het oog op artikel 10a, lid 1, zijn er algemene voorwaarden gesteld.
De voorwaarde over afstemming met de IvD wordt gesteld om te voorkomen dat dieren onnodig worden gebruikt indien de eerste experimenten niet de verwachte resultaten opleveren.

U kunt met uw project "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis" starten. De vergunning wordt afgegeven van 1 februari 2017 tot en met 1 januari 2022. De looptijd van de vergunning wijkt af omdat de startdatum in de aanvraag in het verleden ligt.

Overige wettelijke bepalingen blijven van kracht.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie DEC-UM gevoegd. Dit advies is opgesteld op 15 december 2016. Bij de

beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet.
Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Dit advies van de commissie nemen wij over, inclusief de daaraan ten grondslag liggende motivering. Er worden aanvullende voorwaarde(n) gesteld.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

Bezwaar
Als u het niet eens bent met deze beslissing, kunt u binnen zes weken na verzending van deze brief schriftelijk een bezwaarschrift indienen.
Een bezwaarschrift kunt u sturen naar Centrale Commissie Dierproeven, afdeling Juridische Zaken, postbus 20401, 2500 EK Den Haag.

Bij het indienen van een bezwaarschrift vragen we u in ieder geval de datum van de beslissing waartegen u bezwaar maakt en het aanvraagnummer te vermelden. U vindt deze nummers in de rechter kantlijn in deze brief.

Bezwaar schorst niet de werking van het besluit waar u het niet mee eens bent. Dat betekent dat dat besluit wel in werking treedt en geldig is. U kunt tijdens deze procedure een voorlopige voorziening vragen bij de Voorzieningenrechter van de rechtbank in de woonplaats van de aanvrager. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisend belang.

Voor de behandeling van een voorlopige voorziening is griffierecht verschuldigd. Op http://www.rechtspraak.nl/Organisatie/Rechtbanken/Pages/default.aspx kunt u zien onder welke rechtbank de vestigingsplaats van de aanvrager valt.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
1 februari 2017
Aanvraagnummer:
AVD107002016783

Centrale Commissie Dierproeven
namens deze:

[redacted]

Algemeen Secretaris

Bijlagen:
- Vergunning
  Hiervan deel uitmakend:
  - DEC-advies
  - Weergave wet- en regelgeving

Centrale Commissie Dierproeven

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam:	Universiteit Maastricht
Adres:	Postbus 616
Postcode en plaats:	6200 MD MAASTRICHT
Deelnemersnummer:	10700

deze projectvergunning voor het tijdvak 1 februari 2017 tot en met 1 januari 2022, voor het project "Therapeutic interventions to improve neonatal pulmonary outcomes in the course of chorioamnionitis" met aanvraagnummer AVD107002016783, volgens advies van Dierexperimentencommissie DEC-UM. Er worden aanvullende voorwaarde(n) gesteld.
De functie van de verantwoordelijk onderzoeker is Professor experimental perinatology.
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 15 december 2016
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 15 december 2016;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 15 december 2016;
c Advies van dierexperimentencommissie d.d. 15 december 2016, ontvangen op 15 december 2016.
d De aanvullingen op uw aanvraag, ontvangen op 2 en 17 januari 2017

Aanvraagnummer:
AVD107002016783

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1 Intra-uterine inflammation				72 ooien en 72 lammetjes (foetussen).
	Schapen (Ovis aries) /	144	50% Terminaal 50% Matig	
3.4.4.2 Ventilation-induced lung injury				48 ooien; 48 lammetjes (foetussen)
	Schapen (Ovis aries) /	96	50% Terminaal 50% Matig	

Voorwaarden

Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen

In artikel 10, lid 1 sub a van de wet, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is. Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van deze vergunning. Indien bovenstaande zich voordoet dient aanvrager dit in afstemming met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarde wijzigen of intrekken.

De vergunning wordt verleend onder de voorwaarde dat go/no go momenten en het voortzetten van de tweede dierproef worden afgestemd met de IvD.

Aanvraagnummer:
AVD107002016783

Weergave wet- en regelgeving

Dit project en wijzigingen
Volgens artikel 10c van de Wet op de Dierproeven (hierna de wet) is het verboden om andere dierproeven uit te voeren dan waar de vergunning voor is verleend. De dierproeven mogen slechts worden verricht in het kader van een project, volgens artikel 10g. Uit artikel 10b volgt dat de dierproeven zijn ingedeeld in de categorieën terminaal, licht, matig of ernstig. Als er wijzigingen in een dierproef plaatsvinden, moeten deze gemeld worden aan de Centrale Commissie Dierproeven. Hebben de wijzigingen negatieve gevolgen voor het dierenwelzijn, dan moet volgens artikel 10a5 de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven.
Artikel 10b schrijft voor dat het verboden is een dierproef te verrichten die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, tenzij hiervoor door de Minister een ontheffing is verleend.

Verzorging
De fokker, leverancier en gebruiker moeten volgens artikel 13f van de wet over voldoende personeel beschikken en ervoor zorgen dat de dieren behoorlijk worden verzorgd, behandeld en gehuisvest. Er moeten ook personen zijn die toezicht houden op het welzijn en de verzorging van de dieren in de inrichting, personeel dat met de dieren omgaat moet toegang hebben tot informatie over de in de inrichting gehuisveste soorten en personeel moet voldoende geschoold en bekwaam zijn. Ook moeten er personen zijn die een eind kunnen maken aan onnodige pijn, lijden, angst of blijvende schade die tijdens een dierproef bij een dier wordt veroorzaakt. Daarnaast zijn er personen die zorgen dat een project volgens deze vergunning wordt uitgevoerd en als dat niet mogelijk is zorgen dat er passende maatregelen worden getroffen.
In artikel 9 staat dat de persoon die het project en de dierproef opzet deskundig en bekwaam moet zijn. In artikel 8 van het Dierproevenbesluit 2014 staat dat personen die dierproeven verrichten, de dieren verzorgen of de dieren doden, hiervoor een opleiding moeten hebben afgerond.
Voordat een dierproef die onderdeel uitmaakt van dit project start, moet volgens artikel 10a3 van de wet de uitvoering afgestemd worden met de instantie voor dierenwelzijn.

Pijnbestrijding en verdoving
In artikel 13 van de wet staat dat een dierproef onder algehele of plaatselijke verdoving wordt uitgevoerd tenzij dat niet mogelijk is, dan wel bij het verrichten van een dierproef worden pijnstillers toegediend of andere goede methoden gebruikt die de pijn, het lijden, de angst of de blijvende schade bij het dier tot een minimum beperken. Een dierproef die bij het dier gepaard gaat met zwaar letsel dat hevige pijn kan veroorzaken, wordt niet zonder verdoving uitgevoerd. Hierbij wordt afgewogen of het toedienen van verdoving voor het dier traumatischer is dan de dierproef zelf en het toedienen van verdoving onverenigbaar is met het doel van de dierproef. Bij een dier wordt geen stof toegediend waardoor het dier niet meer of slechts in verminderde mate in staat is pijn te tonen, wanneer het dier niet tegelijkertijd voldoende verdoving of pijnstilling krijgt toegediend, tenzij wetenschappelijk gemotiveerd. Dieren die pijn

kunnen lijden als de verdoving eenmaal is uitgewerkt, moeten preventief en postoperatief behandeld worden met pijnstillers of andere geschikte pijnbestrijdingsmethoden, mits die verenigbaar zijn met het doel van de dierproef. Zodra het doel van de dierproef is bereikt, moeten passende maatregelen worden genomen om het lijden van het dier tot een minimum te beperken.

Einde van een dierproef
Artikel 13a van de wet bepaalt dat een dierproef is afgelopen wanneer voor die dierproef geen verdere waarnemingen hoeven te worden verricht of, voor wat betreft nieuwe genetisch gemodificeerde dierenlijnen, wanneer bij de nakomelingen niet evenveel of meer, pijn, lijden, angst, of blijvende schade wordt waargenomen of verwacht dan bij het inbrengen van een naald. Er wordt dan door een dierenarts of een andere ter zake deskundige beslist of het dier in leven zal worden gehouden. Een dier wordt gedood als aannemelijk is dat het een matige of ernstige vorm van pijn, lijden, angst of blijven schade zal blijven ondervinden. Als een dier in leven wordt gehouden, krijgt het de verzorging en huisvesting die past bij zijn gezondheidstoestand.
Volgens artikel 13b moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.
Uit artikel 13d volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13c is vastgesteld dat proefdieren geadopteerd kunnen worden, teruggeplaatst in hun habitat of in een geschikt dierhouderijsysteem, als de gezondheidstoestand van het dier het toelaat, er geen gevaar is voor volksgezondheid, diergezondheid of milieu en er passende maatregelen zijn genomen om het welzijn van het dier te waarborgen.
De Minister heeft vrijstelling ontheffing verleend volgens artikel 13c, die de afwijkende methode van doden op basis van wetenschappelijke motivering ten minste even humaan acht als de in de richtlijn opgenomen passende methoden.