	Inventaris Wob-verzoek W16-04s								
		wordt verstrekt				**weigeringsgronden**			
nr.	**document**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
	NTS 20151225								
1	Aanvraagformulier				x		x	x	
2	Projectvoorstel			x					
3	Niet technische samenvatting oud			x					
4	Bijlage dierproeven 1				x			x	
5	Bijlage dierproeven 2			x					
6	Bijlage dierproeven 3			x					
7	Ontvangstbevestiging				x		x	x	
8	DEC-advies				x		x	x	
9	Brief aan CCD 14-9-2015				x		x	x	
10	E-mails 21-10-2015				x		x	x	
11	Niet-technische samenvatting nieuw	x							
12	Advies aan CCD		x						x
13	Beschikking en vergunning				x		x	x	

Centrale Commissie Dierproeven

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

☐ Nee > *U kunt geen aanvraag doen*

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

Naam instelling of organisatie	Maastricht University	
Naam van de 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	

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.

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	Onderzoeker	
Afdeling	Kindergeneeskunde	
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 - 12 - 2015
Einddatum	01 - 12 - 2020

3.2 Wat is de titel van het project?

Therapeutic interventions to improve neonatal outcomes in the course of perinatal stress

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	Secretariaat.dec@maastrichtuniversity.nl

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 741,00 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

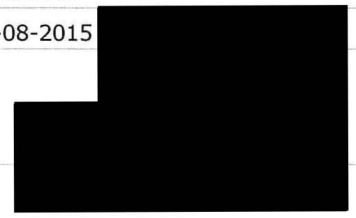
6 Ondertekening

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] – Universiteit Maastricht
Plaats	Maastricht
Datum	21-08-2015
Handtekening	

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'.	
1.2	Provide the name of the licenced establishment.	Maastricht University
1.3	Provide the title of the project.	Therapeutic interventions to improve neonatal outcomes in the course of perinatal stress

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.

Chorioamnionitis

Chorioamnionitis is characterized by microbial invasion of the amniotic cavity. The microorganisms responsible for this invasion, comprising *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Gardnerella vaginalis*, 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) 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).
Since (persisting antenatal) inflammation is clinically a major contributor to surfactant inactivation and RDS, we aim to assess inactivation of synthetic and natural surfactant preparations in a clinical relevant animal model of RDS in which preterm ovine fetuses are exposed to antenatal inflammation (chorioamnionitis) and treated postnatally with surfactant replacement therapy.

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 (e.g. 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, 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 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.
PDE4 inhibitors are very promising as a new treatment option for BPD. At present, PDE4 inhibitors are under development which and appear to be effective in treating different lung diseases like COPD and asthma (19). Because of their anti-inflammatory properties and their protective effect on remodelling processes, PDE4 inhibitors are promising as a potential new therapeutic option for the treatment of

preterm infants with BPD. Studies in small animal models demonstrated a protective effect of PDE4 inhibitors on BPD development through anti-inflammatory action and reversal of aberrant remodeling processes combined with prolongation of survival (20-23). However, a major challenge in a treatment using PDE4 inhibitors 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 inhaled PDE4 inhibitors, of which the compound GSK256066 yielded the most promising results (24). So far, GSK256066 has been investigated in clinical studies relating to asthma (25) and COPD (26). It was well tolerated and produced no systemic adverse effects (in adults) (24, 26). 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 PDE4 treatment.

Hypoxic-ischemia

Fetal hypoxia-ischemia (HI) is a severe condition and defined as a period of insufficient blood gas exchange leading to progressive hypoxia, hypercapnia, metabolic and/or respiratory acidosis and eventually ischemia resulting from a disturbed fetal-maternal circulation(1).

HI affects different organ systems. However, due to its high metabolic rate and energy need, the brain is one of the most vulnerable organs with limited regenerative capacity and HI can cause severe hypoxic-ischemic encephalopathy (HIE). Despite the high prevalence of neurological sequelae, therapeutic options to improve the neurodevelopmental outcome in **preterm** infants after HIE are unavailable. In mild cases of HIE in **term** infants whole body cooling therapy has been shown to improve neurodevelopmental outcomes. Cooling therapy is an independent risk factor for adverse neurological outcomes in **preterm** infants and therefore is not standard clinical care for this vulnerable patient group, indicating the demand for new therapeutic options for this vulnerable patient group.

In the last decade, stem cell therapy has emerged as a putative treatment for neonatal ischemic injury (27). Bone marrow-derived stromal cells (MSC) have great therapeutic potential in the field of neonatal regenerative medicine due to their immune modulatory and regenerative capacities (28). The immune modulatory effects of MSCs consist of modulation of both innate and adaptive immunity in favor of anti-inflammatory properties (28, 29). Therefore, MSCs have been studied as therapeutic intervention in many neonatal diseases in which inflammation or pathogenic immune responses play a key role in pathophysiology including bronchopulmonary dysplasia and brain injury (27). Although the exact mechanisms remain largely unknown, general consensus is that MSC-mediated immunomodulation is predominantly caused by the **paracrine effect** mediated by soluble factors secreted by MSCs (28).This concept is the motivation to move towards a new treatment of preterm babies with cell preparations or cell free excretions of highly specified stem-cell populations. Our concept will identify mechanisms and proof efficacy which will form the basis for the subsequent choice of stem cells that are then tested against cell-free preparations of secreted vesicles from bone marrow derived stromal cells. The effectiveness of such a preparation is the current limit of knowledge which we intend to test. The treatment with secreted vesicles rather than livable stem cells is a simplification of treatment which would make the move into clinical trials for the benefit of patients much faster and easier since concerns

of immunogenic compatibility and cell fate need not to be addressed.

Previously we have demonstrated that bone marrow-derived stromal cells are effective for the treatment of HI-induced injury of the preterm brain (30, 31). Based on the results of, amongst others, this study we postulate that the protective effects of cell-based therapies are **spleen-mediated**; splenic immune cells migrate towards the brain after global HI. Stem cells alter the composition and (re)activity of splenic immune cells (both innate and adaptive) and prevent their migration towards the brain (30-32).

In order to improve our current therapeutic approach, we aim to further explore the splenic response towards the brain after HI and/or cell-based therapies.

Besides brain injury, fetuses which suffered from hypoxia-ischemia are at high risk to develop adverse clinical outcomes of the fetal intestine such as feeding intolerance, altered intestinal motility (33) and necrotizing enterocolitis (NEC) (34), the most severe, life threatening gastrointestinal pathology in preterm neonates. Intestinal hypoxia-ischemia leads to impaired barrier epithelial integrity and delayed gastrointestinal transit (unpublished data). This is a relative new concept of so called gut-brain axis which describes the link between brain innervation of the gut and function of the gut. In order to evaluate gut function, a sugar-based permeability test will be performed. A solution composed of small (evaluate intracellular passage) and a large (evaluate paracellular passage) sugar probes will be infused into the stomach of the fetuses by gastric tube and the concertation of the large/small sugars will be measured non-invasively *in vivo*, in plasma to assess changes in gastrointestinal permeability between the groups. The motility of gastrointestinal tract will be assessed by evaluating the distribution of rhodamine-B-labeled in the bowel of the fetuses. Rhodamine will be infused into the stomach of fetuses by gastric tube and differences in the rhodamine-containing gut content between the groups will be quantified at sacrifice.

Administration of sugars for gut motility and absorption purposes does not interfere with our proposed cell-based therapies, but will be a read-out for the effects of our therapies with respect to the gut-brain axis. The addition of this read-out will prevent the use of additional animals for gut motility and absorption purposes only and reduces the overall number of animals.

Lungs

BPD has been addressed in preclinical trials to test the efficacy of stem cell therapy in different animal models. Recent experimental trials demonstrated that systemic administration of MSCs had direct positive effects on the degree of inflammation. Moreover, tissue remodeling could be demonstrated after administration of MSCs. Furthermore, markers of surfactant homeostasis were found to be increased after administration of MSCs. Moreover, protection from further progression of lung remodeling could be demonstrated (27).

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 perinatal management by developing novel

therapeutic strategies to improve neurological and pulmonary outcomes.

AIM 1: Identifying a surfactant replacement therapy (CHF 5633 vs. Surfaxin vs. Curosurf vs. Control) resistant to inactivation by inflammation/infection.

AIM 2: To assess the effects of pharmacological PDE-4 inhibition (GSK256066) of developmental pathways on BPD development. vs control

AIM 3: Improve structural and functional brain injury with cell-based therapy

AIM 3a: To assess the temporal dynamics of the cerebral and peripheral immune response in the pathophysiology of hypoxic-ischemic brain injury.

AIM 3b: To assess the role of the spleen in the pathophysiology of hypoxic-ischemic brain injury.

AIM 3c: To test intravenous administration of cell-based therapies from different sources: bone marrow-derived stromal cells, cell-free preparations of bone marrow-derived stromal cells, preconditioned stem cell preparations for their effectiveness in reducing hypoxic-ischemic brain injury.

AIM 3d: Define the optimal dosing strategy for the most effective cell-based therapy.

AIM 3e: Assess long-term effects of cell-based therapies on neurodevelopmental outcomes.

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. In the Netherlands. 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.

Epidemiology:
Preterm birth: 13649 (7,7% or 77 per 1000 live and dead born children)

Of the 13649 preterm infants, 1148 infants died due to preterm birth alone or complications of preterm birth (e.g. asphyxia, respiratory insufficiency)
Extreme preterm: 2637 (20% of preterms) of whom 1/3 dies; 1/3 survives with handicap and 1/3/ survives without morbidity.

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

Gestational age (weeks)	**Live and dead born**	
	number	**percentage**
22-24	**630**	**0,4**
25-31	**2.007**	**1,1**
32-36	**11.012**	**6,2**

≥ 37	**162.625**	**91,5**
unknown	1.439	0,8
Total	**177.713**	**100,0**

Brain:
Cognitive, socialization, attentional and/or behavioral disorders: 25-50% of preterm infants
Spastic motor deficits (e.g. cerebral palsy): 5-10% of preterm infants

Lung:
Bronchopulmonary dysplasia: 20% of preterm infants

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:

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.

1. Increasing resistance of surfactant to inactivation will result in maintenance of tissue oxygenation and, therefore, prevent possible hypoxic insults that are detrimental to the developing brain.

Phosphodiesterase 4 (PDE4) inhibition

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. PDE4 inhibition results in reduction of inflammation and stimulation of airway remodelling processes.

1. **PDE4 prevents BPD** and its long term consequences
2. Inflammation is a key process resulting in adverse neurodevelopmental outcomes. **PDE4 reduces inflammation** and, therefore, potentially prevent brain injury.
3. Prevention of BPD results in **improved tissue oxygenation**, thus meeting the high oxygen demand of the brain and prevents secondary brain injury.

Cell-based therapies

Cell-based therapies (1) are immune modulating and (2) stimulate regenerative processes. Key process in development of preterm lung and brain injury is inflammation.

1. Cell-based therapies will **reduce inflammation**, thereby preventing its detrimental effects on fetal development
2. Injury to the lungs and brain often is already present before start of therapy. Since cell-based therapies also have **regenerative properties**, this initial damage might be restored.

Cell-based therapies, therefore, prevent continuation of detrimental processes and restore existing injury.

Moreover, transplantation of living cells (bone marrow-derived stromal cells and their derivatives) has proven safe with respect to malignant transformation and is currently being tested in human (adult) clinical studies for other diseases (e.g. graft versus host disease, stroke, inflammatory bowel disease).

Furthermore, the use of stem cells derivatives (e.g. extracellular vesicles) evades this risk since these biological active vesicles are non-self-replicating. However, back-to-back comparisons with living cells remain crucial.

3.4 Research strategy

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

In the current project we have formulated three 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, mechanical ventilation, and global hypoxia-ischemia (HI).

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 (figure1).

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.

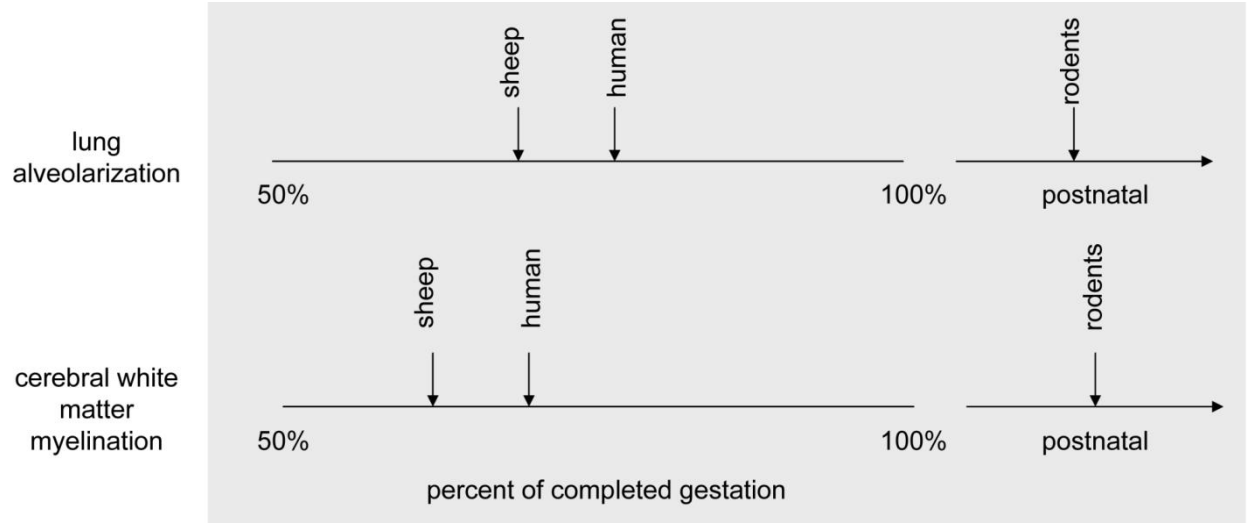


Figure 1 Lung and brain development during gestation in humans, sheep and rodents.

We will focus on the interaction between (**AIM 1**) intra-uterine inflammation (chorioamnionitis) and surfactant replacement therapy during mechanical ventilation, (**AIM 2**) chorioamnionitis, ventilator-induced lung injury and a novel therapeutic agent, and (**AIM 3 a-e**) global hypoxia-ischemia and cell-based therapies.

The objective of this project is to develop novel treatment strategies to reduce the pulmonary and neurodevelopmental sequelae of the most common underlying pathologies in preterm birth; asphyxia and chorioamnionitis. For this purpose, we generated the following three main research questions in this project:

Intra-uterine inflammation:

1. What surfactant preparation is 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 by inflammatory mediators has not been tested in a clinical relevant model of chorioamnionitis-induced preterm birth.

Ventilation-induced lung injury:

2. Is inhibition of pulmonary developmental pathways by a novel phosphodiesterase inhibitor effective in reducing bronchpulmonary dysplasia caused by chorioamnionitis and mechanical ventilation?

Global hypoxia-ischemia:

Is cell-based therapy effective in reducing injury of the preterm brain caused by asphyxia?

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 outcome of this study is oxygenation. Secondary outcomes of this study are lung compliance, lung injury, and survival.

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 a phosphodiesterase inhibitor.

Pulmonary development are comparable between both sheep and humans in terms that alveolarization

starts in late gestation in both species, allowing for preterm birth and subsequent mechanical ventilation. This model was validated in previous experiments in which feasibility of mechanical ventilation with respect to gestational age was tested.
Primary outcome of this study is survival. Secondary outcomes are lung inflammation, lung compliance, and lung injury.

Global hypoxia-ischemia
Preterm ovine fetuses are chronically instrumented and subsequently subjected to *in utero* hypoxia-ischemia and intravenous administration of cell-based therapeutics at predetermined time-points. The gestational age of the fetus at occlusion is determined previously and correlated to the gestational age at which human fetuses are most vulnerable to brain injury caused by pre- and perinatal adverse events (figure 1) (36).
The primary outcome of this study is structural and functional brain injury. Secondary outcomes are brain inflammation, immune modulation, and function and injury of lungs and gastro-intestinal tract.

We have chosen Texel sheep for the following reasons:

1. In the current proposal, all lambs are born preterm through surgical delivery (Caesarean section), thereby, avoiding the risk of spontaneous labor. Therefore, parturition is not the focus of the experiments.
2. 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.

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 program 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, infection (i.e. chorioamnionitis), hypoxia-ischemia (i.e. asphyxia), and iatrogenic stressors (i.e. mechanical ventilation). 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 inflammation; chorioamnionitis, hypoxia-ischemia, and mechanical ventilation all induce a detrimental inflammatory response in the brain and lungs. Therefore, we select therapeutics with high anti-inflammatory potential.
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 synthetic surfactant does not prove to be superior over natural surfactants, we will not pursue further experimentation with this surfactant.
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 PDE4 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.

In the model of HI we seek to improve structural and functional brain injury with cell-based therapy (**AIM 3**). Previous studies of our group have indicated that cerebral and peripheral inflammatory responses play a crucial role in the etiology of hypoxic-ischemic brain injury. However the temporal dynamics of these responses require further elucidation (**AIM 3a**). AIM 3a will be a milestone in the understanding of the dynamics of HI brain injury, and will provide more insight into timepoints at which cell-based therapies should be administered.

The spleen is considered a key immunological organ contributing to brain injury by proving immune effector cells that invade the brain. We therefore postulate that cell-based interventions should modulate this splenic inflammatory response. Splenectomy will enable us to study the contribution of the splenic inflammatory response in the etiology of hypoxic-ischemic brain injury, which would be a milestone in understanding the pathophysiology (**AIM 3b**) Moreover, we will compare the effects of splenectomy to administration of Multipotent Adult Progenitor Cells (MAPC), as subset of mesenchymal stromal cells that have previously been shown to modulate neuroinflammation through interactions with splenic immune cells in adults. AIM3b will be a milestone a milestone and a go or no go. If the splenic involvement cannot be demonstrated in our study, we will pursue other targets (need to be determined on the basis of the experiment), but we will not continue targeting the spleen with cell-based therapies.

We will test clinical-grade stem cells or biological preparations derived from these cells (e.g. microvesicles and preconditioned cells) for their effectiveness in reducing in the preterm brain after asphyxia (**AIM 3c**). AIM 3c will be a milestone in terms of determining the superior cell-based therapy based on the outcome parameters determined in AIM 3B. However, if cell-based therapies a not effective we will put future experiments on hold.

Improving functional and structural outcome of the preterm brain after asphyxia is a huge milestone in neonatal medicine creating a chance to improve the neurodevelopmental outcome of many preterms. Subsequently optimal dosing strategy (**AIM 3d**) and long-term treatment effects (**AIM 3e**) will be determined.

Go or no go's and milestones

AIM 1:

The end of the experiment for aim 1 will serve as a milestone (proof of principle) and as a go or no go: if synthetic surfactant does not prove to be superior over natural surfactants, we will not pursue further experimentation with this surfactant.

AIM 2:

The end of the experiment for aim 2 will serve as a milestone (proof of principle) and as a go or no go: proof of principle that PDE4 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.

AIM 3:

AIM 3a will be a milestone in the understanding of the dynamics of HI brain injury, and will provide more insight into time points at which cell-based therapies should be administered.

AIM 3b will be a milestone and a go or no go. If the splenic involvement cannot be demonstrated in our study, we will pursue other targets (need to be determined on the basis of the experiment), but we will not continue targeting the spleen with cell-based therapies.

AIM 3c will be a milestone in terms of determining the superior cell-based therapy based on the outcome parameters determined in AIM 3B. However, if cell-based therapies a not effective we will put future experiments on hold.

AIM 3d will be a milestone in determining the optimal dosing strategy that will be tested for long term effects in **AIM 3e.**

If our cell-based therapy has long-term effectiveness (**AIM 3e**), this will be a major milestone for future experiments and clinical trials.

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	Global hypoxia-ischemia
4	
5	
6	
7	
8	
9	
10	

Proposal

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Appendix 1

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Appendix 2

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Format
Niet-technische samenvatting

- Dit format gebruikt u om uw niet-technische samenvatting te schrijven
- Meer informatie over de niet-technische samenvatting vindt u op de website www.centralecommissiedierproeven.nl.
- Of neem telefonisch contact op. (0900-2800028).

1 Algemene gegevens

1.1	Titel van het project	Therapeutische maatregelen om de uitkomst van vroeggeboren (premature) kinderen te verbeteren.
1.2	Looptijd van het project	5 jaar
1.3	Trefwoorden (maximaal 5)	Vroeggeboorte, hersenschade, longschade, therapie

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

- ☒ Fundamenteel onderzoek
- ☒ Translationeel of toegepast onderzoek
- ☐ Wettelijk vereist onderzoek of routinematige productie
- ☐ Onderzoek ter bescherming van het milieu in het belang van de gezondheid
- ☐ Onderzoek gericht op het behoud van de diersoort
- ☐ Hoger onderwijs of opleiding
- ☐ Forensisch onderzoek
- ☐ Instandhouding van kolonies van genetisch gemodificeerde dieren, niet gebruikt in andere dierproeven

3 Projectbeschrijving

3.1 Beschrijf de doelstellingen van het project (bv de wetenschappelijke vraagstelling of het wetenschappelijk en/of maatschappelijke belang)

Gezondheid op volwassen leeftijd start bij de ontwikkeling van de baby in de baarmoeder. Het is dus begrijpelijk dat als de ontwikkeling wordt onderbroken door vroeggeboorte, dit een grote invloed kan hebben op de gezondheid op latere leeftijd. Vroeggeboorte kan niet altijd voorkomen worden en soms is het zelfs beter voor de baby om geboren te worden omdat de situatie in de baarmoeder de gezondheid van de baby ernstig in gevaar brengt. Deze baby's hebben speciale zorg nodig die de ontwikkeling van deze

		zeer kwetsbare groep patiënten ondersteunt. Dankzij deze goede zorg is de overlevingskans van deze kinderen sterk gegroeid, maar nog altijd gaat vroeggeboorte vaak gepaard met complicaties (op de lange termijn), zoals longproblemen en hersenschade. Om deze schade te beperken zullen nieuwe therapieën en aanpassingen van reeds bestaande therapieën getest moeten worden alvorens deze in de kliniek kunnen worden toegepast. Het doel van dit project is derhalve om nieuwe therapieën te ontwikkelen om de gevolgen van hersen- en longschade bij vroeggeboorte te verminderen.
3.2	Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?	<u>Wetenschappelijk belang:</u> Dit onderzoek levert inzichten op over nieuwe therapeutische strategieën ter behandeling van schade aan organen na vroeggeboorte. <u>Maatschappelijk belang:</u> Het verminderen van hersen- en longschade rondom vroeggeboorte is van groot maatschappelijk belang, omdat hier mee een grote last op patiënten, de ouders van patiënten en de maatschappij wordt verminderd.
3.3	Welke diersoorten en geschatte aantallen zullen worden gebruikt?	Volwassen en foetale schapen Aantallen: 516 volwassen en 516 foetale schapen
3.4	Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?	De schapen zullen een medische ingreep aan de uterus ondergaan die kan leiden tot wondpijn. Ook zal een deel van de ooien gedurende het experiment fysiek afgezonderd staan in een individueel hok. Hierdoor wordt de bewegingsvrijheid beperkt en is sociaal gedrag met soortgenoten niet mogelijk. Een deel van de foetussen zal een chirurgische ingreep met inhechten van electrodes en catheters ondergaan, hetgeen het welzijn van de foetus kan beïnvloeden. Echter, tijdens de zwangerschap is het bewustzijnsniveau van de foetus gering, waardoor eventueel ongerief niet als zodanig door de foetus wordt ervaren. Ook worden de foetussen na vroeggeboorte kunstmatig in leven gehouden. Dit kan leiden tot pijn en benauwdheid. Door adequate pijnstilling en kunstmatige slaap worden deze negatieve gevolgen voor het welzijn beperkt.
3.5	Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?	Ooi: 20% matig (zonder chirugische ingrep); 80% ernstig (met chirurgische ingrep) Foetus: 80% ernstig; 20% non-recovery (end point van proof al bereijkt met

		verzamelen van monsters)
3.6	Wat is de bestemming van de dieren na afloop?	Zowel de ooi als de foetus zullen na afloop van het experiment worden gedood waarna de effecten van de nieuwe therapieën op de long- en hersenschade uitgebreid geanalyseerd zullen worden.

4 Drie V's

4.1	**Vervanging** Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden.	Onderzoek door middel van analyse van verzamelde gegevens en weefsels van patiënten, aangevuld met data uit reeds uitgevoerde dierexperimenten en laboratoriumexperimenten hebben geleid tot een selectie van therapieën die geschikt zijn voor verantwoorde preklinische studies die de situatie in de mens zoveel mogelijk benaderen. Het ontstaan van complicaties veroorzaakt door vroeggeboorte is namelijk een complexe samenhang van verschillende orgaansystemen (o.a. centrale zenuwstelsel en het immuunsysteem) waarvoor geen proefdiervrije alternatieven beschikbaar zijn.
4.2	**Vermindering** Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.	Goede statistische onderbouwde studies die gebaseerd zijn op voorgaande experimenten en een gefaseerde uitvoering waarin de experimenten logisch op elkaar aansluiten, gekoppeld aan jarenlange ervaring van een gespecialiseerd onderzoeksteam, staan garant voor een wetenschappelijk verantwoorde studie met een minimum aan schapen en een minimum aan ongerief.
4.3	**Verfijning** Verklaar de keuze voor de diersoort(en). Verklaar waarom de gekozen diermodel(len) de meest verfijnde zijn, gelet op de doelstellingen van het project.	De ontwikkeling van een schaap in de baarmoeder is zeer vergelijkbaar met de ontwikkeling van de mens. Bovendien maakt de lange draagtijd en de grootte van de ooi en de foetus het mogelijk om complexe processen in een compleet organisme tijdens en na de zwangerschap te bestuderen.
	Vermeld welke algemene maatregelen genomen worden om de negatieve (schadelijke) gevolgen voor het welzijn van de proefdieren zo beperkt mogelijk te houden.	Alle dieren (ooi en foetus) krijgen adequate verdoving, pijnstilling en antibiotica om ongerief te voorkomen/beperken(?). Bovendien zullen de dieren zo lang mogelijk in hun natuurlijke omgeving gehouden worden om eventuele stress en angst te verminderen. Alle schapen zullen dagelijks worden gecontroleerd op welzijn.

5 In te vullen door de CCD

Publicatie datum	
Beoordeling achteraf	
Andere opmerkingen	

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'.	
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 24 hours during which they are treated with different surfactant preparations. We have formulated the following 4 experimental groups:

1. UP + Control
2. UP + Curosurf (clinical standard, porcine origin)
3. UP + CHF 5633 (novel synthetic surfactant)
4. UP + Surfaxin (approved synthetic surfactant)

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.

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.

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. Postnatal treatment has serious side effects on the neurodevelopmental outcome. Therefore, we are looking at two different treatments: one PRENATALLY to the pregnant woman which is beneficial, one POSTNATALLY to the preterm baby which has side effects.

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 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.

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 can 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 24-hour period of ventilation is the critical outcome in this experiment.

Based on the *in vivo* data the expected treatment effect is 30%, with a spread of 40% (Seehase et al., 2012).
With a power of (π) 80% and alpha 0.05, significant differences in number of surfactant re-dosing of 30% (δ=30) with a SD of 40% (σ=40) can be detected with a sample size of **9** ($n = 15.7 * (30/40)^2 = 8.83$).

Loss of animals (reaching human endpoints, intra-uterine inflammation) for inclusion in the experiments and experimentation are considered to be 25%. Therefore we will add 3 animals, making the total number of animals per experimental group **12** (a - 025a = 9; .075a = 9; a = 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.

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 surfactant replacement therapies and a control group. If there are twin pregnancies less pregnant ewes will be needed. The gestational age at which the lambs are delivered (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.

We have chosen Texel sheep for the following reasons:

1. In the current proposal, all lambs are born preterm through surgical delivery (Caesarean section), thereby, avoiding the risk of spontaneous labor. Therefore, parturition is not the focus of the experiments.
2. 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.

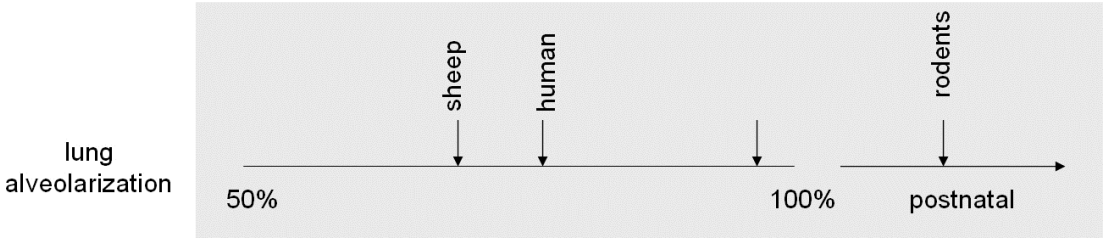

Figure 1 Time-points are shown when lung alveolarization, an important hallmark of lung maturation, occurs in different species. Lung maturation in sheep occurs prenatally, as in humans. Lung maturation in rodents takes place after birth.

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: 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.

Moreover, 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 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 (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: 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. 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).

Fetus

Previous experiments have demonstrated that fetal cortisol levels do not change in the course of chorioamnionitis (3). 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.

The use of **synthetic surfactant** has been tested, and proven superior compared to biological surfactant preparations, in non-infectious models of prematurity (ovine models) in which surfactant inactivation was established by administration of exogenous compounds. However, clinically, surfactant is inactivated by due to the **endogenous** production of inflammatory mediators that arise from chorioamnionitis and/or a subsequent infection. Previously, we have used lipopolysaccharide as 'inflammatory trigger'. Despite its proinflammatory potential, it does not cause **chronic inflammation** which persists **postnatally** and can influence ventilation and oxygenation. Moreover, UP is most commonly associated with chorioamnionitis.

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: induction of anesthesia through thiopental i.v., continuation with isofluorane 1-2% and remifentanil i.v. Local analgesia of surgical wounds with lidocaine.

Lamb: whilst on placental circulation, the lamb is sedated through anesthesia of the ewe. Ex utero experiments are performed with ketamine and midazolam i.v.

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. 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 be euthanized immediately after delivery of the fetus to minimise post-operative pain and complications.

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:** Elevation of the body-temperature, elevation of the heart-rate, 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'.	
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, sedated and mechanically ventilated for a maximum of 8 days since we previously showed that a minimum of 8 days of mechanical ventilation is necessary to develop (histological) BPD [1]. During mechanical ventilation the lambs are treated with a PDE4 inhibitor. The following primary and secondary outcome parameters will assess feasibility, safety and efficacy of a topical administered PDE4 inhibitor:

Primary outcome parameters:

1. Survival. Both intrauterine inflammation and preterm birth are major risk factors for neonatal death due to complications that arise from underdeveloped organ systems. Treatment might improve survival compared to controls.

Secondary outcome parameters:

2. Lung inflammation, lung compliance, and lung injury

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.

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.

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 which is repeated if necessary (based on oxygenation). The lambs remain under ventilatory support for 8 days while anesthesia is maintained. During these days, repetitive doses of iPDE4 or sham treatment will be administered endotracheally.

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 6-8 animals for evaluations of structural changes in elastin deposition in the fetal lung (Kuypers et al., 2012; Collins et al., 2013).

Based on these data, with a power (n) of 80% and an alpha of 0.05, significant different structural changes between the control and experimental group of 20% (δ=20) with a Standard deviation of 15% (σ=15) can be detected with a sample size of **9** ($n = 15.7 * (15/20)^2 = 8.83$ (L. Sachs))

We take into account a loss of 25% of the animals due to complications of intra-uterine inflammation, premature birth and human endpoints. Therefore the total number of animals per experimental groups will be **12** (a - 025a = 9; .075a = 9; a = **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.

This experiment will be performed with a maximum of **48** pregnant sheep (Texel breed) and their respective singleton fetuses (> 2/3 gestation), that are randomly allocated to their respective treatment group (figure 1).

The gestational age at which the lambs 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.

We have chosen Texel sheep for the following reasons:

1. In the current proposal, all lambs are born preterm through surgical delivery (Caesarean section), thereby, avoiding the risk of spontaneous labor. Therefore, parturition is not the focus of the experiments.
2. 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.

Figure 1 [redacted]

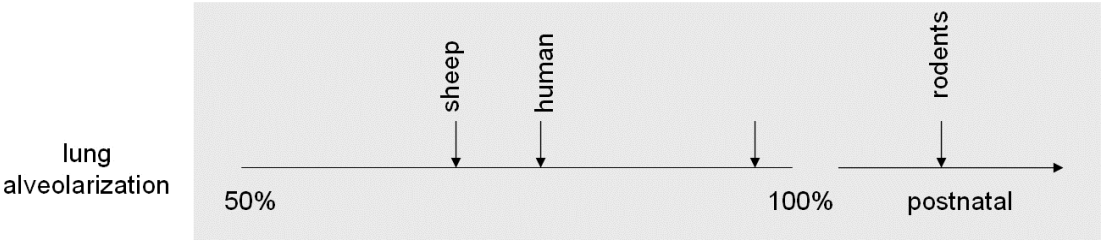

Figure 2 Time-points are shown when lung alveolarization, an important hallmark of lung maturation, occurs in different species. Lung maturation in sheep occurs prenatally, as in humans. Lung maturation in rodents takes place after birth.

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: For this innovative technique of PDE4 inhibitor administration, 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.

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 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 inhalable PDE4 inhibitors have been tested in vitro and in vivo (adult models of asthma and COPD), from which GSK256066 yielded the most promising results [2-6]. Therefore, we will only test

GSK256066 in our current model at dosages defined on this pre-existing data.

Refinement: 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.
Based on previous experiments [1], we have defined that a minimum of 8 days of postnatal ventilation is necessary to result in a phenotype similar to BPD. Moreover, the fetus will receive nutitional support (glucose) and analgesia will be given during mechanical ventilation (anesthesia)

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).

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.

Induction of intra-uterine inflammation in pregnant sheep with Ureaplasma Parvum is an existing model. However, this model has not been used thus far for pre-clinical testing of topical administration of iPDE4. Moreover, for the first time a combination of different factors including antenatal and postnatal hits (intra-uterine inflammation, mechanical ventilation; hyperoxia) is used to induce lung injury. These conditions are all new and therefore do not entail repetition of experiments. State of the art therapy

standards are also included in the study design which better reflects the human clinical situation and makes translation easier.

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: induction of anesthesia through thiopental i.v., continuation with isofluorane 1-2% and remifentanil i.v. Local analgesia of surgical wounds with lidocaine.

Lamb: whilst on placental circulation, the lamb is sedated through anesthesia of the ewe. Ex utero experiments are performed with ketamine and midazolam i.v.

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 be euthanized immediately after delivery of the fetus to minimise post-operative pain and complications.

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:** Elevation of the body-temperature, elevation of the heart-rate, 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.

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').

10% based on previous experiments. Chorioamnionitis and/or preterm birth are major risk factors for fetal death, regardless of treatment. Therefore, sham-treated animals might die during the experiments

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

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'.	
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: 3 Type of animal procedure: Global hypoxia-ischemia

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.

Ovine fetuses will be chronically instrumented. After a four day recovery period the fetuses are subjected to 25 minutes of (sham) umbilical cord occlusion. After occlusion the fetus will receive an intravenous bolus of cell-based therapy. During the entire experiment electrophysiological and hemodynamic recordings are made continuously.

We have formulated the following experiments and experimental designs:

Table 1 Experimental groups

Aim	Nr. groups	sham/HI	Experimental condition	Cell-based therapy	N	Total N
3a	6	sham	1 day	-	N=12	N=60
			3 days	-	N=12	
			7 days	-	N=6	
		HI	1 day	-	N=12	
			3 days	-	N=12	
			7 days	-	N=6	
3b	8	sham	SPLX	saline	N=12	N=96
				MAPC	N=12	
			Sham SPLX	saline	N=12	

				MAPC	N=12	
		HI	SPLX	saline	N=12	
				MAPC	N=12	
			Sham SPLX	saline	N=12	
				MAPC	N=12	
3c	12	sham	-	saline	N=6	N=108
			-	MSC	N=6	
			-	MSC-EV	N=12	
			-	MAPC	N=6	
			-	MAPC-EV	N=12	
			-	preconditioned	N=12	
		HI	-	saline	N=6	
			-	MSC	N=6	
			-	MSC-EV	N=12	
			-	MAPC	N=6	
			-	MAPC-EV	N=12	
			-	preconditioned	N=12	
3d	10	sham	saline		N=6	N=108
			Superior therapy	Dose 1, timing 1	N=12	
			Superior therapy	Dose 1, timing 2	N=12	
			Superior therapy	Dose 2, timing 1	N=12	
			Superior therapy	Dose 2, timing 2	N=12	
			saline		N=6	
		HI	Superior therapy	Dose 1, timing 1	N=12	
			Superior therapy	Dose 1, timing 2	N=12	
			Superior therapy	Dose 2, timing 1	N=12	
			Superior therapy	Dose 2, timing 2	N=12	
3e	4	sham	saline		N=12	N=48
			Superior therapy		N=12	
		HI	saline		N=12	
			Superior therapy		N=12	
SPLX = splenectomy						**N=420**

Primary outcomes:

1. Structural brain injury (determined post-mortem with MRI and immunohistochemistry)
2. Brain function (analysis of electro graphical data)

Secondary outcomes:

1. Brain inflammation
2. Immune activation
3. Function and injury of lungs and gastro-intestinal tract

Ovine fetuses are the most appropriate animals to address our hypotheses for a number of reasons:

1. Ovine neurodevelopment is comparable to human neurodevelopment in terms of white matter myelination; myelination starts prenatal in humans and sheep compared to rodent in which myelination starts postnatally.
2. Preterm lambs (106 days gestational age , ~30 weeks human neurodevelopment) are large enough to perform chronic instrumentations which allow the investigator to perform continuous

measurements in vital parameters throughout gestation.
The long gestational period (~147 days) allows for close investigation of specific developmental processes.

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

Fetuses of time-mated Texel ewes (102 days gestational age) will be **chronically instrumented** under strict sterile conditions: under general anesthesia the fetus will be exposed through a median laparotomy. While remaining on placental circulation an arterial catheter for blood pressure measurements and blood sampling, a venous catheter for administration of therapeutics and ECG electrodes for cardiac monitoring are placed. EEG electrodes are paced on the dura to monitor brain activity and seizures. An inflatable vascular occluder is placed around the umbilical cord to induce transient global hypoxia-ischemia. The fetal spleen is removed in 10-20% (AIM3b) of the animals through a subcostal incision. A gastric tube is placed (oral) for studies of gastro-intestinal absorption and motility. The fetus is placed back in utero and upon closure of the uterus a catheter is placed in the amniotic cavity for pressure recordings. All catheters and leads are exteriorized trough a trocar hole in the flank of the ewe.
After a 4 day recovery period the fetuses are subjected to **25 minutes of umbilical cord occlusion** (106 days gestational age) by rapid inflation of the vascular occluder. After occlusion a **reperfusion period** will follow of maximal 30 days while the fetus will remains in utero during which **cell-based therapy** is administered intravenously (maximal 2 repeated dosages). Moreover, arterial blood samples are taken (maximal 1 sample per 48 hours). 24 hours before the end of the experiment rhodamine-labelled dextran is given through the gastric tube for post-mortem evaluations of gastro-intestinal absorption and motility. At the end of the experiment, the ewe and the fetus will be euthanized (intravenous pentobarbital, simultaneously), followed by C-section and tissue sampling for *ex vivo* analysis.

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

In previous experiments on hypoxia-ischemia and cell-based therapies in ovine fetuses we have successfully used group-numbers of 6-8 animals (Jellema et al., 2013a, 2013b, 2013c).
Based on these data, with a power (n) of 80% and an alpha of 0.05, significant different structural changes and functional changes between the control and experimental groups of 30% (δ=30) with a Standard deviation of 40% (σ=40) can be detected with a sample size of **9** ($n = 15.7 * (30/40)^2 = 8.83$ (L. Sachs))
We take into account a loss of 25% of the animals due to complications of fetal instrumentation and umbilical cord occlusion. Therefore the total number of animals per experimental groups will be **12** (a - 025a = 9; .075a = 9; a = **12**).

Since some groups will be repeated throughout all aims, we will, if supplementing with historical controls is possible, not repeat these experiments, but limit our animal number to 6. We have to repeat control experiments to be able to correct for differences between breeding seasons (personal communication: differences in outcome parameters were detected between different breeding seasons).

B. The animals

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

This experiment will be performed with a maximum of **420** pregnant sheep and maximally **420** of their respective singleton fetuses (> 2/3 gestation). We have planned 5 individual experiments (AIMS 3a-e) which will following in logical order and which implementation is dependent on the results of previous experiments ("milestones"). Group sizes have been calculated with the formula of Sachs in which structural brain injury and brain function (obtained from previous experiments) were primary outcome parameters.

The sheep used in this study are bred by a highly experienced farmer who also breeds for other sheep experiments. 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.

We have chosen Texel sheep for the following reasons:

1. In the current proposal, all lambs are born preterm through surgical delivery (Caesarean section), thereby, avoiding the risk of spontaneous labor. Therefore, parturition is not the focus of the experiments.
2. 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.

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: These animal experiments cannot be performed otherwise since:

1. We aim to mimic the clinic a much as possible on order to make an easy translation towards clinical application.
2. The pathophysiology of global hypoxia-ischemia involves complex interplay between different organ systems connected to each other by the vascular and nervous system. This interplay cannot be mimicked outside the body.

3. We give a systemic therapy which will engage multiple facets of the pathophysiology of global hypoxia-ischemia.

We intend to perform these experiments in sheep since:

1. Ovine prenatal organ development (lung, brain and gut) closely mimics human organ development compared to rodents.
2. The long gestational period of sheep (~147 days) allows for more detailed and better timed studies of developmental processes during gestation compared to rodents.
3. The relative large size of sheep allows for chronic instrumentation and (electro) physiological monitoring of vital clinical relevant functions during gestation.

Reduction:

We strive to minimize the number of animals used in the proposed study as follows:

1. We will complement control groups with historical controls in order to prevent repetition of experiments.
2. Based on the go's and no go's and milestones we will reduce the number of cell-based therapies, and therefore animal numbers, in experiments 4 and 5.
3. The addition of gut-function as an additional functional read-out for our cell-based interventions will prevent the use of additional animals for gut motility and absorption purposes only and reduces the overall number of animals, without increasing discomfort.

Refinement: It is vital that the sheep are housed solitary in a confined space since the catheters and leads that exit the ewe's flank are connected to monitoring equipment and infusion pumps. Solitary housing will prevent that other animals can gnaw through the vascular catheters (personal experience) and cause early termination of the experiment (human endpoint). However, the sheep will be within auditory, visual and olfactory reach of other sheep. Moreover, behavioral observation and cortisol measurements have determined that this type of housing is well tolerated by the sheep and does not induce significant stress.

The stress for sheep has been assessed in different manners like hormone (cortisol), heart rate, breathing rate, blood pressure, behavior and food intake - among which the cortisol level has been relatively reliable as an indicator of stress. Other indicators of stress depend on the time of the day, environment and facilities. Keeping the sheep in groups rather than alone is the most important prevention of stress in this species which is consistently done at the facilities in Maastricht. Stress is in addition a confounding factor in our experiments which we want to avoid at all costs. Given our experience with the models and the expertise in the facility in Maastricht, we are convinced that the stress level is kept to a minimum.

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

Ewe

Before instrumentation-surgery the sheep are kept in their natural environment as long as possible with access to food and water *ad libitum*. During the experiment the sheep will be kept within visual, auditory

and olfactory range of other sheep and will have a normal day-night cycle.
The sheep will be checked daily for discomfort due to surgery and hypoxia-ischemia. Discomfort is described in the humane endpoint section. Post-operative antibiotics and analgesia will be administered in order to prevent (wound) infection and pain. All wounds are assesses and, if necessary, treated with antiseptic wound spray daily.
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).

Fetus

All sedation that is administered to the ewe is able to cross the placental barrier. Therefore, the fetus is also sedated during surgery. Upon closure of the uterus, a bolus of antibiotics is administered into the amniotic cavity in order to prevent *in utero* infection.

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.

For this project repetition of previously performed experiments is necessary for the following reasons: Previous studies of our group indicated that cell-based therapy may be a promising candidate to treat injury in the preterm brain. In the proposed experiments we will study the pathophysiology of this injury in further detail by adding new techniques (e.g. splenectomy) which have not been performed before. Furthermore we will test different cell-based therapies which have more potent anti-inflammatory and regenerative properties in vitro then the stem cells previously tested.

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.

The sheep are housed in a confined space with limited room for movement in order to prevent fetal loss due to injury to the externalized fetal leads and catheters, which are connected to monitoring equipment and infusion pumps. However, the sheep will be housed near other sheep in olfactory, visual and auditory distance. This practice has been done successfully and has been evaluated for maternal stress hormones which appear to be low.

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.

Sheep will be operated under full-anaesthesia. Post-operatively they will receive analgesia.

I. Other aspects compromising the welfare of the animals

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

1. The ewe can experience post-operative wound infection
2. After surgery the sheep a placed in confined sollitary housing
3. The sheep can go into preterm labor

Explain why these effects may emerge.

Although the sheep are operated on aseptically and have a permanent wound on the abdominal wall throught which catherters and leads are exteriorized the chance for post-operative infection is small (personal experience). However, the sheep deficate in their own pen which always poses a risk for infection.

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

1. Sheep receive prophylactic antibiotics which are continued post-opertively for 5 days and longer if necessary. Moreover, the wounds are inspected and treated daily with chlorotetraspray. Moreover, the pen is cleaned daily.
2. Although the sheep are placed in solitary confined housing they are within auditory, visual and olfactory distance of other sheep.
3. Preterm labor cannot be prevented but is (based on experience) a very rare event. Moreover, preterm labor is a human endpoint.

The fetus displays an acute physiological reaction illustrative of discomfort during and after asphyxia. However, due to a continuous "dormant" status of the fetal brain during gestation, the fetus is not aware of discomfort and will not experience it as such (1).

Opioids will affect receptors that are activated by different stimuli such as pain. In the disease situation of hypoxia-ischemia an abrupt shortage of oxygen and blood supply occurs. This occurs in a very immature fetus which has not yet developed the neuronal links of responding to such a shortage of blood and oxygen which is shown by the responses of the cardiovascular system. The neuronal system lacks the perception and response mechanisms due to immaturity.

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:

1. Untreatable pain:
   - o 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.

The sheep receive post-operative pain medication. If a sheep does not respond to the maximum amount of pain medication within 24 hours, we consider the pain to be untreatable and define this as a human end-point.

2. Infection: The animals will be monitored via analyses of blood (pH, PaO_2, $PaCO_2$, base excess, lactate, glucose, Hb, Ht, HCO_3^-, O_2 saturation) and vital parameters (temperature, ECG, blood pressure). However, instant analysis of blood for cellular components is not possible.
   Therefore, we need to rely on clinical parameters described below:
   - o Local (site of surgical wound): redness, pain, and swelling with or without pus that cannot be treated with local antiseptics or antibiotics are considered a human endpoint.
   - o Systemic: Elevation of the body-temperature, elevation of the heart-rate, lethargy, excessive time lying down.
3. Intra-uterine fetal death: Fetal death can be determined by evaluation of the fetal ECG, blood-pressure, and blood gas analysis
4. Pending labor: A sheep showing behaviour of pending labor will be taken out of the experiment
5. Abdominal hernia: Abdominal hernia will be assessed by palpation of the abdominal wound. When an abdominal hernia is determined, the sheep will be euthanized.

Humane endpoints for fetus:

Persistent bradycardia of less than 30 beats/min and a lactic acidosis with a pH<6.8 over a period of 6h not responding to fluid treatment

Indicate the likely incidence.

Based on previous experiments we have determined the incidence of reaching a human endpoint at 20%, which is mostly determined by *in utero* fetal death.

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: Severe

The ewe will undergo abdominal surgery with permanent exteriorization of cables and leads while being housed solitary in a confined space.

Lamb: Severe
The fetus will undergo chronic instrumentation and induction of hypoxia-ischemia but will not constantly receive sedation and analgesia.
The fetus displays an acute physiological reaction illustrative of discomfort during and after asphyxia. However, due to a continuous "dormant" status of the fetal brain during gestation, the fetus is not aware of discomfort and will not experience it as such (1). However, we still consider this as a serious discomfort since it is acute abrupt and results in changes of brain activity, heart rate, and blood pressure, which are associated discomfort.

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 (especially the brain) is crucial to determine the effects of our treatment(s).
The ewe will be euthanized since the discomfort during the experiment is severe, making re-use not possible. Moreover, undoing the effects of chronic instrumentation will cause and additional increase in discomfort.

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

7.

> Retouradres Postbus 20401 2500 EK Den Haag

Maastricht University
[redacted]
Postbus 616
6200 MD MAASTRICHT

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

Onze referentie
Aanvraagnummer
AVD107002015225

Bijlagen
2

Datum 24-08-2015
Betreft Ontvangstbevestiging Aanvraag projectvergunning Dierproeven

Geachte heer/mevrouw [redacted]
Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 21 augustus 2015.
Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD107002015225. 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. Zodra uw aanvraag compleet is, ontvangt u binnen veertig werkdagen een beslissing op uw aanvraag. 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 wordt uw aanvraag buiten behandeling 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.zbo-ccd.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.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Gegevens aanvrager

Uw gegevens	
Deelnemersnummer NVWA:	10700
Naam instelling of organisatie:	Maastricht University
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	50169181
Straat en huisnummer:	Mindersbroedersberg 4-6
Postbus:	616
Postcode en plaats:	6200 MD MAASTRICHT
IBAN:	NL04INGB0679510168
Tenaamstelling van het rekeningnummer:	Universiteit Maastricht

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

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	Onderzoeker
Afdeling:	Kindergeneeskunde
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 december 2015
Geplande einddatum:	1 december 2020
Titel project:	Therapeutische interventions to improve neonatal outcomes in the course of perinatal stress
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:	Secretariaat.dec@maastrichtuniversity.nl

Betaalgegevens

De leges bedragen:	€ 741,-
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

Ondertekening

Naam:	[redacted]
Functie:	[redacted] - Universiteit Maastricht
Plaats:	Maastricht
Datum:	21 augustus 2015

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD107002015225

Bijlagen
2

Datum 24-08-2015
Betreft Ontvangstbevestiging Aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 24 augustus 2015
Vervaldatum: 23 september 2015
Factuurnummer: 201570225

Omschrijving	**Bedrag**
Betaling leges projectvegrunning dierproeven Betreft aanvraag AVD107002015225	€ 741,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL28RBOS 056.99.96.066 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 20401, 2500 EK te 's Gravenhage.

8.

DEC-advies projectvoorstel 2015-005

Maak bij de toepassing van dit format gebruik van de bijbehorende toelichting, waarin elke stap in het beoordelingsproces wordt toegelicht

A. Algemene gegevens over de procedure

1. Aanvraagnummer: 2015-005
2. Titel van het project: *Therapeutic interventions to improve neonatal outcomes in the course of perinatal stress*.
3. Titel van de NTS: *Therapeutische maatregelen om de uitkomst van premature kinderen te verbeteren*.
4. Type aanvraag:

   X nieuwe aanvraag projectvergunning

   ☐ wijziging van vergunning met nummer
5. Contactgegevens DEC:
   - naam DEC: DEC-UM
   - telefoonnummer contactpersoon: [redacted]
   - mailadres contactpersoon: [redacted] of secretariaat.dec@maastrichtuniversity.nl
6. Adviestraject (data dd-mm-jjjj):

   X ontvangen door DEC: 10-07-2015

   ☐ aanvraag compleet

   X in vergadering besproken: 17-07-2015

   ☐ anderszins behandeld

   X termijnonderbreking(en) van22-07-2015 tot 12-08-2015

   Van 12-08-2015 tot 14-08-2015

   ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen

   ☐ aanpassing aanvraag

   ☐ advies aan CCD
7. Eventueel horen van aanvrager
   - Datum
   - Plaats

- Aantal aanwezige DEC-leden
- Aanwezige (namens) aanvrager
- Strekking van de vraag / vragen
- Strekking van het (de) antwoord(en)
- Het horen van de aanvrager heeft geleid tot aanpassing van de aanvraag

8. Correspondentie met de aanvrager
   - Datum dd. 22-07-2015/14-08-2015/17-08-2015

Strekking van de vragen:

Proposal:

1. 3.4.2. Waarom wordt het Texelse schapenras, bekend staand als ongemakkelijk lammerend, gekozen? De keuze van een gemakkelijk lammerend schapenras zou als verfijning ten aanzien van ongerief kunnen worden beschouwd. De DEC-UM wenst een motivatie waarom voor dit ras gekozen is.
2. 3.4.2. Is het valide om aan te nemen dat het deelonderzoek betreffende stamcellen translationeel relevant is gezien de mogelijkheid tot het ontwikkelen van maligniteit van deze stamcellen?
3. Ter verduidelijking van de grootte van het probleem, prevalentie en incidentie weergeven? Hoeveel van de 13500 preterm births hebben er last van?
4. 3.4.3: Hierin staat veel herhaling. De DEC-UM verzoekt u hier met name de samenhang tussen de verschillende doelen te onderbouwen.
5. De DEC vindt de onderbouwing voor de aantallen en de groepen onduidelijk en onvoldoende. U verwijst naar Sachs, we vernemen graag wat de kritische uitleesparameters zijn. Het gebruik van schapen met meer dan één foetus komt niet tot uiting in de totale aantallen.
6. Bij punt **aim 3b** staat een typo. A milestone - a miletstone. Gaarne corrigeren.

Appendix 1:

1. In het projectvoorstel wordt gemeld dat “But due to the risk of short- and long-term side effects, including impairment of neurological development, the routine use of glucocorticoids has been drastically reduced in BPD therapy in the last years”, terwijl in bijlage 1 wordt gesteld dat “Moreover, maternal intramuscular dexamethasone administration mimics the clinical standard nowadays”. In hoeverre lijkt dit een discrepantie?
2. U geeft aan dat “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”. Vergroot deze toediening van dexamethason de kans op een abortus bij het schaap en in hoeverre is deze toediening van invloed op de immuunrespons ten aanzien van de Ureaplasma parvum-infectie?
3. Hoe wordt in de nutritionele ondersteuning van het premature lam voorzien met name ook ten aanzien van het risico op hypoglycemie?
4. Met behulp van echografie zou toch inzicht moeten kunnen worden verkregen in kennelijke één– of meerlingdracht?

5. K: is de classificatie voor het ongerief van het lam correct (non-recovery in plaats van anderszins) daar het lam wel bij bewustzijn is tot aan de euthanasie? De DEC-UM verzoekt aan te geven of de lammeren onder anesthesie of sedatie zijn? Wanneer de lammeren onder sedatie zijn lijkt de term non-recovery niet op zijn plaats.
6. De DEC verzoekt replacement punten te noemen die wel ter zake doen.
7. H: Gaarne uitleggen welke methode van anesthesie u toepast, u hebt geen toelichting gegeven.
8. De ooien krijgen post operatieve antibiotica. Het is niet helder op welke procedure deze behandeling volgt. Na de intra-uterine injectie?
   Wanneer worden de ooien precies gedood?
9. De omschrijving van “adverse effects” ontbreekt voor het lam.

Appendix 2:

1. De lammeren zullen van 5 tot 10 dagen worden geventileerd. Wat bepaalt de uiteindelijke duur van ventilatie? Het beperken van deze periode zal het risico op mogelijke complicaties, met daaruit volgend mogelijk ongerief en uitval verminderen.

Appendix 3:

1. Er wordt gesteld dat: “We cannot do biochemical analysis of ovine blood, White blood cell count is not reliable due to post-operative elevation”. Dit lijkt een valide uitspraak voor een ziekenhuislaboratorium, maar een veterinair laboratorium zou er geen moeite mee moeten hebben. Er lijkt weinig grond niet ook de dieren te monitoren middels bloedonderzoek. Dit lijkt ook voor glucose erg belangrijk om het overlevingspercentage van de lammeren op niveau te houden.
2. De doelen omschreven hebben allen betrekking op het brein van het lam. Er wordt in de omschrijving van de procedures ook ondernomen om gastro-intestinale absorptie en motiliteit te kunnen bestuderen.
   Het extra uitvoeren van extra implantatie en handelingen verhoogd de kans op ongerief en uitval. De DEC-UM wenst een aanvullende onderbouwing.
3. Refinement. Moet uit deze omschrijving geconcludeerd worden dat de ooi altijd in de aanwezigheid van een andere ooi zal zijn? De DEC-UM gaat er vanuit dat de ooien met meerdere dieren gehuisvest worden (met meerdere dieren in één ruimte).
4. Refinement. Is de cortisol waarde alléén een voldoende argument voor de stelling dat de schapen geen stress hebben? De DEC-UM is van mening dat dit wel degelijk een significante aantasting van het welzijn is.
5. Wat is bekend over het ongerief voor de foetus gedurende het globale hypoxie-ischemisch insult?
   Kunt u aangeven waar u op baseert dat zij geen ongerief ervaren? Zou ongerief kunnen worden gereduceerd door toediening van opiaten voorafgaand aan het hypoxie-ischemisch insult?

6. **Appendix 2 en 3.**
   Sectie L: De onderbouwing voor het doden van de dieren ontbreekt. De DEC-UM wenst een onderbouwing.

NTS:

De DEC-UM heeft een vraag gesteld over de non recovery bij appendix 1. Graag de appendix en de NTS in overeenstemming brengen.

- Datum antwoord dd. 12-08-2015

- Strekking van het (de) antwoord(en) **Deze hebben de vragen van de DEC niet geheel verhelderd.**
- De antwoorden hebben geleid tot **gedeeltelijke** aanpassing van de aanvraag.

Dd. 14-08-2015 heeft de DEC-UM nog gevraagd om een aantal tekstuele aanpassingen.

Dd. 17-08-2015 heeft de DEC-UM de volgende vragen nog gesteld:

- In de experimenten met de prematuren worden de lammeren tot geboorte gebracht na 132 dagen zwangerschap. Op deze leeftijd wordt anesthesie wenselijk/noodzakelijk geacht. Bij aim 3 worden andere experimenten, in de uterus, uitgevoerd. Het argument dat de lammeren in deze experimenten geen pijn lijden is dat zij nog geen volledig ontwikkeld neuraal systeem hebben (appendix 3 vraag 5). In de appendix 3 staat niet vermeld in welke periode van de dracht het hypoxie-ischemie insult wordt veroorzaakt. Wel wordt de 147 dagen drachtperiode benoemd als argument voor de keuze voor de diersoort. Kunt U aangeven op welke dag van de dracht het insult precies wordt gegeven?

- In antwoord op vraag 9 geeft U aan dat "Pain will be prevented by continuous sedation and analgesia". Dient aangenomen te worden dat ook hier anesthesie wordt bedoeld in plaats van sedatie (ook bij de beantwoording van vraag 5 lijkt anesthesie welhaast gelijk te worden gesteld aan sedatie als anesthesie = sedatie + analgesie)?

De antwoorden hebben geleid tot aanpassing van de aanvraag.

9. Eventuele adviezen door experts (niet lid van de DEC) **Geen advies elders gevraagd.**

- Aard expertise
- Deskundigheid expert
- Datum verzoek
- Strekking van het verzoek
- Datum expert advies
- Expert advies

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet): **JA**
2. De aanvraag betreft een **nieuwe aanvraag.**
3. De DEC is competent om hierover te adviseren: **JA**

4. Vanwege betrokkenheid bij het betreffende project is een aantal DEC-leden, met het oog op onafhankelijkheid en onpartijdigheid, niet betrokken bij de advisering **NVT**

C. Beoordeling (inhoud):

1. Het project is:

   X uit wetenschappelijk oogpunt verantwoord
   - uit onderwijskundig oogpunt verantwoord
   - uit het oogpunt van productiedoeleinden verantwoord
   - wettelijk vereist

2. De in de aanvraag aangekruiste doelcategorie(ën) is / zijn in overeenstemming met de hoofddoelstelling(en): **JA**
3. De DEC onderschrijft het belang van de doelstelling. Het wordt ingeschat als een substantieel belang. **Het belang van de doelstelling wordt door de DEC-UM erkend, te weten: De effectiviteit van therapeutische maatregelen om de uitkomst van premature kinderen te verbeteren.**
4. De gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project: **Naar de overtuiging van de DEC-UM beschikt de aanvrager over voldoende expertise en voorzieningen om de projectdoelstelling met de gekozen strategie / aanpak binnen de gevraagde termijn te realiseren.**
5. Er is sprake van de volgende bijzonderheden op het gebied van categorieën 10g, 13, 13c3 van dieren. De keuze hiervoor is voldoende wetenschappelijk onderbouwd: **JA**

x Huisvesting en verzorging

x Locatie: instelling vergunninghouder (10.g)

x Toepassing verdoving/pijnbestrijding (13)

x Dodingsmethode (13.c.3)

6. Het ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd: **JA**
7. Er zijn geen methoden die de voorgestelde dierproeven geheel of gedeeltelijk zouden kunnen **vervangen.**

8. In het project wordt optimaal tegemoet gekomen aan de vereiste van de **vermindering** van dierproeven. Het maximale aantal te gebruiken dieren is realistisch ingeschat. De aanvrager beschikt over voldoende expertise en informatie om, bij wettelijk vereist onderzoek, te voorkomen dat onnodige duplicatie plaatsvindt.
9. Het project is in overeenstemming met de vereiste van de **verfijning** van dierproeven en het project is zo opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd. Er is geen sprake van belangwekkende milieueffecten
10. De niet-technische samenvatting is een evenwichtige weergave van het project en begrijpelijk geformuleerd

D. Ethische afweging

De doeleinden van het project rechtvaardigen het voorgestelde gebruik van dieren (niet), de schade in de vorm van lijden, pijn en angst bij dit aantal dieren wordt (niet) gerechtvaardigd door het verwachte resultaat. Het is uit wetenschappelijk oogpunt verantwoord en het is waarschijnlijk dat de doeleinden worden gehaald. Op termijn kan het project (geen) voordelen opleveren voor mens, dier of milieu.

Ethische afweging DEC-UM:
Het project "*Therapeutic interventions to improve neonatal outcomes in the course of perinatal stress"*, wordt door de DEC-UM beoordeeld als een relevante, nieuwe aanpak in de optimalisatie van de behandeling van vroeggeboren kinderen. Dit onderzoek heeft zowel een wetenschappelijk als maatschappelijk belang. Resultaten kunnen te zijner tijd ten goede komen aan vroegeborenen en hun familie. De gekozen strategie en de concrete doelstellingen lijken passend en haalbaar en kunnen op dit moment niet zonder dierproeven worden behaald. Het project behelst het gebruik van schapen - ooien en lammeren - die maximaal matig ongerief zullen ondervinden en die veelal in het kader van de proef worden gedood.
Bij de voorgestelde dierproeven en de verzorging, behandeling en huisvesting van de proefdieren wordt adequaat invulling gegeven aan de vereisten op het gebied van de vervanging, vermindering en verfijning van dierproeven. De betrokken onderzoekers zijn zeer ervaren en goed op de hoogte van de wetenschappelijke ontwikkelingen in het veld. Men bouwt voort op onderzoek dat al eerder is beoordeeld. Er is geen sprake van duplicatie.

Conclusie: De DEC-UM acht enerzijds het project "*Therapeutic interventions to improve neonatal outcomes in the course of perinatal stress"* van substantieel belang. Anderzijds onderschrijft zij de intrinsieke waarde van het dier. Gezien het belang en de kwaliteit van het voorgestelde project acht de DEC-UM het gebruik van dieren hier ethisch aanvaardbaar.

E. Advies

1. Advies aan de CCD
   - ☐ De DEC adviseert de vergunning niet te verlenen vanwege:
     - o De vaststelling dat het project niet vergunningplichtig is
     - o De volgende doorslaggevende ethische bezwaren
     - o De volgende tekortkomingen in de aanvraag
   - ☐ De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden
     - o Op grond van het wettelijk vereiste dient de projectleider bij beëindiging van het project een beoordeling achteraf aan te leveren die is afgestemd met de IvD.
     - o Voor de uitvoering van dit project is tevens een ministeriële ontheffing vereist
     - o Overige door de DEC aan de uitvoering verbonden voorwaarden

   X De DEC adviseert de vergunning te verlenen.

2. Het uitgebrachte advies is gebaseerd op consensus.

Op grond van alle voor de afweging relevante argumenten komt de DEC-UM tot de conclusie dat dit onderzoek ethisch toelaatbaar is.

9.

Aan: Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
[redacted] 124-15	0[redacted]	14-09-2015

DEC-UM
Voorzitter DEC-UM
[redacted]
p/a secretariaat DEC-UM

Secretariaat DEC-UM
[redacted]

Bezoekadres
[redacted]
[redacted]Maastricht

Postadres
Postbus 616
6200 MD Maastricht

Betreft: reactie op uw mail dd. 11-09-2015 betreffende aanvraagnummer AVD107002015225, ons kenmerk: 2015-005.

Geachte mevrouw [redacted]

In antwoord op Uw vragen het volgende:

Uw vraag 1: In het advies geeft de DEC aan, bij punt 5, dat er sprake is van bijzonderheden op enkele gebieden in de aanvraag. Zou u meer uitgebreid willen uitleggen wat de bijzonderheden zijn, en hoe de DEC tot zijn oordeel is gekomen dat deze bijzonderheden voldoende gemotiveerd zijn?

Antwoord DEC-UM: Het betreft de volgende bijzonderheden 1) Huisvesting en verzorging met als locatie: instelling vergunninghouder (10.g), 2) Toepassing verdoving/pijnbestrijding (13) en 3) Dodingsmethode (13.c.3). Daar de aanvraag betrekking heeft op een kuddedier als het schaap heeft de DEC-UM zich ervan vergewist dat alhoewel de ooien individueel worden gehuisvest binnen de UM zij contact kunnen hebben met andere ooien middels reuk, gehoor en visuele waarneming. Ten aanzien van de verdoving/pijnbestrijding/dodingsmethode hebben wij ook nadere aanvulling gevraagd. Er blijkt te gelden dat de ooien worden geanaestheseerd juist voorafgaand aan de *sectio caesarea* (induction of anesthesia through thiopental i.v., continuation with isofluorane 1-2% and remifentanil i.v. as well as local analgesia of surgical wounds with lidocaine). Tevens geldt voor de lammeren *in utero* sedatie via anesthesie van de ooi, terwijl *ex utero* experimenten worden uitgevoerd middels ketamine and midazolam i.v. Zowel ooien als lammeren worden geeuthanaseerd middels een overdosis thiopental i.v. te weten onder anesthesie aan het einde van een *sectio caesarea* respectievelijk tijdens sedatie (bijlagen 1 en 2) of *in utero* (bijlage 3).
De dieren uit de experimenten, beschreven in bijlage 3, worden niet geëuthanaseerd onder algehele anesthesie, maar zullen wel sedatie ontvangen voordat euthanasie zal plaatsvinden.

Uw vraag 2: Bovenop geeft de DEC aan dat het ongerief realistisch ingeschat en geclassificeerd is, maar in de aanvraag, in bijlage dierproef 2 staat het ongerief niet vermeld. Weet de DEC wat het ongerief van de dieren in dierproef 2 is?

Antwoord DEC-UM: Voor de dierproeven 1 en 2 binnen de projectaanvraag geldt dat “during the mechanical ventilation, the lambs are continuously sedated and will not regain full conscience. At the end of the experiment they will be euthanized while sedated. (Therefore, the classification of the experiment is considered to be non-recovery.) Abusievelijk is dit niet vermeld in bijlage 2, maar daar geldt hetzelfde als vermeld onderaan bijlage 1.

Hopenlijk zijn Uw vragen hiermee naar wens beantwoord.

[redacted]

Voorzitter DEC-UM

i.o

10.

[redacted]

Van: [redacted]
Verzonden: woensdag 21 oktober 2015 14:38
Aan: [redacted]
Onderwerp: RE: verzoek tot aanpassing NTS bij aanvraag AVD107002015225
Bijlagen: DEC-UM 2015-005_Niet technische samenvatting_V4.0_after approval.docx

Categorieën: Dossier: [redacted]

Dear [redacted]

Please find the revised summary in the enclosure.

Best regards
[redacted]
Maastricht University Medical Center

From: Info-zbo [info@zbo-ccd.nl]
Sent: Monday, October 19, 2015 9:33 AM
To: [redacted]
Cc: [redacted]
Subject: verzoek tot aanpassing NTS bij aanvraag AVD107002015225

Geachte [redacted],

Op 21 augustus 2015 hebben we uw aanvraag met titel "Therapeutic interventions to improve neonatal outcomes in the course of perinatal stress" en aanvraagnummer AVD107002015225 ontvangen.

De CCD heeft uw aanvraag beoordeeld, en besloten deze gedeeltelijk goed te keuren (alleen voor de dierproeven 1 en 2). Daarom hebben wij een herziene versie van de Niet Technische Samenvatting van u nodig, waarin u alleen het vergunde gedeelte van uw project beschrijft.

De commissie is van mening dat dit project bestaat eigenlijk uit twee aparte projecten: project 1) de dierproeven 1 en 2, en project 2) de dierproef 3. De enige samenhang bestaat uit het feit dat alle dierproeven gericht zijn op onderzoek naar efficiënte therapieën bij pre-term geboorte.
De eerste twee dierproeven 'Intra-uterine inflammation' en 'Ventilation-induced lung injury' vormen een toetsbare eenheid aangezien zowel de experimentele opzet, als de onderzoeksvraag en doelstelling vergelijkbaar zijn. Beide dierproeven maken gebruik van hetzelfde infectiemiddel, zijn gefocust op longontwikkeling. Bovendien wordt dezelfde onderzoeksprocedure gebuikt in beide dierproeven.
De derde dierproef 'Global hypoxia-ischemia' heeft een andere experimenteel opzet, bevat onderzoek naar problemen in het brein, milt en andere organen. Bovendien, dierproef 3 dient zelf 5 doelstellingen. Tot slot, de dieren in de derde dierproef zullen ernstig ongerief ondergaan waardoor een beoordeling achteraf vereist zijn. Voor dierproef 3 zou een aparte aanvraag moeten worden ingediend.

Zodra wij de nieuwe versie van de NTS hebben ontvangen sturen wij de beschikking en vergunning aan u toe. Tot die tijd kunt u niet met uw project "Therapeutic interventions to improve neonatal outcomes in the course of perinatal stress" beginnen.

We hopen snel van u dit document te ontvangen.

Met vriendelijke groet,

[redacted]

Uitvoeringsexpert

Centrale Commissie Dierproeven
www.centralecommissiedierproeven.nl<http://www.centralecommissiedierproeven.nl/>
.....

Postbus 20401 | 2500 EK | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl<mailto:info@zbo-ccd.nl>
Let op: vanaf nu heeft de CCD een nieuw e-mailadres info@zbo-ccd.nl<mailto:info@zbo-ccd.nl>. Heeft u ons oude e-mail adres in uw adressenboek, dan vragen we u om dat aan te passen.

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Maastricht University
[redacted]
Postbus 616
6200 MD MAASTRICHT

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

Onze referentie
Aanvraagnummer
AVD107002015225

Datum 22 OKT. 2015
Betreft Aanvraag projectvergunning Dierproeven

Geachte [redacted]
Op 21 augustus 2015 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Therapeutische interventions to improve neonatal outcomes in the course of perinatal stress" met aanvraagnummer AVD107002015225. Wij hebben uw aanvraag beoordeeld.

Op 21 oktober 2015 heeft u uw aanvraag aangevuld. U heeft een aangepaste versie van de Niet-technische samenvatting naar de CCD gestuurd.

Beslissing
Wij keuren uw aanvraag gedeeltelijk goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning. U kunt met uw project "Therapeutische interventions to improve neonatal outcomes in the course of perinatal stress" starten, met de dierproeven opgenomen in de vergunning. De vergunning wordt afgegeven van 1 december 2015 tot en met 30 november 2020. Deze termijn is anders dan in uw aanvraag, omdat de looptijd van de vergunning voor maximaal 5 jaar is.
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 21 augustus 2015. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet. Wij hebben de DEC om aanvullende informatie gevraagd. Op 15 september 2015 heeft de DEC gereageerd op onze vragen. De vragen hadden betrekking op de bijzonderheden in de aanvraag en op het ongerief van de dieren te gebruiken in dierproef 2.
Wij kunnen ons niet geheel vinden in de inhoud van het advies van de Dierexperimentencommissie. De CCD is van mening dat dit project bestaat

eigenlijk uit twee aparte projecten: project 1) de dierproeven 1 en 2, en project 2) de dierproef 3. De enige samenhang bestaat uit het feit dat alle dierproeven gericht zijn op onderzoek naar efficiënte therapieën bij pre-term geboorte.
De eerste twee dierproeven 'Intra-uterine inflammation' en 'Ventilation-induced lung injury' vormen een toetsbare eenheid aangezien zowel de experimentele opzet, als de onderzoeksvraag en doelstelling vergelijkbaar zijn. Beide dierproeven maken gebruik van hetzelfde infectiemiddel, zijn gefocust op longontwikkeling. Bovendien wordt dezelfde onderzoeksprocedure gebruikt in beide dierproeven.
De derde dierproef 'Global hypoxia-ischemia' heeft een andere experimenteel opzet, bevat onderzoek naar problemen in het brein, milt en andere organen. Bovendien, dierproef 3 dient zelf 5 doelstellingen. Wij nemen dit advies van de commissie niet geheel over, inclusief de daaraan ten grondslag liggende motivering.
Dit 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).

Met vriendelijke groet,

Centrale Commissie Dierproeven
namens deze:

[redacted]

mr. drs. H.M. van der Gaag-Halbertsma
plv Algemeen Secretaris

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

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

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

deze projectvergunning voor het tijdvak 01 december 2015 tot en met 30 november 2020, voor het project "Therapeutische interventions to improve neonatal outcomes in the course of perinatal stress" met aanvraagnummer AVD107002015225, gedeeltelijk volgens advies van Dierexperimentencommissie DEC-UM. Hierbij is afgeweken van het DEC-advies omdat de CCD van mening is dat dit project bestaat eigenlijk uit twee aparte projecten: project 1) de dierproeven 1 en 2, en project 2) de dierproef 3. De enige samenhang bestaat uit het feit dat alle dierproeven gericht zijn op onderzoek naar efficiënte therapieën bij pre-term geboorte.

De functie van de verantwoordelijk onderzoeker is [redacted]
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 21 augustus 2015
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 21 augustus 2015;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 21 augustus 2015 en aangepast op 21 oktober 2015;
c Advies van dierexperimentencommissie d.d. 21 augustus 2015, ontvangen op 21 augustus 2015.

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
Intra-uterine inflammation	Schapen (Ovis aries) / volwassenen en foetale schapen	96	Matig / moderate	
Ventilation-induced lung injury	Schapen (Ovis aries) / volwassenen en foetale	96	Terminal / non-recovery	

Voorwaarden
Op grond van artikel 10a1 lid 2 Wod zijn aan een projectvergunning voorwaarden te stellen
De vergunning wordt verleend onder de voorwaarde dat eventuele go/no go momenten worden afgestemd met de IvD.

In artikel 10, lid 1a 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 overleg met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarden wijzigen of intrekken.

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.

Beoordeling achteraf

Volgens artikel 10a1, lid 1d en lid 3 van de wet worden projecten waarbij niet-menselijke primaten worden gebruikt, projecten die als ernstig ingedeelde dierproeven omvatten of een dierproef die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, achteraf beoordeeld worden. In dit project worden dierproeven toegepast waarbij die vallen in de categorie ernstig volgens artikel 10b van de wet en wordt daarom voorzien van beoordeling achteraf. Deze beoordeling zal uiterlijk plaatsvinden. Er zal dan beoordeeld worden of de doelstellingen van het

project werden bereikt. Daarnaast wordt bekeken of de schade die de dieren hebben ondervonden, het aantal en soorten proefdieren en de ernst van lijden van de proevendieren conform de vergunning waren.