Begeleidingsformulier aanvraag dierproef DEC- UM

Versie 2006

Herziene versie

DECNR: 2011-135

Ontvangen: 26-10-2011

DEC datum goedkeuring#	Type aanvraag [2]
27-10-2011	Nieuw

VROM/GGONR[3]	LNV/CBDNR[4]

Hoofdproject	CARIM	NUTRIM	Hersenen en gedrag	GROW	biomaterialen	Ander UM	Geen UM
Deelproject	1. 2. 3.	1. 2. 3. 4.	1. 2. 3.	1. 2. 3.			

Financieel beheerder		Budgetnummer 30080000N	

Titel van het onderzoek: Fetal sheep maturation database

startdatum 01-11-2011 einddatum [9] 31-10-2015 *Duur van de proef [10]:* *2 dagen*

	Naam	Tel (+ Tel privé enkel VO, VVO en VM)	E-mailadres	Bevoegd-heid[5]	Cap. groep /afdeling
1. Verantwoordelijk onderzoeker (VO)				Art.9	
2. Vervanger VO (VVO)				Art.9	
3. Verantwoordelijk medewerker (VM) GGO[7]				Art. 9 Art. 9 Art. 9 Art. 9 Art. 9	
4. overige uitvoerenden					
5.				Art.12	CPV

Diergroep	1	2	.	.	.	.	.
ctrl/exp/sham	EXP	EXP					
Diersoort	44	44					
Stam	NA	NA					
Construct / mutatie ?	Normal	Normal					
Herkomst (leverancier) *	03	03					
Aantal	108	216					
Geslacht	F	M/F both					
Dieren immuuncompetent ?	ja	ja/	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]
Leeftijd/gewicht	Pregnant ewes	Lambs					
Doel van de proef *	33	33					
Belang van de proef *	01	01					
Toxicologisch onderzoek *	01	01					
Bijzondere technieken *	01	01					
Anesthesie *	01	01					
Pijnbestrijding *	01	01					
Mate ongerief *	02	02					
Toestand dier einde exp*	01	01					

* VHI-coderingen zie bijlage

1 Verantwoording

Aanvraag dierproef DEC-UM (**kaders zijn licht flexibel, maar het geheel is max. 5 pag. versie 2006**)
Titel: Fetal sheep organ maturation database

1. Doel van de proef.
The aim of this study is to create a detailed fetal sheep maturation database. We want to establish a detailed database of normal fetal organ maturation and the development of the fetal immune system with short time intervals (5 days) starting from early-gestation (60 days) until term (145 days).

2. Maatschappelijke relevantie en/of wetenschappelijk belang
Previous findings of our and other international groups have found that perinatal pathologies such as chorioamnionitis and perinatal asphyxia interfere with normal maturation of fetal organs. Chorioamnionitis interferes with normal lung and gut development, alters the immune system, and causes brain injury. These studies revealed how little we know of the dynamics of normal fetal organ maturation and the development of fetal immunity. Understanding these physiological processes during gestations is highly relevant both for clinicians and scientists.

3. Alternatieven
After consultation with our international colleagues in the field of sheep research we found out that no maturation sheep database exists. We use sheep to document fetal maturation, because their developmental biology is comparable to that of humans (see figure 1).

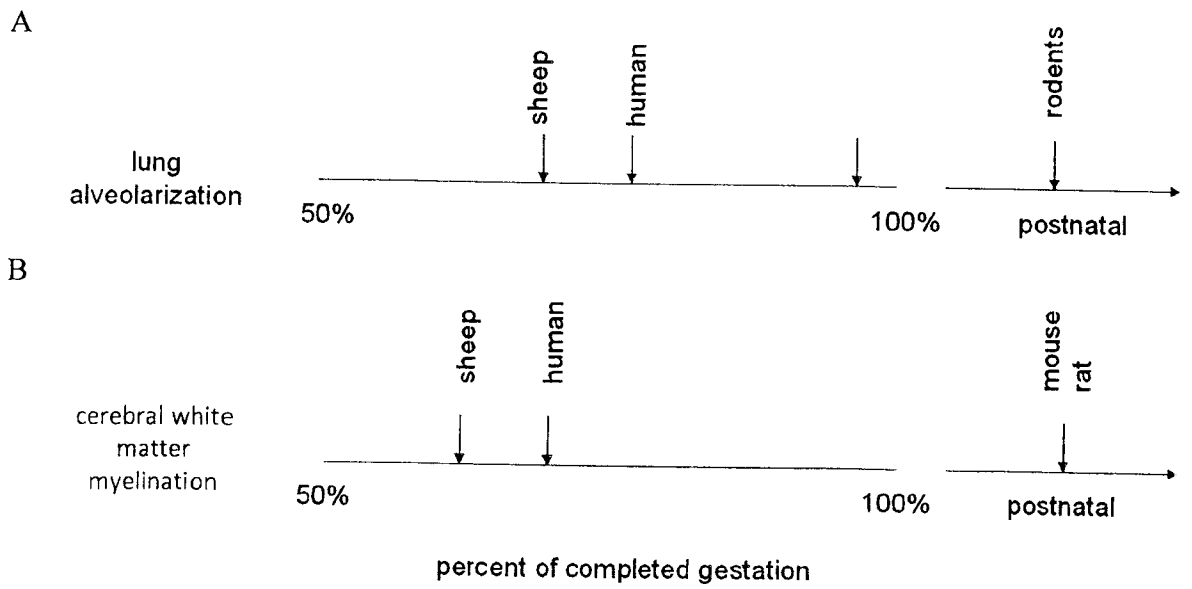


Figure 1. Lung alveolarization (A) and Cerebral white matter myelination (B) in humans and sheep is completed before birth, whereas maturation of these organs systems in rodents is completed postnatally.

4. Ethische afweging
The experiments proposed are needed to give more insight into fetal organ system development under non-pathological conditions. Our current knowledge of maturation and the development of the fetal immune system is based on limited time points in gestation, and was determined in control groups of several protocols with different sham treatments (i.e. fetal instrumentation, intra-amniotic saline injection, mechanical ventilation), which do not represent physiological conditions. Therefore, we want to establish a detailed database of 'untouched' fetal organ maturation with short time intervals (5 days) starting from early-gestation (60 days) until term (145 days).
The knowledge obtained from this unique database justifies the discomfort of the animals in the experiment.

Wetenschap

5. Wetenschappelijke onderbouwing

Previous findings of our and other international groups have found that perinatal pathologies such as chorioamnionitis and perinatal asphyxia interfere with normal maturation of fetal organs.
In a translational ovine chorioamnionitis model, we recently showed that antenatal exposure to endotoxin, ureaplasma or IL-1 resulted in a pro-inflammatory response of the fetal gut. More importantly, this inflammatory process prevented maturation of the gut, potentially predisposing the fetus to intestinal pathologies after birth (Wolfs, Buurman et al. 2009; Wolfs, Kallapur et al. 2011).
We also demonstrated that endotoxin-induced chorioamnionitis modulated the innate immunity of monocytes in preterm sheep (Kramer, Ikegami et al. 2005), making them more susceptible to respiratory distress syndrome or bronchopulmonary dysplasia (Kramer, Kallapur et al. 2008).
Beside changes in gut and lung development endotoxin-induced chorioamnionitis also resulted in an increase in central nervous system injury in fetal sheep (Gavilanes, Strackx et al. 2004, Gavilanes, Gantert et al. 2010)
As described above, our current knowledge of fetal sheep organ maturation and the development of fetal immunity is based on limited time points in gestation, and was determined in control groups of several protocols with different sham treatments (i.e. fetal instrumentation, intra-amniotic saline injection, mechanical ventilation), which do not represent physiological conditions. Therefore, we want to establish a detailed database of 'untouched' fetal organ maturation with short time intervals (5 days). We start documenting maturation on day 60 since it is assumed that fetuses become immunocompetent around 75 days of gestation (Liechty, MacKenzie et al. 2000).This gives us the unique opportunity to study the development of the fetal immune system.

6. Wetenschappelijke beoordeling
The DEC request was reviewed and approved by 1

7. Proefdier keuze

7a. Soort, stam / herkomst / eindbestemming

Sheep fetuses of gestational age between 60 and 145 days (term is 150 days) will be included in this study. The sheep used in this study are bred by a highly experienced farmer who also breeds for other sheep experiments.

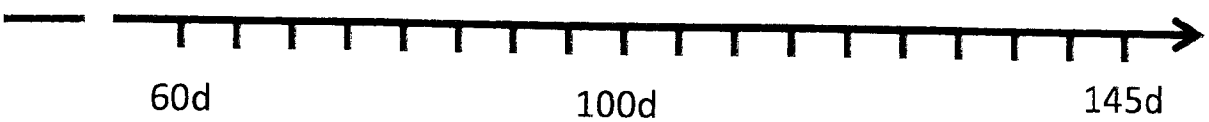
7b. Sexe

We will use singleton and twin preterm ovine fetuses of both sexes.

7.c. Aantallen

We will document maturation at 18 different time-points starting on 60 days of gestation with 5 day intervals until 145 days of gestation (see figure). **We choose for 5 day intervals since previous studies of our group have shown that the inflammatory response after intra-amniotic LPS injection or systemic hypoxic-ischemia in the lung, gut, spleen, thymus and brain differs strongly between animals of different gestational ages. Animals only a few days older than their counter mates mount different inflammatory response to the same pro-inflammatory stimulus. This indicates that maturation of these organs and the fetal immune system play a pivotal role in the extent of inflammation that is caused by chorioamnionitis or hypoxic-ischemia. Therefore we want to study the maturation of the immune system and vital organs in detail to be able to understand these age-dependent responses.**

We will include 6 animals per gestational age group in order to investigate fetal organ maturation in both perfusion fixated animals (n=3) for MRI analysis and immunohistochemistry and freshly sampled animals (n=3) for protein and RNA analysis. The total number of animals will therefore be 6 x 18 = 108 animals.



Dierproef

8. Experiment
Pregnant ewes will be humanely euthanized at different gestational ages by intravenous pentobarbital (150-200 mg/kg) injection. The fetus will be immediately exteriorized and euthanized by injecting pentobarbital (150-200 mg/kg) into the umbilical cord vein. Then the fetus will be either perfusion fixated before sampling or organ samples will be freshly snap frozen.

9. Experimentele condities

9a. Anesthesie
No anesthesia will be used.

9b. Pijnbestrijding
No analgesia will be used

9c. Euthanasie en Humane eindpunten

- Ewes will be euthanized at different gestational ages with a lethal overdose of pentobarbital (150-200 mg/kg).
- Exteriorized lambs will be euthanized directly with a lethal overdose of pentobarbital (150-200 mg/kg).

Human endpoints:
The wellbeing of the ewe is assessed daily. Untreatable pain, discomfort or fever, intra-uterine fetal death and pending labor are considered human endpoints. If indicated the ewe will be euthanized with a lethal dose of pentobarbital (150-200 mg/kg).

Zorg

10a. Ongerief

Ewe

1. Transport: **gering (01),** duration 10 min
2. Euthanasia: **gering/matig (02),** duration 2 min

Lamb

3. Euthanasia: **gering/matig (02),** duration 2 min

10b. Welzijnsevaluatie
The welfare of the pregnant ewes will be recorded daily by certified personnel.

11. Verzorging en huisvesting
At least one week before the experiment ewes are transported from the breeder to the CPV paddock to allow adaptation. The pregnant ewes will be transported to the CPV 1-2 days prior to the experiment. The pregnant ewes will have ad libitum access to food and water will be housed at standard CPV conditions for sheep.

12. Deskundigheid
Our research team, headed by , has broad experience in the handling of sheep.

13. Standard Operation Procedures (SOP)

SOP 1: Euthanasia

Relevante literatuur

Gavilanes AW, Gantert M, Strackx E, Zimmermann LJ, Seeldrayers S, Vles JS, Kramer BW Increased EEG delta frequency corresponds to chorioamnionitis-related brain injury. Front Biosci (Schol Ed). **2010**;2:432-8.

Gavilanes AW, Strackx E, Kramer BW, Gantert M, Van den Hove D, Steinbusch H, Garnier Y, Cornips E, Steinbusch H, Zimmermann L, Vles J. Chorioamnionitis induced by intraamniotic lipopolysaccharide resulted in an interval-dependent increase in central nervous system injury in the fetal sheep. Am J Obstet Gynecol **200**(4): 437 e1-8.

Kramer BW, kallapur S, Newnham J, Jobe AH. Prenatal inflammation and lung development. Semin Fetal Neonatal Med. **2009** ;14(1) :2-7

Kramer BW, Ikegami M, Moss TJ, Nitsos I, Newnham JP, Jobe AH. Endotoxin-induced chorioamnionitis modulates innate immunity of monocytes in preterm sheep. Am J Respir Crit Care Med. **2005**;171(1):73-7

Liechty, K. W., T. C. MacKenzie, et al. (2000). "Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep." Nat Med **6**(11): 1282-1286.

Wolfs TG, Kallapur SG, Polglase GR, Pillow JJ, Nitsos I, Newnham JP, Chougnet CA, Kroon E, Spierings J, Willems CH, Jobe AH, Kramer BW IL-1α mediated chorioamnionitis induces depletion of FoxP3+ cells and ileal inflammation in the ovine fetal gut. PLoS One. **2011**;6(3):e18355

Wolfs TG, Buurman WA, Zoer B, Moonen RM, Derikx JP, Thuijls G, Villamor E, Gantert M, Garnier Y, Zimmermann LJ, Kramer BW.Endotoxin induced chorioamnionitis prevents intestinal development during gestation in fetal sheep. PLoS ONE **2009**(6): e5837.

SOP 1: Euthanasia

1. The ewe is transported from the paddock to the animal facility one or two days in advance of the experiment.
2. In the animal facility the ewe is examined in order to assess whether she is healthy and fit for inclusion.
3. The ewe is transported to the Operating Room in a holding pen.
4. The ewe will be placed in shearing position.
5. A venous access will be placed in one of the ewe's front legs.
6. Pentobarbital (150-200 mg/kg) will be gently infused.
7. Death will be confirmed by absence of heart sounds upon auscultation and absence of corneal reflex.
8. The dead ewe will be placed on an operating table and the fetus will be quickly exteriorized.
9. The fetus will be euthanized by a pentobarbital (150-200 mg/kg) injection in the umbilical cord vein.

University Maastricht

Faculty of Health, Medicine
and Life Sciences

Dierexperimenten Commissie

DEC

Aan:

..., voorzitter
p/a Secretariaat DEC-UM
Postbus 616
NL-6200 MD Maastricht
Telefoon: 043

Uw referentie:

Onze referentie : .

Maastricht, 26-10-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Fetal sheep maturation database*", is op de DEC vergadering van 21 oktober 2011 besproken.

De DEC heeft één enkele vraag/opmerking:

- Punt 7c- Waarop berust de keuze voor de tijdsinterval van 5 dagen?

Gelieve eventuele vragen te beantwoorden in **een brief en indien noodzakelijk Uw project aan te passen en duidelijk de aanpassingen grijs te markeren.**

Uw project staat bij de DEC geregistreerd onder nummer **2011-135**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Aan: DEC-UM

Betreft: wijzigingsverzoek DEC 2011-135

26 oktober 2011

Geachte leden van de DEC,

Naar aanleiding van onze project aanvraag "*Fetal sheep maturation database*" had u één één enkele vraag/opmerking:

- Punt 7c- Waarop berust de keuze voor de tijdsinterval van 5 dagen?

Zoals u in de gewijzigde DEC aanvraag kunt zien (grijs gedrukt), hebben wij gekozen voor dit tijdsinterval omdat voorgaande studies laten zien dat de sterkte van de inflammatoire respons na blootstelling aan LPS of hypoxie-ischemie afhankelijk is van de foetale leeftijd. Foetussen die slechts enkele dagen in leeftijd verschillen met anderen vertonen een verschillende immuun respons. We vermoeden dat deze verschillen worden veroorzaakt door verschil in maturatie van organen en het immuunsysteem. Met behulp van deze gedetailleerde database van (immunologische) organen willen we de leeftijdsafhankelijke inflammatoire respons beter leren begrijpen.

Ik hoop dat hiermee uw vraag voldoende is beantwoord.

Met vriendelijke groet,

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
	043-	27-10-2011

Project: *Fetal sheep maturation database.*

Verantwoordelijk onderzoeker (VO):

Namens de Vergunninghouder van de DEC-UM, delen wij u mede dat voornoemd project aan de ethische toetsingscriteria voor proefdiergebruik voldoet.
De DEC maakt geen bezwaar tegen uitvoering van dit project zoals aangevraagd en geeft een **positief advies**.

Projectnummer:	**2011-135**
Diersoort:	**schaap**
Aantal dieren:	**108 ooien en 216 lammeren**
Einddatum:	**27-10-2015**

Uw project staat bij de DEC en CPV geregistreerd onder bovenstaand nummer. Gelieve dieren, die voor dit project bestemd zijn, ook onder dit nummer aan te vragen.

Voorzitter DEC-UM

Vicevoorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM
T (043)

Bezoekadres

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