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Begeleidingsformulier aanvraag dierproef DEC- UM Herziene versie	DECNR: 2011-057 Ontvangen: 27-05-2011

DEC datum goedkeuring#	Type aanvraag [2]
10-06-2011	Nieuw / Herz.versie / Pilot

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:

Improving preterm gut maturation in chorioamnionitis

startdatum 1-6-2011 einddatum [9] 31-3-2015 *Duur van de proef[10]: 15 dagen*

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

Diergroep	1	2					
ctrl/exp/sham	**Exp**	**Exp**					
Diersoort	VHI:44	VHI:44					
Stam	N/A	N/A					
Construct / mutatie ?	normal	normal					
Herkomst (leverancier) *	03	03					
Aantal	80	80					
Geslacht	F	M/F					
Dieren immuuncompetent ?	ja	ja					
Leeftijd/gewicht	pregnant ewes	fetus (0.8 GA)					
Doel van de proef *	33	33					
Belang van de proef *	01	01					
Toxicologisch onderzoek *	01	01					
Bijzondere technieken *	01	01					
Anesthesie *	04	04					
Pijnbestrijding *	04	04					
Mate ongerief *	04	03					
Toestand dier einde exp*	01	01					

* VHI-coderingen zie bijlage

1 *Verantwoording*

Aanvraag dierproef DEC-UM

Titel: Improving preterm gut maturation in chorioamnionitis

1. Doel van de proef

In a translational ovine chorioamnionitis model, we recently showed that antenatal exposure to either endotoxin or IL-1 resulted in a pro-inflammatory response of the fetal gut and this inflammatory process interfered with maturation of the gut (Wolfs, Buurman et al. 2009; Wolfs in preparation).

In the latter study, strong suggestive evidence was provided that a imbalance may contribute to the intestinal inflammatory response that disturbs maturation of the organ.

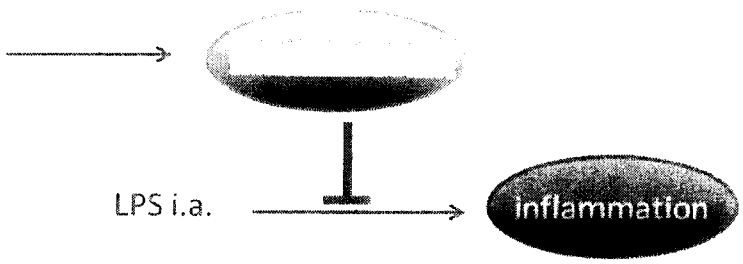


Figure 1. Experimental concept

Hypothesis

- The administration of increases the number of in sheep fetuses
- Mobilized reduce the inflammatory damage in the gut and the lung after intra-amniotic LPS injection.

Objectives

- to titrate optimal
- to determine the number of mobilized after administration
- to determine inflammation in the gut and the lung

2. Maatschappelijke relevantie en/of wetenschappelijk belang

The prevalence of preterm birth (approximately 12.5% in the western countries), constitutes a public health problem, but unlike many health problems, the rate of preterm birth has increased in the last decade (Eichenwald and Stark 2008).

The birth of preterm infants results in significant health consequences to the infant and emotional and economic costs for families and communities. An organ that is held responsible for severe outcomes when developed immature is the gut. Immaturity of the gut is associated with poor nutritional uptake, failure to gain weight, postnatal growth restriction and intestinal pathologies such as necrotizing enterocolitis.

Although chorioamnionitis is the most important cause for preterm labor, the adverse outcomes caused by prematurity and/or antenatal inflammation are predominantly studied after birth. Therefore, our project aims to elucidate the developmental changes of the preterm gut in utero using a unique ovine chorioamnionitis model. Such increased understanding is necessary for the development of therapeutic interventions, intended to accelerate maturation of the intestinal immune system and the gut barrier function in premature infants to prevent intestinal disorders upon birth.

The results will be translated into clinical research, ultimately aimed at the improvement of the life of preterm babies.

3. Alternatieven

In contrast to that of rodents, the developmental biology of sheep is very similar to the human situation. For this reason we use sheep to study different pathologies in perinatal medicine. Findings in our animal model can be easily translated into clinical practice.

4. Ethische afweging

Chorioamnionitis and subsequent inflammatory damage of the gut is a very relevant condition in perinatology with far reaching consequences (such as necrotizing enterocolitis). Striving for a treatment of this condition justifies the discomfort of the animals in this study. Exploring new interventions mandates a reliable and representative animal model to assess efficacy and safety of potential therapies. Unfortunately, it is not possible to perform the proposed experiments in a rodent model or in vitro models (see 3. Alternatieven).

5. Wetenschappelijke onderbouwing

In a translational ovine chorioamnionitis model, we recently showed that antenatal exposure to either 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 in preparation). Following intraamniotic ureaplasma or IL-1 delivery, strong suggestive evidence was provided that a imbalance might be responsible for the detrimental inflammatory response of the fetal gut. More precisely, these results indicate that loss of the cells on disturbs resulting in excessive immune activation and tissue damage.
Recent work from showed that the absolute numbers of were greatly elevated when mice were injected daily with
We hypothesize that chronic stimulation of fetal lambs by results in which protects these animals against the detrimental inflammatory response in the gut after intra-amniotic (IA) LPS delivery. The acquired knowledge is essential to develop interventions aimed at improving development of the preterm gut and to prevent adverse outcomes after preterm birth.
Animals will be exposed to LPS for 2 or 7 days occurs within the first 2 days after intraamniotic LPS infusion. At 7d following IA LPS injection, the strongest inflammatory response occurs. By repetitive we want to investigate whether seen 2d after IA LPS delivery, can be prevented. Subsequently, we want to test whether the potential following reatment will reduce the inflammatory response in the fetal gut after intraamniotic LPS exposure for 7d.
Major immunoprotective nediated effects following xposure were seen after 4d of treatment (Helfand, Soergel et al. 1994; Margolin, Atkins et al. 2007; Punt, Jansen et al. 1992)

6. Wetenschappelijke beoordeling

The DEC request was reviewed and approved by

7. Proefdier keuze

7a. Soort, stam / herkomst / eindbestemming

The experiments will be performed on instrumented sheep fetuses at 110-115 days of gestation. Term is 145 days.

We choose sheep because developmental biology closely resembles the human situation (see chapter 3. Alternatieven). We have broad experience in fetal surgery and maintenance of instrumented fetuses and their mothers. We can instrument either singletons or twins.

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

At the end of the experiment the ewe will be euthanized. Then the fetus is delivered with C-section for sampling of organs and tissue.

7b. Sexe

We will use preterm ovine fetuses of both sexes

7c. Aantallen

In this experiment we will use a total of 80 ewes and 80 of their corresponding fetuses.

We have successfully used group numbers of 6-8 animals for evaluations of inflammation in the gut and the lung. ; with a power of 80% and alpha 0.05 significant different inflammation in the gut between the control and experimental group of 20% (δ=20) with a SD of 12% (σ=12) can be detected with a sample size of n = 6 (n = 15.7 * (12/20)2 = 5.7).(van Wilgenburg and van Schaick Zillesen 2003)

Mortality rate with fetal instrumentation is about 25%. Therefore we want to take a loss of 2 animals per experimental group into account. Therefore group size will be n = 8 and total number of animals n = 80 (see figure 2).

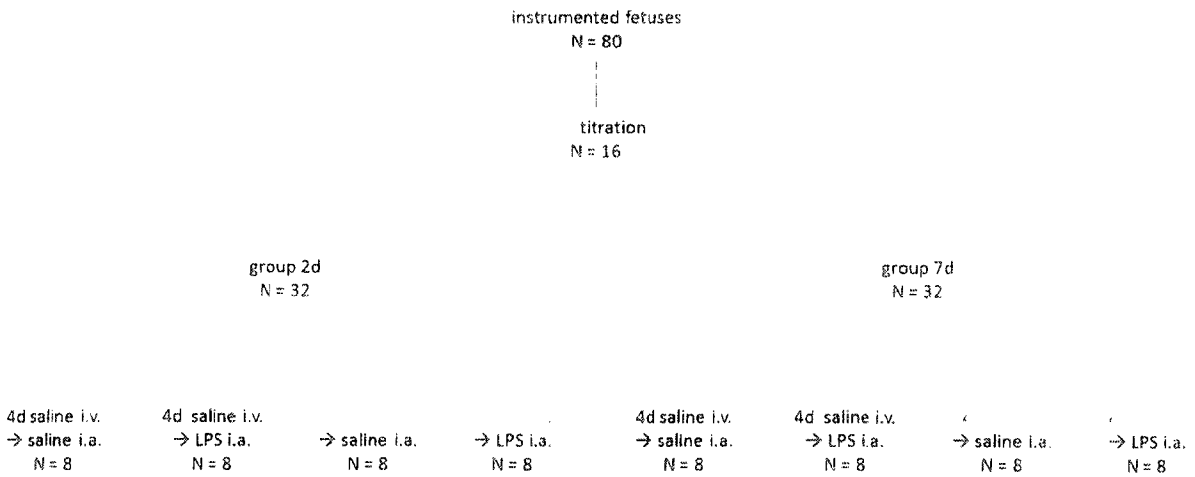


Figure 2. Number of animals

Before we start the inclusion of the experimental groups we will perform a titration experiment to find the optimal ... . which results in the highest the gut and the circulation. We will test four different dosages:

1. kg body weight/ day
2. kg body weight/ day
3. kg body weight/ day
4. kg body weight/ day

For each dosage three animals will be used to correct for biological variation. Taking into account a drop-out rate of 25% a total number of 16 animals will be needed for the titration

8. Experiment

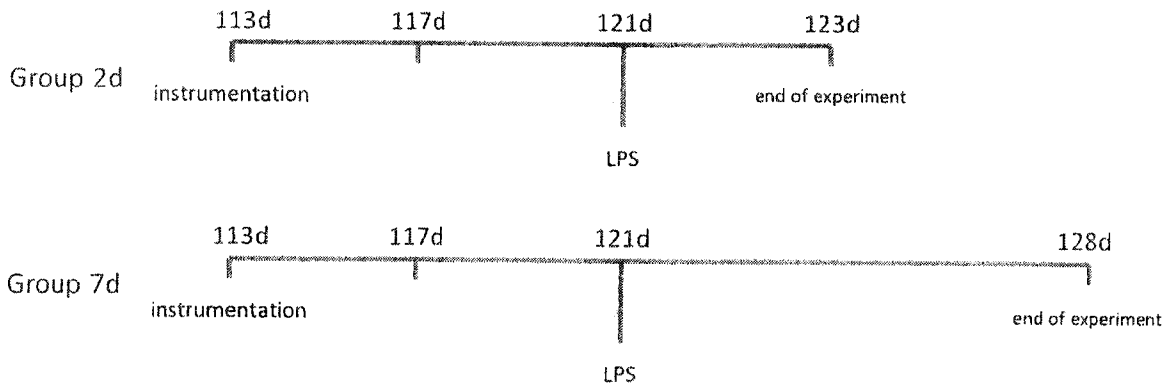
Fetal instrumentation

At a gestational age of 110-115 days the fetus will be exposed by median laparotomy under general anesthesia. Venous and arterial catheters are introduced in the fetus. A catheter is placed in the amnion. The uterus is closed again. The catheters exit the abdominal cavity in the flank of the ewe through a trocar hole. A venous line is placed in one of the hind legs of the ewe for blood sampling and administration of antibiotics. All catheters are connected to perfusion pumps for continuous heparinized saline infusion. After fetal instrumentation there is a recovery period of 4 days (see figure 3).

Experiment

At 121 days the LPS is injected in the amnion through the amniotic catheter.

In group 2d the ewe and fetus are sacrificed two days after the inflammatory stimulus (LPS). In group 7d the animals are sacrificed 7 days after LPS injection (see figure 3).



9. Experimentele condities

9a. Anesthesie

General anesthesia

1. Induction: thiopental i.v. (0.5-1.0 g/ 50 kg), guided by depth of sedation.
2. Sedation: isofluorane inhalation (1-2%), guided by depth of sedation.
3. Pain management: remifentanyl i.v. (0.75 µg/kg/min).(Webster, Cara et al. 2005)

9b. Pijnbestrijding

pain management after surgery

1. wound infiltration with a long-acting local anesthetic, Marcain (bupivacaine hydrochloride 0.25% with adrenaline 1: 400 000

pain management on indication

1. buprenofine (Temgesic: 0,3 mg/ml) 6 µg/kg i.m. or,
2. flunixine meglumine (Finadyne: 50mg/ml) 1 mg/kg i.m.

Infection prohylaxis

1. Surgical wounds are treated with wound spray daily
2. Augmentin i.v. (1000 mg) daily, during first 5 days.

9c. Euthanasie en Humane eindpunten

- At the end of the experiment the ewe will be euthanized by a lethal dose of pentobarbital (200mg/kg). The fetus will be immediately delivered with C-section for sampling. Pentobarbital crosses the placenta, but not immediately in a lethal dose. Therefore the fetus will be euthanized with pentobarbital (200 mg/ kg) once exteriorized.
- The well being 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 (200mg/kg).

10a. Ongerief

Ewe

1. Transport: **gering (01), 2 hours**
2. Fetal instrumentation under general anesthesia: **matig (03), 2-4 hours**
3. Recovery from surgery: **matig (03), 12 hours**
4. Housing in confined space: **matig (03), 10-15 days**
5. Daily intravenous antibiotics and wound care: **gering/matig (02), 5 days**
6. Daily fetal monitoring and continuous flushing of catheters: **gering/matig (02), 10-15 days**
7. IL-2 and endotoxin administration **gering/matig (02), 1 hour**
8. Numerous procedures and manipulation; total discomfort level: **matig/ernstig (04), 11 days (group 2d), 15 days (group 7d).**

Fetus

1. Fetal instrumentation under general anesthesia: **matig (03), 2-4 hours**
2. Intravenous IL-2 administration and intra-amniotic LPS administration: **gering/matig (02), 1 hour**
3. Euthanasia: **gering/matig (02)**

Total discomfort

- **matig/ernstig (04)** for ewes
- **matig (03)** for fetuses

NOTE: preterm ovine fetuses are not viable when delivered

10b. Welzijnsevaluatie

The well-being of the ewe and instrumented lamb will be assessed daily by the investigators and/or the CPV staff. After fetal instrumentation vital parameters (heart rate and blood gas) of the fetus are recorded daily. The arterial and venous lines will be continuously flushed with heparinized saline with an infusion pump.
The clinical impression, food and fluid intake, output, heart rate, respiratory rate and temperature of the ewe will be recorded daily.

11. Verzorging en huisvesting

During the experiment (11-15 days) the ewe is kept in a confined space (see figures below). This allows for continuous perfusion of the catheters and minimal handling during daily monitoring. The ewes are always placed in front of a buddy sheep and have *ad libitum* access to water and food. In this type of experiment, with intensive fetal monitoring, risk of infection and need for continuous catheter perfusion, this way of housing is mandatory. We have tried in the pilot experiments to allow the ewe to walk freely around which resulted in fetal death.

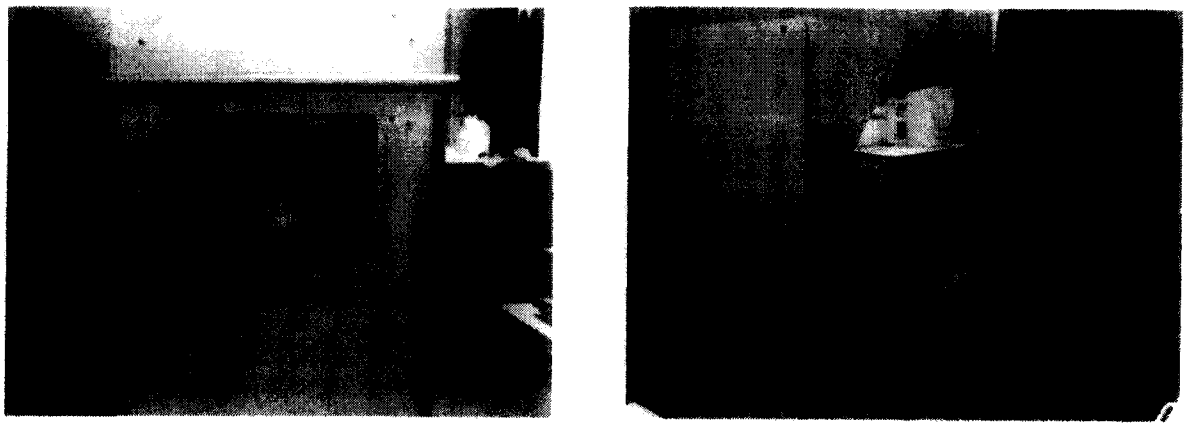


Figure 6. Housing in confined space

The ewes will be intensively monitored for signs of stress by the investigators and personnel of the animal facility. In case of problems the principal investigator or his substitute will be available at any time.

12. Deskundigheid

Our research team has broad experience in sheep experiments in general and especially with this fetal instrumentation model. In July 2010 I from the Auckland University, New Zealand, visited our facility. She developed this model. We are in close contact for continuous optimalization of the experimental set-up according to international standards.

13. Standard Operation Procedures (SOP)

1. fetal instrumentation under general anesthesia
2. postoperative care
3. fetal monitoring
4. administration of endotoxin (LPS)

Literature

Chougnet, C. A., P. Tripathi, et al. "A major role for Bim in regulatory T cell homeostasis." J Immunol **186**(1): 156-63.

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van Wilgenburg, H. and P. G. van Schaick Zillesen (2003). Sample Size and Power Calculation.

Webster, V. L., D. M. Cara, et al. (2005). "Description of a technique for anaesthetizing pregnant ewes for fetal surgery." Lab Anim **39**(1): 94-9.

Wolfs, T. G. (in preparation).

Wolfs, T. G., W. A. Buurman, et al. (2009). "Endotoxin induced chorioamnionitis prevents intestinal development during gestation in fetal sheep." PLoS ONE 4(6): e5837.

SOP 1 Fetal instrumentation under general anesthesia

1. The ewe is examined in order to assess whether she is healthy and fit for inclusion in the experiment when arriving in the animal facility.
2. The wool of the ewe is completely sheared. In this way the ewe will be more comfortable inside the animal facility
3. Prior to surgery, the ewe will be placed in the confined space (car) for a few hours, so she can get used to this environment.
4. The ewe is weighed and transported to the Operating Room in a transport trolley.
5. A venous access is inserted in both front legs. The ewe is sedated with **thiopental** i.v. (0.5-1.0 g/ 50 kg, guided by depth of sedation). The ewe is placed supine on a laparotomy trolley. The ewe is intubated and mechanically ventilated. Sedation is maintained with continuous inhalation of **isofluorane** 1-2%, guided by depth of sedation and **remifentanyl** i.v. (0.75 μg/kg/min) for pain management.
6. Prior to surgery, antibiotic prophylaxis is given: 1000 mg **Augmentin** i.v.
7. All operation procedures on the ewe and the fetus are performed under strict aseptic conditions.
8. On indication, an arterial line can be inserted in the ewe's carotid artery for peri-operative maternal blood pressure measurement.
9. A central venous line is placed in the one of the ewe's hind legs. The catheter is tunneled subcutaneously to exit above the hip joint of the ewe.
10. The ewe's abdomen is cleaned and the incision site is infiltrated with a long-acting local anesthetic, **Marcain** (bupivacaine hydrochloride 0.25% with adrenaline 1: 400 000
11. The abdomen is cut open by median laparatomy. A hand is introduced into the abdomen to palpate the uterus and identify the location of the fetal head. The uterus is gently manipulated and the fetal head and overlying uterus are exteriorized through the maternal abdominal incision. The uterus and fetal membranes are then incised, with care taken to avoid cutting of cotyledons. The fetus is exposed.
12. Arterial catheters are introduced in the axillary or femoral artery and securely tightened with 3-4 sutures. A blood gas is taken to check the condition of the fetus.
13. Venous catheters can be inserted in brachial or femoral vein
14. ECG and EEG electrodes are put into place. A little cut is made in the skin and then the electrode is placed under the skin and secured by two sutures and glue.
15. A vascular occluder is placed around the umbilical cord. The occluder is inflated to check how much liquid is needed to completely stop blood flow in the cord.
16. An amniotic fluid catheter is left in the amniotic cavity
17. The fetus is put back inside the uterus. Before closure warm saline is poured in the uterus to replace lost amniotic fluid. The uterus is sewed in two series.
18. **Gentamicin** (80 mg) is flushed in the uterus via the amniotic catheter
19. The electrodes and catheters exit the abdominal cavity through a trocar hole at the flank of the ewe. The electrodes are placed in a sterile glove which is placed in a bag sewed to the skin of the ewe. The catheters are connected to an infusion pump for continuous heparinized saline infusion
20. The median laparatomy is closed
21. The anesthesia is stopped. Once spontaneous breathing has initiated the ewe is extubated.
22. The ewe is placed in the confined space (car), where she can recover.

SOP 2 postoperative care

1. Extra pain killing is only given on indication:
    a. buprenofine (**Temgesic**: 0.3 mg/ml) 6 µg/kg i.m.
    b. flunixine meglumine (**Finadyne**: 50mg/ml) 1 mg/kg i.m. is given daily.
2. The ewe will receive Augmentin i.v. (1000 mg) daily during 5 consecutive days.
3. The arterial and venous catheters are continuously flushed with heparinized saline (25 U/ mL heparin).

SOP 3 fetal monitoring

1. The vital signs of the fetus will be monitored daily by connecting the ECG and EEG electrodes to the monitor.
2. A blood sample is taken from both the fetus and the mother.
3. The fetal arterial line can be connected to a blood pressure sensor in order to measure blood pressure and heart frequency.

SOP 4 administration of endotoxin (LPS)

On day 4 endotoxin (LPS 100 ng/kg) is administered to the fetus via a fetal catheter

- Preparation
    1. Endotoxin (LPS) is prepared under sterile conditions.
    2. LPS (100 ng/kg body weight of the fetus) is dissolved in 1,0 mL of saline in a 1,0 mL syringe.
- Infusion
    3. The stopcock on the fetal catheter is closed
    4. The catheter is detached from the perfusion pump
    5. The stopcock and catheter are cleaned with 70% ethanol
    6. The LPS solution (100 ng/kg) is slowly infused through the fetal catheter
    7. The catheter is flushed slowly with 2,5 mL of sterile saline
    8. The catheter is connected to the perfusion pump to allow for continuous perfusion.

SOP 5 administration of

is given to the fetus through the fetal catheter during 4 consecutive days starting on day 4.

- Preparation
    1. is prepared under sterile conditions in our lab
- Infusion
    1. The stopcock on the fetal catheter is closed
    2. The catheter is detached from the perfusion pump
    3. The stopcock and catheter are cleaned with 70% ethanol
    4. The is slowly infused through the fetal catheter
    5. The catheter is flushed slowly with 2,5 mL of sterile saline
    6. The catheter is connected to the perfusion pump to allow for continuous perfusion.

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

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

Aan:

Uw referentie: | Onze referentie | Maastricht, 03-05-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Improving preterm gut maturation in chorioamnionitis*", is op de DEC vergadering van 29 april 2011 besproken.

De DEC heeft één enkele vraag/opmerking:

- De DEC wenst een aparte onderbouwing voor de berekening van de groepsgrootte van de groepen.

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 **2011-057**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Aan: DEC-UM

Betreft: wijzigingsverzoek DEC 2011-057

27 mei 2011

Geachte leden van de DEC,

Naar aanleiding van mijn project aanvraag "*Improving preterm gut maturation in chorioamnionitis*" had u één verzoek tot wijziging. De DEC wenst een aparte onderbouwing voor de berekening van de groepsgrootte van de roepen.

Zoals u in de gewijzigde DEC aanvraag kunt zien (grijs gedrukt), vragen wij 16 dieren aan om onze interventie allereerst nauwkeurig te titreren alvorens we met de inclusie in de studie beginnen. We testen 4 concentraties in elk 3 dieren. Rekening houdend met 25% uitval komen we op een totaal van 16 dieren voor de titratie.

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-	10-06-2011

Project: *Improving preterm gut maturation in chorioamnionitis*

Verantwoordelijk onderzoeker (VO):

Hierbij 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:	2011-057
Diersoort:	schaap
Aantal dieren:	80 ooien en 80 lammeren
Einddatum:	10-06-2015

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

Vice-Voorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM
T (043)

Bezoekadres

Postadres
Postbus 616
6200 MD Maastricht