**THE UNIVERSITY OF WESTERN ONTARIO**
**BIOLOGICAL AGENTS REGISTRY FORM**
**Approved Biohazards Subcommittee: July 9, 2010**
**Biosafety Website: www.uwo.ca/humanresources/biosafety/**

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website:

| | |
|---|---|
| PRINCIPAL INVESTIGATOR | Dr Barbra de Vrijer |
| DEPARTMENT | Obstetrics and Gynaecology |
| ADDRESS | St Joseph's Health Care London Rm B3-041 |
| PHONE NUMBER | 519 646 6106 |
| EMERGENCY PHONE NUMBER(S) | 519 860 1767 / 519 649 5991 |
| EMAIL | |

Location of experimental work to be carried out: Building(s) _WSB_____ Room(s)_____

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES:__Labatt Heart Centre Innovation Fund _____
GRANT TITLE(S): _1. Creating a Fetal Lamb Model of Hypoplastic Left Heart Syndrome _____
__2. Acute Atrial and Ventricular Epicardial and Endocardial Pacing of the Fetal Lamb _____
_____

List all personnel working under Principal Investigators supervision in this location:

| Name | UWO E-mail Address | Date of Biosafety Training |
|---|---|---|
| Brad Matushewski | bjmatush@uwo.ca | |
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**Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.**

Studies involve pregnant sheep; Level 3 biological hazard as they potentially are a reservoir of *coxiella burnetti*. All researchers involved in these studies will adhere strictly to the ACVS requirements for training and safety regulations.

Studies involve minimal-invasive surgery (through an ultrasound-guided needle into the fetus); to either alter the structure of the fetal heart (project 1) or to alter the fetal heart rate (project 2). At autopsy, only heart tissue will be collected and stored in the Level 3 unit in either formalin or ethanol until Q-fever test results are available and the facility is cleared and tissues can be removed from the sheep unit.

The remainder of the waste; sheep carcasses, fetuses, products of conception, and all disposables will be double bagged and spray disinfected, labeled and stored in the cold room for incineration. Non-disposable items will be autoclaved and needles/sharps will be disposed of into approved biomedical sharps containers.

**Please include a one page research summary or teaching protocol.**

**Protocol 1. Acute Atrial and Ventricular Epicardial and Endocardial Pacing of the Fetal Lamb**

**Project Description:**
Repeated intravenous, intramuscular and intraperitoneal fetal injections of amiodarone, digoxin and adenosine, in addition to the conventional therapy, are performed to resolve drug-refractory fetal reentrant supraventricular tachycardia (SVT) complicated by fetal hydrops which is associated with a 25% mortality rate. While this approach may be life-saving, direct injection of drugs into the fetal circulation leads to an increase in fetal volume load and may further deteriorate cardiac failure and contribute to loss of the fetus. We hypothesize that the conversion of fetal SVT and atrial flutter, the most common causes of fetal tachyarrhythmias, to a normal cardiac rhythm could safely be achieved by direct fetal cardiac pacing similar to transesophageal overdrive pacing after birth. Moreover, fetal pacing could be helpful to treat transient bradycardia. Severe fetal bradycardia at around 50 beats per minute occurs in half of fetuses during intracardiac interventions once the ventricle is punctured by a needle. The preventive injection of atropine or epinephrine does not always overcome the life-threatening slowing of heart rate. Indeed, drug-resistant bradycardia was the main cause of demise of all 3 fetal cases that underwent technically successful cardiac interventions between 19 and 23 gestation weeks.

**Proposed project**
This is a pilot project to assess if ultrasound-guided fetal pacing is technically feasible and could be safely applied in the treatment of the human fetus. We will test the utility of a 1.5F (0.5 mm diameter) microcatheter (PATHFINDER, Cardima) that is approximately one-fourth the size of standard electrophysiology catheters. The catheter has a highly flexible, atraumatic tip attached to its leading end which easily conforms to the contours of the heart wall, thereby maintaining controlled, regular contact even in a fast-beating heart.

We will study 14 pregnant ewes at around 110 gestation days (term 145 days). Animals will receive care in accordance with the animal care regulations. The pregnant ewes will be fasted 24 hours prior to the procedure. On the day of the intervention, anaesthesia will be induced using intravenous Thiopental (40 ml) administered through the jugular vein of the ewe, followed by intubation and ventilation with 1.0-3.0 % Isoflurane and oxygen at 5-6 liters/min.The fetal presentation will be determined by transabdominal ultrasound imaging. Using an ultrasound-guided, strictly percutaneous approach, we will first advance a 20 G needle with a trajectory aiming at the anterior atrial or ventricular cavity, respectively, of the fetus. After advancement of the needle in immediate proximity of the atrial or ventricular epicardium, the mandrel will be slowly retrieved and the microcatheter carefully advanced through the lumen of the needle. Once in contact with the epicardium, we will connect the pacing lead with an external pacemaker system. Endocardial pacing will then be attempted after advancement of the needle across the atrial or ventricular wall. After entering the atrial or ventricular cavity, the catheter will be advanced until its looped distal end is in direct contact with the endocardial surface.
In two groups consisting of each 7 animals, we will either attempt epicardial atrial and endocardial ventricular pacing (group 1) or endocardial atrial and epicardial ventricular pacing (group 2). This will allow us to test 4 different pacing sites and to assess the damage that may be caused by pacing or by the needle. Pacing thresholds and voltage strength-duration curves will be measured and the effect of different pacing sites and increasing heart rates (200–300 beats/minute) on fetal cardiac function evaluated by echocardiography. After removal of the pacing system, the fetal lambs will be followed for another hour for bleeding and effusions. Postmortem examination for any tissue damage will be performed at the end of the procedure.

**Protocol 2. Creating a fetal lamb model of hypoplastic left heart syndrome**

**Project Description:**
Despite almost thirty years of experience with three-stage palliative surgery for hypoplastic left heart syndrome (HLHS) overall morbidity and mortality remain high and hypoplastic left heart syndrome is the leading single cause of cardiac death in the first year of life. Only 60-65% of a cohort of neonates with HLHS will reach the final stage of their palliative surgery at two years of age.The improvements in outcome in the first six months of life have come in small increments, and there is concern that a plateau has been reached.

It has been suggested that HLHS could be produced in fetal lambs by supravalvar aortic banding or inflow obstruction (Fishman 1978, Levin 1980). More recent studies have demonstrated that supravalvar aortic banding produces severe LV hypertrophy rather than LV hypoplasia. The discrepancy was attributed to Fishman et al using autopsy data to demonstrate cavity hypoplasia which in retrospect was due to post-mortem myocardial contracture, whereas Beekman's group used fetal echocardiography (Eghtesady 2007). Chick models have suggested that diminished flow (atrial ligation) can result in a hypoplastic ventricle, and this can be partially reversed by restoring flow (creation of an atrial shunt; de Almeida 2007).

**Proposed project**
This is a pilot project to assess if there is any potential for inducing even mild ventricular hypoplasia with left ventricular inflow obstruction. It is based on the premise that normal ventricular growth is dependent on blood flow through the chamber (Hove 2003), and that disturbances of flow can result in altered development of ventricles. The flow disturbances can arise from a variety of causes which may have a gentic basis e.g abnormal valve development.

We aim to mimic mitral stenosis/mitral atresia by creating inflow obstruction by partially occluding the LV cavity by deploying detachable coils in the LV cavity (n=12) and comparing their ventricular growth with sham-operated controls (n=12). The instrumentation will occur at 70-90 days using a wholly percutaneous transabdominal approach in ewes pregnant with twins, with apical puncture of the fetal left ventricle. Intracardiac pressure swill be measured. Four ewes will be studied at each of 65, 75 and 85 days.The fetal lambs will be followed for 4-6 weeks at which point they will have a repeat ultrasound to measure left ventricular function and dimensions, intracardiac pressure will be measured, and they will then be sacrificed. The heart, aorta and pulmonary artery will be harvested and snap frozen, for later analyses of gene expression. Fetal myocardial tissue will be made available to other Heart Centre projects.

This is a technically demanding study and very few groups have the expertise to attempt these experiments. We are confident that we can safely instrument the fetal lambs, as our team (Greg Ryan, Mt Sinai Hospital; Edgar Jaeggi, Rajiv Chaturvedi, HSC) have already completed a fetal lamb study involvling stenting of the atrial septum (Jaeggi 2007) and we have also performed human fetal cardiac interventions. Greg Ryan is one of the most experienced fetal interventionalists in the world. Barbra deVrijer, our co-investigator in the University of Western Ontario has extensive expertise of working with fetal lambs.

## 1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O YES X NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES O NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:
_____
_____
_____

1.2 Please complete the table below:

| Name of Biological agent(s)* | Is it known to be a human pathogen? YES/NO | Is it known to be an animal pathogen? YES/NO | Is it known to be a zoonotic agent? YES/NO | Maximum quantity to be cultured at one time? (in Litres) | Source/ Supplier | PHAC or CFIA Containment Level |
|---|---|---|---|---|---|---|
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 2+ O 3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 2+ O 3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 2+ O 3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 2+ O 3 |

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

## 2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? O YES X NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

| Cell Type | Is this cell type used in your work? | Source of Primary Cell Culture Tissue | AUS Protocol Number |
|---|---|---|---|
| Human | O Yes O No | | Not applicable |
| Rodent | O Yes O No | | |
| Non-human primate | O Yes O No | | |
| Other (specify) | O Yes O No | | |

2.3 Please indicate the type of established cells that will be grown in culture in:

| Cell Type | Is this cell type used in your work? | Specific cell line(s)* | Supplier / Source |
|---|---|---|---|
| Human | O Yes O No | | |
| Rodent | O Yes O No | | |
| Non-human primate | O Yes O No | | |
| Other (specify) | O Yes O No | | |

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 O 2 O 2+ O 3

**3.0 Use of Human Source Materials**

3.1 Does your work involve the use of human source materials? O YES X NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

| **Human Source Material** | **Source/Supplier /Company Name** | **Is Human Source Material Infected With An Infectious Agent? YES/NO** | **Name of Infectious Agent (If applicable)** | **PHAC or CFIA Containment Level (Select one)** |
|---|---|---|---|---|
| Human Blood (whole) or other Body Fluid | | O Yes<br>O Unknown | | O 1 O 2<br>O 2+ O 3 |
| Human Blood (fraction) or other Body Fluid | | O Yes<br>O Unknown | | O 1 O 2<br>O 2+ O 3 |
| Human Organs or Tissues (unpreserved) | | O Yes<br>O Unknown | | O 1 O 2<br>O 2+ O 3 |
| Human Organs or Tissues (preserved) | | Not Applicable | | Not Applicable |

**4.0 Genetically Modified Organisms and Cell lines**

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? O YES X NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NO

| Bacteria Used for Cloning * | Plasmid(s) ** | Source of Plasmid | Gene Transfected | Describe the change that results from transformation or tranfection |
|---|---|---|---|---|
| | | | | |

* Please attach a Material Data Sheet or equivalent if available.
** Please attach a plasmid map.

4.3 Will genetic modification(s) involving viral vectors be made? O YES, complete table below O NO

| Virus Used for Vector Construction | Vector(s) * | Source of Vector | Gene(s) Transduced | Describe the change that results from transduction |
|---|---|---|---|---|
| | | | | |

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O NO

4.5 Will virus be replication defective? O YES O NO

4.6 Will virus be infectious to humans or animals? O YES O NO

4.7 Will this be expected to increase the containment level required? O YES O NO

**5.0 Human Gene Therapy Trials**

5.1 Will human clinical trials be conducted involving a biological agent? O YES X NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES O NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

**6.0 Animal Experiments**

6.1 Will live animals be used? X YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____SHEEP_____

6.3 AUS protocol # ___2009-066 and 2010-257_____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: __X NO

6.5 Will the agent(s) be shed by the animal: O YES O NO, please justify:

_____

_____

**7.0 Use of Animal species with Zoonotic Hazards**

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? X YES O No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats X YES, please specify species ____SHEEP_____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O NO

**8.0 Biological Toxins**

8.1 Will toxins of biological origin be used? O YES X NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the $LD_{50}$ (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide details:_____ O NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

**9.0 Insects**

9.1 Do you use insects? O YES X NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____

9.7 Do you use insects that require a permit from the CFIA permit? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

**10.0 Plants**

10.1 Do you use plants? O YES X NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree…)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

**11.0 Import Requirements**

11.1 Will any of the above agents be imported? O YES, please give country of origin_____ X NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES O NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

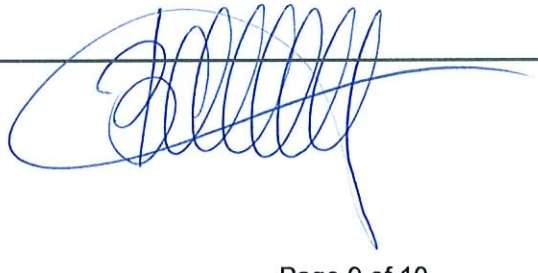
**12.0 Training Requirements for Personnel Named on Form**

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____



**13.0 Containment Levels**

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 O 2 O 2+ X 3

13.2 Has the facility been certified by OHS for this level of containment?

X YES, permit # if on-campus_____
O NO, please certify
O NOT REQUIRED for Level 1 containment

**14.0 Procedures to be Followed**

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at

SIGNATURE _____ Date:_____2010-10-22 _____

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

_____
_____
_____

14.3 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury:

_____
_____

See E-mail

**15.0 Approvals**

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE:_____
Date:_____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE:_____
Date:_____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

E-mail

-------- Original Message --------
**Subject:**Re: Biological Agents Registry Form (deVrijer)
**Date:**Wed, 10 Nov 2010 10:40:08 -0500
**From:**Barbra deVrijer <Barbra.deVrijer@sjhc.london.on.ca>
**To:**Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

14.2 I am not using an additional agent? Find the form confusing. If you want an answer - it is 'none' - the whole sheep unit is set up as a level 3 just because of the Q-fever and all SOPs will be adhered to.

Safety procedure SF-03, SF-04 and SF-05 and SOP WV 115-02, 144-02, 169-02, 621-01, 622-02, 625-02, 626-02, 628-02.

14.3 All incidents, including animal bites and scratches or cuts from cages or other equipment will be documented and the employee should report to Workplace Health for medical assessment and follow-up. If an animal is found to be Q-fever positive exposure through aerosols is highly likely and follow-up with Q-fever titres is recommended.

Can't help but comment - I may hope you have these SOPs and therefore have the distinct impression this form is a test or a quiz and not a form. I know, don't shoot the messenger...
Barbra