**IACUC Training Exercise #2 – 2019 (Which Category – Part 2)**

The following exercise may be useful in stimulating discussion regarding compliance with PHS Policy and VA Handbook 1200.07. To facilitate discussion, pages 1 and 2 of the exercise may be distributed to the IACUC members prior to a meeting. After a few minutes of discussion about the exercise during the meeting, the remainder of the exercise may be distributed to provide ideas for the committee’s consideration.

Dr. Samantha Hollingsworth, a cardiologist, will be joining the Hometown VAMC in a few months. In preparation for her move, she has submitted two protocols to the IACUC for review and approval. The primary focus of Dr. Hollingsworth’s research is to develop more effective treatments for peripheral vascular disease (PVD), which affects as many as 20% of people in the US over the age of 60, including veterans. The causes of PVD include both acute events (blood clots that block arteries) and more commonly gradual narrowing related to atherosclerosis (fatty plaque deposition inside vessel walls). Dr. Hollingsworth wants to bring with her a breeding colony of Watanabe rabbits that have heritable hyperlipidemia (high levels of fat in the blood) and spontaneous progressive atherosclerosis (<https://www.sciencedirect.com/science/article/pii/S0021915009002366>), which makes their vascular system similar to that of many veterans. Dr. Hollingsworth’s next research project is to study a new agent, XXX, which promotes the development of collateral arteries after sudden occlusion of an existing artery. Some have raised doubts that XXX will still work if gradual narrowing of the arteries has already initiated collateral development before the occlusion occurs. The Watanabe rabbits will allow Dr. Hollingsworth to study XXX in the settings of acute occlusion, progressive narrowing, or both.

These are the protocols that the IACUC members received:

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| Protocol number | Summary | USDA Pain & Distress Category |
| 101-2019 | This protocol describes all aspects of colony management, including veterinary care. Dr. Hollingsworth specifically discusses that some rabbits will likely need treatment for malocclusion, gastrotomy for surgical removal of trichobezoars (hairballs), treatment of fight wounds, etc. | D |
| 102-2019 | To reduce the need for surgical interventions for PVD, Dr. Hollingsworth is working on other ways to restore blood flow. Her hypothesis is that XXX works by a different mechanism than other drugs that mediate an arteriogenic (formation of new blood vessels) response to gradually restricted flow, so she expects XXX will be equally effective, regardless of the time course over which the flow restriction develops. This protocol will involve eight experimental groups of 10 rabbits each, comparing young adult and geriatric NZW rabbits with young adult and geriatric Watanabe rabbits, with half the rabbits receiving XXX and half receiving vehicle: 1. NZW young adult rabbits receiving vehicle
2. NZW young adult rabbits receiving XXX
3. NZW geriatric rabbits receiving vehicle
4. NZW geriatric rabbits receiving XXX
5. Watanabe young adult rabbits receiving vehicle
6. Watanabe young adult rabbits receiving XXX
7. Watanabe geriatric rabbits receiving vehicle
8. Watanabe geriatric rabbits receiving XXX

Each rabbit will be trained to exercise on a treadmill (fed low calorie treats while on treadmill to encourage exercise), and then anesthetized for surgical ligation of the right femoral artery (the left femoral artery will not be ligated - sham control). Standard post-operative analgesics for 72 hours will be provided for incisional pain. The ligation is not expected to result in any readily evident changes in appearance or function of the right hindlimb at rest, but the rabbits may be reluctant for a week or two after the ligation to exercise on the treadmill. Flow conductance in each leg will be calculated from perfusion and pressure measurements made during a terminal study 4 weeks after the ligation. | D |

When the IACUC convened for its monthly meeting and discussed Dr. Hollingsworth’s two protocols, questions were raised about the USDA pain and distress category assignments of the rabbits on each protocol. For the breeding protocol (101-2019), Dr. Diaz, the attending veterinarian, explained that it is highly unlikely that every rabbit on the protocol would require anesthesia to treat a wound or other condition. She suggested the IACUC approve one-third of the total number of breeding colony rabbits for Category D, while the rest could be assigned to Category C. For protocol 102-2019, the Non-Scientific Member brought up his own experience with intermittent claudication (pain associated with decreased blood flow to the legs, especially during exercise), a common symptom of PVD. He wondered whether the experimental rabbits should be assigned to Category E because there was no indication in the protocol that analgesics would be administered to relieve possible pain associated with impaired blood flow to the right hindlimb.

To which categories do you think the IACUC should assign the rabbits?

The key to making decisions about the assignment of animals to USDA categories is the language in the Animal Welfare Regulations, and on the USDA APHIS form 7203 for annual reports of registered institutions, defining the categories:

Category D is for “animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used”;

Category E is for “animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests.”

For the breeding protocol (101-2019), several procedures may require anesthesia for wound repair, hairball removal, etc. but these procedures would only be performed for clinical veterinary care reasons. According to guidance provided by the USDA, and summarized in VA Guidance Document AR2019-001 (<https://www.research.va.gov/programs/animal_research/guidance.cfm>), procedures performed to benefit the individual animal should not be taken into account in decisions about the assignment of animals to USDA categories. Therefore, all of the animals on the breeding protocol should be assigned to Category B.

For the research protocol (102-2019), each of the rabbits will undergo surgical ligation of the right femoral artery under anesthesia, so all of them should be assigned to at least Category D. The question is then whether the potential for the rabbits to experience pain as a result of the limitation of blood flow to the right hind limb requires their assignment to Category E. The potential for pain alone is not sufficient grounds for assignment to Category E. Category E requires that (1) there are appropriate analgesic, anesthetic, or tranquilizing drugs to manage the pain, and (2) that those pain-relieving drugs be withheld because their administration would adversely affect the “procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests”.

The literature indicates that femoral artery ligation is known to limit blood flow without causing any tissue damage, grossly impaired function, or even detectable ischemia at rest (<https://academic.oup.com/cardiovascres/article/49/3/609/309441>). The NZW rabbits and the young adult Watanabe rabbits (with little atherosclerosis) may only experience discomfort related to the limitation of blood flow when they attempt to exercise on the treadmill, and this would be addressed by allowing them to stop when they show any sign of reluctance to exercise. Geriatric Watanabe rabbits, with both advanced atherosclerosis and femoral ligation, are more likely to experience intermittent claudication pain, even at rest. In humans, symptomatic relief of the pain of intermittent claudication is achieved by limiting the activity that increases the demand for blood flow to more than can be supplied, and by administration of drugs to improve blood flow. Administering these drugs would interfere with the limitation of blood flow under study, so they would be withheld in this protocol because of their adverse effects on the research, but these are not “analgesic, anesthetic, or tranquilizing drugs.” Give the concern about pain relief for the rabbits, especially the geriatric Watanabe rabbits, the IACUC asked Dr. Diaz for advice about the clinical signs indicating that the rabbits were painful and what pain-relieving drugs might be used. Dr. Diaz suggested that if a rabbit refused to walk on the treadmill for treats or if the rabbit abruptly stopped walking on the treadmill that these behaviors would be good indicators of pain. As for which drugs could be used, Dr. Diaz said NSAIDs like ketoprofen, an opioid drug, such as buprenorphine, or maybe even a tranquilizer could be used but she thought Dr. Hollingsworth would be concerned about these drugs compromising her study because:

NSAIDs, as whole, raise blood pressure by constricting blood vessels (<https://www.health.harvard.edu/press_releases/nsaid-side-effects>).

Opioids, including buprenorphine, a mixed opioid agonist-antagonist, can cause peripheral vasodilation (<https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=23aa1bb3-cecf-4e62-29bb-48488bb66fc3>).

Tranquilizers, such as acepromazine, would help keep the rabbits quiet and calm but they also have vasodilating effects (<https://www.sciencedirect.com/topics/neuroscience/acepromazine>).

Based on the information provided by Dr. Diaz, the IACUC voted that modifications were required to secure approval; they wanted a justification from Dr. Hollingsworth as to whether or not, she could treat the rabbits that appeared to be in pain with analgesics or tranquilizers. If she agreed to treat the rabbits with pain-relieving agents, category D would be appropriate; however, if Dr. Hollingsworth adequately justified that the use of pain-relieving drugs would compromise her study, then the withholding of these drugs would require assignment to category E.

Regardless of the category assignments, it is important to keep in mind that the charge of the IACUC is to oversee animal welfare. The IACUC’s responsibility is to consider the potential for pain and distress and if pain and distress is possible to then clarify how the pain and distress will be managed. The committee may want to consult with outside experts to fully understand the effects of femoral ligation with and without atherosclerosis and seek advice on clinically appropriate treatments for relieving any resulting pain or distress. In this case, the Hometown VA-IACUC acted appropriately by requiring Dr. Hollingsworth to make it clear in the protocol how the rabbits will be monitored for pain, and what measures will be taken, if possible, to minimize the pain that they experience without compromising the scientific objectives.