Title: Addressing animal welfare issues in fetal blood collection for FBS production

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Abstract

There is ethical debate over whether fetal calves suffer when their dam is slaughtered and fetal blood extracted by cardiac puncture for FBS production, yet the serum industry does not follow recommended best practice to avoid fetal distress. We discuss the key elements of this debate and recommend how the serum industry can alter its practices to improve animal welfare.

Introduction

Fetal bovine serum (FBS) is used widely as a media additive for the *in vitro* culture of human-derived cells. There are several important issues that scientists should consider regarding its use in scientific research and bioprocessing. Firstly, the use of high levels of FBS (up to 10%) in the culture medium of human-derived cells raises questions regarding its impact on the behavior and phenotype of those cells, and thus their relevance as models of human biology and disease. Furthermore, the variation in composition of FBS between batches seriously undermines the validity of its use in many scientific applications. Secondly, the global beef industry will come under increasing commercial pressure during the next two decades, due to concerns of its impact on climate change, and the growth of markets for meat alternatives - whether these be increasing vegetarianism/veganism or the commercial development of laboratory-grown, FBS-free, meat sources. FBS will thus become an increasingly scarce, and therefore expensive, commodity. Finally, there is significant ethical debate about the extraction of blood from a calf fetus for FBS production: specifically, whether the fetus feels pain, and whether it is dead or alive at the time of blood collection.

The animal welfare and ethics issues surrounding the practices of collection of fetal blood for FBS production have recently been discussed in two commentary articles (1, 2, published in the April 2021 edition of ALTEX). They describe many of the issues and areas of disagreement between the serum industry and animal welfare advocates. Whilst we welcome the International Serum Industry Association (ISIA)'s reiteration that it "shall establish, promote and assure compliance with uncompromised standards of excellence and ethics in the business practices of the global animal serum and animal derived products supply industry"(1), we fail to understand how its reliance on the application of unenforceable guidelines provided by the World Organisation for Animal Health (OIE) and the European Food Standards Agency (EFSA) equate with these "uncompromised standards of … ethics". These articles summarize the scientific work that underpins the regulation of practices for the slaughter industry, including the retrieval of fetal material from slaughterhouses. We believe there are additional important areas to address for the open and rational evaluation of the ethics of collecting fetal blood and using FBS. We will attempt to address these areas here.

Sentience and Consciousness

It is broadly accepted that, for an organism to perceive positive or negative sensations (more correctly termed "affective states"), it must be both sentient (able to perceive itself and its surroundings) and conscious (aware of its surroundings) (3, 4). If a sentient organism is unconscious it cannot feel pain, which is a good thing for patients undergoing surgery. Equally, if a non-sentient organism is conscious, it is generally accepted that it cannot suffer, and we do not consider that unicellular organisms, for example, feel pain.

Fetal calves, as most other mammals, are generally considered to have acquired the anatomical capacity for sentience by the end of the second trimester of pregnancy at the latest (5). Brain electrical activity is detectable, as are responses to external stimuli. It is very difficult to establish whether the presence of appropriate functional neuroanatomical structures in a fetus can be said definitively to equate to sentience. However, many jurisdictions have legislation stating that mammals (and other animals) are sentient and worthy of protection, and that this protection extends to fetal forms in the second half of gestation (6). Ethically, it is wise to err on the side of caution and assume that, if there is connection of nociceptors to sub-cortical structures of the brain, and electrical activity in the brain, then the fetus should be considered to be sentient.

Consciousness ought to be a more straightforward condition to establish, but this also remains a controversial issue. We must consider the status of the calf fetus from before slaughter, through the slaughter process, and until the moment that all blood has been extracted from the fetus by cardiac puncture. The serum industry maintains that fetal calves are unconscious in the womb during transport and slaughter of the dam, and then dead when blood extraction takes place (1). Clearly there can be no rational argument that says that a dead animal suffers when a procedure is conducted on it and, as described above, if an organism is unconscious it cannot feel pain. Several questions arise: 1) Is the calf truly unconscious in the uterus prior to its death? 2) If not, does the calf undergo a humane death? 3) How is death ensured prior to extracting blood?

Guidelines for handling pregnant livestock and for humane treatment of the fetus are heavily reliant on the work of Professor David Mellor, Professor Emeritus of Animal Welfare Science and Bioethics at Massey University, New Zealand. In a series of papers and reviews

(7, 8), Professor Mellor and his colleagues reported a body of evidence suggesting that ungulate fetuses are maintained in a "sleep-like" state that ensures their quiescence during pregnancy. They contend that a series of endocrine and physiological factors, including relatively low fetal oxygen tension, high levels of circulating adenosine, pregnanolone, prostaglandin D_2, and an unidentified placental factor, all suppress fetal cortical activity and "awake" behavior. Much of this work was undertaken on lamb fetuses, and it is reasonably assumed that calf fetuses undergo a similar developmental sequence at equivalent stages of pregnancy.

In terms of fetal welfare, the key message propounded by Professor Mellor's work is that "the fetus displays EEG activity indicating that it is continuously asleep *and therefore* unconscious" (8; our emphasis). This conclusion is used to argue that an unborn fetus cannot suffer at any time during gestation, birth, slaughter of the dam, or extraction of blood *via* cardiac puncture. This extrapolation, in which being asleep is equated with being unconscious, goes against many peoples' personal experience, and there seems no reasonable justification to apply it to fetuses. Clearly being asleep is not equivalent to being unconscious – no one would want to be operated on when asleep! While many studies are cited to highlight how very difficult it is to demonstrate that fetuses spend time awake and conscious (8), it is far from established that fetal sleep is a truly unconscious state in which the animal cannot experience pain or negative affective states. Merker (9) and Panksepp (10) argue that fetuses can indeed experience sub-cortical consciousness while apparently "asleep". Bellieni (11) cites data that human fetuses, which are born at an earlier developmental stage than calves, are not always in a sleep-like state, and that they can be aroused from sleep by external stimuli. Therefore, fetuses may well experience negative

affective states. This possibility is explicitly described by the EFSA (12). Following the principle of maximum precaution, we believe that FBS producers and legislators should assume:

- That third trimester calf fetuses can spend time in a wakeful state
- That they might experience sub-cortical consciousness, and
- That they could be aroused from a sleeping state by noxious stimuli.

In modern, well-regulated, and correctly monitored slaughterhouses (from which the ISIA maintains that commercial FBS derives), cows are stunned prior to slaughter. As such they cannot feel physical pain at the time of death. There are exceptions to this general rule of stunning animals prior to slaughter, for example when meeting kosher and some halal religious requirements. Having been rendered insensate, the cow is killed, usually by having its throat cut or its heart opened, leading to a catastrophic loss of blood pressure and death within 60 to 90 seconds. Whatever one's opinions about the ethics of farming animals and eating meat, if an animal is rapidly (<1 second) rendered unconscious before it is killed, it cannot suffer distress at the moment of its slaughter. The fetus, however, is not stunned, rather it is left to die of asphyxiation in the uterus, as a result of the lack of oxygen supply from the now-dead dam. Is its death humane?

Is the death of a fetal calf humane?

The arguments supporting the idea that the fetus' death is humane, derived from the previously cited work of Mellor and colleagues (3, 7, 8), is that the fetus is maintained in an unconscious state when its dam is killed, and that lack of oxygen leads to its death without arousal from unconsciousness. The counter-arguments are: that the fetus is not always

asleep during pregnancy; that it may be aroused by the dam's distress at being in a slaughterhouse; that the natural sedatives produced in the fetus (adenosine etc.) are not analgesic and that fetuses can and do feel pain; and that the lack of oxygen is likely to elicit fetal distress during the course of the several minutes of its death (5, 11).

The time taken for fetuses to die from asphyxiation is said to take somewhere between 5 and 15 minutes (see 13, but no evidence is presented for this). Versteengen *et al* (1) maintain that slaughterhouse procedures operate at such a pace that it routinely takes at least 20 minutes between killing the dam and collection of the fetal blood. However, there is no legal or ISIA requirement for a minimum time before extracting a fetus from the uterus. Furthermore, there is no requirement to ensure that the fetus is indeed dead, so the risk remains that blood is being collected from a living fetus that could return to consciousness once exposed to air.

The EFSA's recent Scientific Opinion on the 'Welfare of cattle at slaughter' (12) concluded that, based on available scientific evidence, it is not clear whether fetuses of livestock feel pain or not. The Scientific Opinion acknowledges the scientific difference of opinion about the state of consciousness of a fetus when its dam is slaughtered. As a result, it is estimated that there is a two-thirds or greater probability that fetuses do not perceive pain, and up to a one-third probability that they do feel pain during the slaughter process. The Scientific Opinion further suggests two possible scenarios for dealing with the fetus, depending on whether or not one believes that the fetus can feel pain. The *minimum* requirement is to leave the fetus in the unopened uterus for *at least* 30 minutes after killing the dam, to ensure that it is dead by the time it is retrieved from the uterus. Given the significant risk that the fetus might experience pain or other negative affects during this 30 minutes, the

avoidance of harm principle suggests following the EFSA's alternative recommendation that, when a pregnancy is detected in a slaughtered cow, the uterus should *immediately* be opened and the fetus stunned and killed according to EU Reg 1099 (14).

Conclusions

We believe that the ISIA should, at the very least, ensure that its members adhere to the EFSA's minimum requirement. However, if they genuinely wish to "assure compliance with uncompromised standards of … ethics", why would they not require their members only to collect blood from calf fetuses that have been stunned and killed by approved humane practices? Indeed, a former chair of the ISIA has recently acknowledged that challenges facing the serum industry include the need to "demonstrate and certify that safe and ethical collection methods for FBS are used at all collection sites" (15). Following the more conservative EFSA recommendation would be a strong signal that the serum industry took its ethical responsibilities seriously.

Given the ethical issues surrounding fetal blood collection described here and in references 1 and 2, the potentially negative impact of using FBS in experiments using human cell models, and the future limitations to FBS supply, surely now is the time for researchers and funders to adopt more modern, and scientifically- and ethically- relevant approaches to human cell culture-based research, and to move away from the use of FBS.

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