

1) UOM Final Trial Report Context and Review by John Steinfort BVSc.

The Final University of Melbourne Trial Report was finalised at COB 1st April 2021.

The Final Report requires scientific interpretation and statistical significance analysis understanding, to be viewed in conjunction with the Scientist's report, comments and explanations.

For the interests of all industry stakeholders, the science is often required to reflect what is witnessed under field conditions. The Final report and it's science does supports part of the story and as we know it, and we also know that there is generally a requirement for differing foci in science studies to give greater depth to add to our current knowledge. This also applies to SFB and other animal husbandry practices within the Australian wool industry.

This Sheep Freeze Brand process is relatively new to the wool and sheep meat industries and has attracted much attention. I believe after reviewing the findings of the UOM Final Report, many past observers may confer with me that it may not have necessarily captured the exact data according to what is seen and witnessed by many observers. However, it shows scientifically, that there is an important role for SFB as a humane process for the long-term benefit of the sheep breech health with resultant ease of management for the managers and growers. And that is an important step.

The following information is an account of my observations, comments, and scientific understandings of aspects of Sheep Freeze Brand (SFB) process seen in conjunction with the Final Trial Report by the University Of Melbourne (UOM) and with my understanding of animal husbandry procedures in Australia.

- Firstly, in regard to the trial setup, there was a pressing requirement to conduct a scientific trial in 2020, and so some of the confounding variables on the trial property and omissions in trial protocols due to time restraints, unfortunately had to be put aside, for the sake of conducting and completing a scientific trial. As well managing, the Covid 19 restrictions proved to be both manageable and challenging.
- The UOM Final Report in part reflects what we observe in the field: in that there are low numbers of abnormal discomfort/pain related behaviours in the trial. What we do see in the field, is significantly lower numbers of discomfort/pain related behaviours. This is one of the limiting factors of conducting observations in containment yards rather than out in the field. This phenomenon is scientifically understood and relates to the animals modifying their processing of the pain/discomfort given their environment or immediate

circumstances or stress levels. In our work, we might see a very small percentage of lambs or weaners with slight hunched stance whilst in the holding yards, whilst the remaining cohort are standing with normal postures. Given the opportunity to walk back to their paddocks and graze and motherup or group back up as in weaners, normal behavioural responses are seen consistently seen with the grower feedback reporting normal behaviours and with some amazement in their statement "looks like nothing has happened".

- This compares to other animal husbandry situations with significantly high % of recumbent wether lambs expressing abnormal behaviour from castration pain, after lamb marking without pain relief.
- From my observations, the Pain Relief effect on the associated Mulesed cohorts show a reduced impact from Mulesing in the first 3 days.
- However, customers for Australian wool are requesting and paying a differential for wool from Non-Mulesed sheep.
- We see that the blood and open wounds associated with Mulesing is in distinct contrast to the closed skin healing effect of Freeze Brand (FB) groups and as the trial scientist notes, it is difficult to differentiate the breech skin of Castration/Tail (C/T) and the FB lambs. This is a significant differential as FB is bloodless, slow in the skin changes whilst producing a plain breech for life. According to all our growers surveyed, the FB process is minimising flystrike predisposition, and provides ease of shearing and crutching with constantly noted minimal stress shown by the animal when crutched, and easier work for the crutchers.
- Every Scientific Trial has its strengths and limitations. In this trial, there were several extraneous variables as explained by the author. The trial was also limited by low sheep numbers, lacking a sham control group (no processes applied) and the use of small containment yards for observations on Day 1 & 2.
- In our observations, the process of walking back to paddocks, coupled with grazing and mothering activities or mobbing up for weaners reflect a positive animal welfare outcome.
- In consultation with other veterinary scientists, the advice given to myself was to conduct multiple trials over time, where there would be a multiplicity of behavioural, locational and productivity parameters considered, so as to give a broader picture of the animal welfare and animal management benefits.
- Further trial work considered is objective field data, sourced by proximity and mobility metering on lambs and ewes in their paddock. I believe this data with larger numbers will further add to the scientific data and to what is seen and experienced by many wool growers who have utilised the freeze brand process.
- Veterinary perspective and experience in this cryogenic field.
 - In the trial report, there was nil or very little difference in visuals of the breech skin of the C/T and FB groups in first couple of weeks as commented.
 - The freeze thaw cycle does invoke a degenerative cellular response somewhat similar to vaccine responses. This cellular response will cause a variable local and systemic response and when livestock are in containment yards, this response will possibly be more amplified compared to that in their natural environment. This

response doesn't mean the animal is necessarily feeling pain, rather the animal is experiencing a systemic physiological response in mediating an cellular response to freeze thaw cycle that most people have experienced. The difference being that the total targeted skin thickness is undergoing a cellular response, not the superficial or upper layer skin response as seen with wart or skin lesion cryogenics. The difference being the significant nerve deactivation action of full skin thickness freeze thaw cycle.

• I believe the Meloxicam pain relief showed significant value in this trial.

Meloxicam has two pharmacological actions.

- I Anti inflammatory
- II Systemic analgesic.

The anti-inflammatory component slows the bodies reactive physiological responses to the degenerative cellular phase initiated by the freeze thaw cycle. Also reduces the inflammatory responses from all processes, including castration, tail docking and Mulesing.

In our previous work, and undertaken without pain relief, the lambs showed discomfort/pain from the castration ring effect than any effects of the breech skin freeze thaw cycle.

Systemic Meloxicam, either oral or injectable is like an extended action Voltaren providing the optimum outcomes for animal husbandry practices. Utilising either systemic or localised pain relief or both combined for the once off animal husbandry practices, should be a recognisable and rewarded gold standard, reflecting the grower's animal welfare credentials.

2) Interpretation of UOM Final Results

a) Statistical significance and associated P values.

From my understanding,

If the P value is under 0.05, then the results are considered statistically significant and therefore repeatable.

If the P value is under 0.001, then the results are highly statistically significant. Therefore, a high probability of being repeatable.

Hence the bold font for the more statistically meaningful data in the Final Report. The data interpretation according to the P values is very important.

b) <u>Day 2 Data</u>. Dr Ellen describes the low number of abnormal behaviours observed.

If you refer to Table 4, Day 1 behaviours, the 3 values are given.

The first value is the mean, the second the median and third, the mid spread or middle value. (refer to point c) following)

So, on any one scan/observation out of 24 possible scans per day, and out of possible 30 lambs per group, the median numbers noted, say for the (Hunch Severe Standing) HSS observations are recorded as such; one lamb with HSS in C/T group, two lambs in FB, 3 lambs in MM and two lambs in MT. With a P value of <0.001, so high rating for Statistical significance.

So, on overview, the number of discomfort/pain related behaviour for this category are seen in relation to the mob size of x30 lambs.

So, the incidence on that and subsequent observations are low in relation to the whole mob.

Often the focus of results is narrowed to the differential between the stats, rather than understanding the meaning of the values in context of the whole group. The context of the results require an understanding of the interpretation of the data and what the values actually represent, combined with the discussions in the report.

This confirms with our work with the freeze brand process, in that the observable animal impact of a mob of lambs in very low. (Refer to grower's testimonials and references on website).

Interpretation of the Tables.

There are three values given in the Behavioural tables. The interpretation of these values are as follows according to Dr Ellen Jongman.

The values in the table are the mean, median and the Inter Quartile Range in brackets. The IQR is the midspread, or the middle 50%. Of the data is very skewed to one side or there are a lot of 0s, the median and IQR is more meaningful to show where most of the data is. The mean can be heavily influenced by just a few outliers, which can be important in itself, so I have presented these 3 values to give a complete picture of the data.

In regard to the observed behaviours with higher on average P values (indicating lower stat. significance) and then using these factors to combine as aggregates, Dr Ellen Jongman's response is copied her for your understanding with her permission.

Regarding aggregates of behaviour, there are two points. First of all the nature of statistics. Low numbers and/or large variation make it hard to draw any conclusions with 95% certainty, even though the effect may be real. If I throw a dice 4 times and it comes up with a 5 for 3 out of 4 times, is the dice weighted towards 5? By combining a few different measures that all indicate a similar thing, you can strengthen the confidence in your conclusion. So different measures that all indicate discomfort can be combined to come to a more robust conclusion. In addition, different procedures can produce different types of pain, which can be expressed with different behaviours. So Mulesing pain may be a little different from freeze branding pain and different again from castration. In order to compare different procedures, you can combine behaviours that indicate discomfort or pain to come to some sort of conclusion.

c) Further to the trial set up and protocols.

Melbourne Uni. Animal Ethics Committee were unable to add an additional control group to the trial within the trial start time frames.

We understand that approx. 90% of the Australian Non Mulesed (NM) Genetic category lambs go through the lamb marking only process without PR and the majority of producers utilise castration and tail rings. From our field work and observations, there are considerable behavioural changes in lambs with rings on tails and ring castration. This cohort and industry standard comparison, missed out on trial inclusion and would have given a more complete picture of Australian wool/sheep industry animal husbandry practices at lamb marking. This would have given some comparative index for NM Genetics status behavioural outcomes in

comparison to SFB at Lamb marking. Rather than, as with this trial, a comparison with Lamb mark with Meloxicam (low industry practice) in comparison to Mulesing with M (low industry practice) and Mulesing with TS (common industry practice) and FB with M. (most commonly practised with voluntary PR (approx..95%) is utilised.

d) <u>Whole of life animal welfare studies</u> would reflect the lifetime outcomes experienced when NM Genetic sheep are crutched or become fly struck. It is a well-known fact there is increased animal trauma with loose breech and tail skin and the effects on the comfort/stress of both shearer and animal are noted.

This Final Report with its findings and both its value and confounding factors are as outlined by the author. Sound judgement with scientific knowledge and scrutiny is best employed in its interpretation and in light of the knowledge known for whole of life experiences for NM sheep and their managers.