The effect of premating copper edentate injectin on fertility parameters in dairy cattle

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Abstract

Aim: To investigate the impact of 200mg of calcium copper edetate administered prior to the start of mating to lactating dairy cattle at pasture under New Zealand conditions.

Method: Just over 3000 cows from seven farms across New Zealand were paired and randomly divided into treatment and control groups. 4ml of Cue Injection® (International Animal Health Products Ltd, ACVM #A7711) was administered by subcutaneous injection in the caudal fossa to the treatment group. The control group was left untreated.

Results: There was no impact on survival from Mating Start Date (MSD) to 1st Service. There was however a negative impact on survival from MSD to pregnancy on two farms. (1-way p<0.001 and p=0.051). The impact on one farm in particular was enough to show an overall detriment of treatment when farm results were analysed together (1-way p=0.036).

Conclusions: Treatment with 200mg calcium copper edetate 10 days prior to mating increases the risk of significant reproductive failure through early embryonic loss. Treatment with parenteral copper supplementation within 10 days prior to MSD and during the mating period is not recommended.

Introduction

Trace minerals have long been recognized as having an important role to play in maintaining optimal animal health. In New Zealand (NZ) deficiencies in cobalt, iodine, copper and selenium have been recognized as causing clinical syndromes such as bush sickness in sheep (1893), goitre (1925), swayback in sheep and ill-thrift in calves (1940+) and white muscle disease (1960) respectively. Significant effort in the mid to late 20th century saw a rapid increase in the knowledge of the epidemiology, definition of normal levels, diagnosis of deficiency and the supplementation of these trace minerals. In the case of copper the effect of interactions with sulphur, molybdenum and iron on the uptake of copper from the gut were also elucidated.

Thanks to the research and extension activities undertaken by relevant industries we now find ourselves in a period where trace element deficiencies resulting in clinical disease are less often seen. Current activity in practice largely focuses on the appropriate management of various forms of supplement to maintain freedom from clinical deficiency. In the case of copper, forms of supplement include application of copper sulphate in the fertiliser; orally administered copper sulphates, amino acids and chelates via water systems or as drench components; orally administered copper oxide boluses and parenterally administered copper glycinites and edetates.

Parenteral administration of copper has been associated with unsightly lumps and discomfort at the site of injection. This has been more so with some of the earlier products rather than the more recent. However parenteral administration has the advantage of delivering copper directly into the animal, bypassing the gut and the mineral complexes that adversely affect copper uptake. They are also relatively easy to administer when
compared to oral administration of boluses and require a very small investment in equipment to deliver the copper to the cow.

The amount of copper delivered from a single parenteral administration (2–4ml of currently registered 15-50mg/ml products) is usually lower than a single orally administered bolus requiring repeat doses at strategic intervals to maintain adequate copper status in the animal. Under New Zealand seasonal conditions the following factors put pressure on the copper levels of the typical NZ dairy cow: foetal demand for copper to enable growth; decreased intakes of mid-late gestation cattle; administration of zinc to prevent facial eczema in the late summer-autumn; dry period grazing off the farm at run-offs with varying fertility and/or antagonists such as iron sands; commencement of lactation and reduced ability of the cow to meet her DM intake requirement as she transitions into lactation; seasonal increases in dietary antagonists such as molybdenum over the wetter spring period; application of sulphur in fertiliser to promote pasture/crop growth. The net result of these factors is that copper reserves in the dairy cow are often at their lowest in the early to peak lactation periods when calving, lactation and mating are also placing metabolic demands on the cow. (Hawkins 2004) Subsequently strategic copper supplementation practices revolve around ensuring that copper levels are not limiting at these critical times.

The use of parenteral copper in the early lactation is a regular part of the trace-mineral supplementation programme on large numbers of dairy farms across NZ. In certain circumstances monitoring of trace elements occurs in conjunction with early lactation monitoring of macro-minerals such as magnesium. Subsequently correction of diagnosed copper depletion often takes place in the early lactation and may occur before or during the mating period.

The timing of parenteral copper administration has been a source of varied concern for a number of years. The desire to avoid clinical copper deficiency, to maximise reproductive performance and avoid any negative sub-clinical impacts that depleted copper reserves may cause have to be balanced with the concerns about the impacts of treatment.

One stream of anecdotal evidence from vets and farmers across NZ suggests no impact on either milk production or fertility when parenteral copper treatments have been administered in the early lactation. Indeed Whitaker (1982) demonstrated no impact of treating cows with copper glycinate prior to mating on calving to first service intervals, services per conception and first service conception rates. Conversely concerns have been expressed on the potential for negative impacts on milk production and reproductive outcomes of the herd of parenteral copper injections given in the early lactation. Cummins and Harris (1984) associated reduced fertility with parenteral treatment with copper glycinate in a case study of an Australian dairy herd and a small controlled study in heifers. Severe injection site reactions were also noted in this report and may have impacted the outcome. Wiseman (2002) reports a small controlled study dating from 1983 associating an injectable formulation of calcium copper edetate (now withdrawn from sale in NZ) with fertility reduction in dairy cows. So does parenteral administration of copper reduce fertility? Wakelin (1991) states, “It seems prudent to avoid injection of copper compounds immediately prior to or during mating where feasible, until such time as this question is answered.”

In balancing the duration of effect of different treatment options with the possible side effects of treatment and the desire to eliminate risk of deficiency, the timing of treatment becomes a critical issue.

**Objective**

The objective of this study was to determine whether administration of 200mg of copper calcium EDTA (4mL Cue Injection®) at 10 days prior to PSM has any observable effect on dairy cow reproductive performance and answer the concerns voiced by Wakelin in 1991.

**Materials and methods**

The study was conducted in seven New Zealand commercial dairy herds. Herds were selected from the Waikato, Taranaki, Canterbury and Southland regions. 3084 cows were enrolled in the study.

Cows included in the study were in good health and had not received any form of injectable copper supplementation in the previous three months or any long-acting oral bolus copper supplementation in the preceding six months. No other form of copper supplementation was administered over the duration of the study period.
Cows included in the study had available birth, calving, health and mating records, no evidence of clinical illness or reproductive pathology, were between BCS 3.5–6.0 at enrolment and had calved at least 30 days prior to the start of mating.

Cows were identified by herd tag and were paired on the basis of age, breed and days calved. Within the pairs, cows were randomly allocated to either the treatment or control group. There were equal numbers of treated and control animals on the farms enrolled. Body Condition Score (BCS) variation within herds was reported as very low by attendant veterinarians. Trial animals were grazed as a single herd on each farm. They were grazed, fed and managed under routine conditions and practices standard for the herd.

The treatment group was injected by the herd’s attendant veterinarian with the maximum recommended dose of 4mL Cue Injection® (200mg calcium copper edetate) 10 days prior to PSM. Treatments were administered by subcutaneous injection in the anterior half of the neck or in the caudal fossa. The control group remained untreated.

Heat detection and Artificial Insemination (AI) was conducted for at least 24 days from PSM and all heats and submissions to AI recorded. All cows were pregnancy tested using ultrasound at 12 weeks following PSM to determine pregnancy rates and first service conception rates.

Statistical procedures
Submission rate and conception rate data were collated for each farm and group. Cumulative submission rates and conception rates for each farm and group were plotted. Survival to 1st AI Submission and to Conception was plotted for each group and survival analysis for paired data completed.

P values presented are one way or single tailed values to three decimal places. The rationale for using one way tests is as follows; there was unlikely to be a beneficial effect of supplementation because the herds enrolled in the study have been on copper supplementation programmes over previous years. Individual herds would have been in various stages of depletion resulting in marginal to sufficient copper status at the start of the study but the likelihood of copper deficiency at a herd level in the author’s opinion was low in spite of not receiving copper prior to the study as stated above; previous case reports of Cummins and Harris (1984) and Wiseman (2002) have demonstrated adverse impacts with a similar product at a different dose prior to mating and adverse impacts were therefore the impacts in question; a hypothetical mechanism of detriment to embryo survival exists; and a desire to err on the side of caution rather than on the side of negligence.

Results
There were 2520 cow (1260 paired) data sets available for conception analysis and 2022 cow (1011 paired) data sets available for submission analysis. The difference in the numbers of sets available for analysis in each category related to aged pregnancy testing data providing evidence of conception where no submission was recorded. This was evident in the latter period of mating following AI. For the most part records of bull matings were absent for given ages of pregnancy recorded. Where one cow in a pair had an inconclusive or incomplete data set, the complementary cow’s data was also removed.

Loss of data sets from enrolment to completion was between 11% and 19% (average of 13%) in six out of the seven farms. Farms are identified as ‘A, B, C, D & E’, the North Island farms and ‘X and Y’, the South Island farms. One farm, ‘Farm X’, comprising 19% of the enrolments had a drop-out rate of data sets between enrolment and completion of 37%. The high numbers of discrepancies in this farm were a cause for concern. Significant effort was made to pass a correct judgment on the robustness of this farm’s data set as removal of this farm’s data from the study would have significantly altered the conclusions drawn. Analysis of the cows whose data dropped out compared to the cows whose data remained in the study showed no influence of calving date, health status or body condition on the cows dropping out of this herd. However there was a significant effect of age (p<0.0001 Chi-square) on those cows whose data had to be excluded. Older cows were far more likely to drop out of the study in this herd. Older cows in the herd had correspondingly older tags with increasing tag legibility issues. Irreconcilable discrepancies in the Farm X data set were largely explained by poor tag legibility in this group of cows. As cows were paired and 63% of data sets from this herd were robust, this paper is presented with these cows’ data included in the final analysis.

Survival from Mating Start Date (MSD) to first service across all farms is depicted in Figure 1. No effect of treatment was demonstrated across all farms (p=0.310). On individual farms there was no effect of treatment on submission rates between treatment groups.
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Survival from MSD to viable conception is depicted in Figure 2. There was a negative effect of treatment across all farms on survival to viable conception from MSD. p=0.036 In other words cows that were treated 10 days prior to MSD with 200mgCaCuEDTA s/c were slower to hold conceptions.

In calf rate data by farm is depicted in Figure 3. This shows the variation in In-Calf rates between farms and treatment groups. Five out of the seven farms cluster together in the middle of the graph with no noticeable trend in conception rates between groups. The in-calf rates for farm ‘X’ stand out as being above the group and the early in-calf rates for farm ‘B’ stand out as being below the group. On both farms there is a noticeable difference in the in-calf rates between treatment groups. Survival analysis from MSD to viable conception on farm ‘X’ indicates that the treated cows conceived slower than non-treated cows (p<0.001). Survival analysis from MSD to viable conception on farm ‘B’ indicates that the treated cows also conceived slower than the control cows (p=0.051).
Discussion

Of interest is the difference in the in-calf rates in the face of no statistically significant difference in submission rates. This suggests that the underlying cause of the lower in-calf rates is related to reduced conception or increased embryonic loss.

Recent evaluation of the effects of administering larger doses of parenteral copper to lactating dairy cattle (4ml \textit{Cue Injection}® ACVM #A7711, containing calcium copper edetate 50mg/mL), on milk parameters has taken place (Hawkins, 2002). A transient reduction in milk volume and protein% and an increase in fat% were recorded at herd tests either side of subcutaneous injection of 4ml of calcium copper EDTA. No significant effect was seen on total milk solids production. These changes in milk composition are typically associated with a reduction in feed intake and the impact on milk parameters was attributed to a transient reduction in feed intake following injection in this study. Although this study did not find noticeable injection site lesions the decreased feed intake was attributed to the impact of discomfort at the site of injection.

While nutrition impacts reproduction through the maintenance of adequate Body Condition Score (BCS) at calving, reduction in the amount of BCS lost post calving and in the time taken to reach the BCS nadir post calving, (In-Calf NZ 2007) a transient reduction in feed intake over a 36 hour period prior to mating is highly unlikely to significantly impact herd fertility. Significant negative nutritional impacts prior to the beginning of the mating period tend to present as reduced pre-mating heats or reduced submission rates and consequently reduced in-calf rates. While cows in the treatment groups may have experienced a transient reduction in feed intakes they did not register a reduction in submission rates.

Wiseman (2002) commented that any effect of parenteral copper administration on fertility could be due to toxic effects of serum copper elevation occurring during the period of copper translocation from the site of injection to the liver.

Metallic copper liberated from copper wire and cupric chloride above molar concentrations of $2.5 \times 10^{-5}$, have been shown \textit{in vitro} to be toxic to the 2-cell embryo and blastocysts. Cupric chloride appears to cause dissolution of the zona pelucida (Brinster and Cross 1972). Parenteral administration of various copper salts to various
laboratory animals in early gestation prior to implantation have demonstrated embryotoxic effects on the 2-cell embryo through to the blastocyst; hamster, copper citrate iv, copper sulphate iv (Ferm and Hanlon 1974); hamster, copper citrate iv or ip (Di Carlo 1980); and rat, copper sulphate ip (Giavini et al. 1980). Interestingly 30mg/kg of copper gluconate administered via stomach tube to rats and mice did not result in embryotoxicity (de la Iglesia et al. 1972) whereas 1mg/kg of copper gluconate administered to developing chicken embryos was found to be highly toxic (Verret 1976).

These studies demonstrate that elevations of copper in the reproductive tract prior to implantation can negatively impact embryo survival. They also suggest that copper administered parenterally may be potentially more embryotoxic than copper absorbed through the intestinal lining.

Following oral ingestion of copper, copper ions are absorbed across the gut lining loosely bound to various amino acids and are transported to the liver loosely bound to albumin. In the liver copper is then incorporated into Cu-containing proteins such as caeruloplasmin, the protein responsible for transporting copper to other tissues, or copper metallothionines, the proteins primarily responsible for storage of copper in the liver. Anatomical design ensures that copper absorbed from the gut passes through the liver and can be incorporated into a stable form prior to storage or distribution to other tissues.

Following parenteral administration of copper, copper is translocated from the site of injection into the blood loosely bound to albumin. The rate of translocation (as measured by elevations in serum or plasma copper compared to baseline) depends on a number of factors. The type of copper salt used – sodium copper edetate translocates faster than calcium copper edetate, than does copper glycinate than does copper methionate at the same dose of copper. Anatomical considerations mean that blood copper associated with albumin originating from these sites does not necessarily pass through the liver prior to being circulated throughout the body. It is likely that copper finding its way to the reproductive tract loosely bound to albumin is more able to dissociate from the albumin and become toxic to embryos than copper in the reproductive tract associated with caeruloplasmin. If this is the case then understanding the likely duration of copper translocation is important to ensure appropriate timing of supplementation.

Blood and Radostits (1989) state that following subcutaneous administration of copper calcium edetate or copper oxyquinolone sulfonate in sheep, a rapid increase in the copper concentration of whole blood, serum and urine occurs within the first 24 hours. Bohman et al. (1984) demonstrated rapid translocation into plasma of copper following injection of calcium copper edetate at doses from 20mg to 120mg in 2-4 week old calves averaging 57kg liveweight. Elevations of plasma copper peaked within 2hrs of administration and higher doses were associated with higher peak plasma copper levels. However plasma copper levels approximated pre-injection levels within 24hrs of treatment independent of the dose. Data from the Cox-Witton Study (2002 DOF) indicates that serum copper levels following 2ml of Cue Injection® in cattle, approximate pre-injection levels by seven days with the copper levels peaking earlier in the period. Emslie (2009) indicates that while peaks in calf serum copper levels following s/c injection of 200mg calcium copper edetate are higher than those for 100mg, serum copper levels for both dose rates approximate each other within 24-48 hours.

Blood and Radostits state that there is a steady increase in serum caeruloplasmin activity over period of 10-20 days followed by a slow fall to pre-injection activity by 40 days in sheep treated with injectable copper.

These data suggest that the translocation of copper from the site of administration into the blood progresses rapidly. There is some disagreement between the data as to when peak serum copper levels are reached but they are in decline by day 10 irrespective of treatment and dose. The data sets based on serum copper levels indicate that translocation may be essentially complete within a seven day period. Return of caeruloplasmin activity to baselines in sheep indicates that copper could still be circulating at an increased level out to between 20-40 days in some cases.

Individual animal factors affecting the duration of elevation of serum copper following supplementation are also poorly defined, however injection site reactions delay copper translocation resulting in lower more sustained peak blood levels and elevations above baseline for longer Hawkins (2008). This would result in copper loosely bound to albumin circulating in the animal for a longer period.

Translocation as assessed by serum/plasma copper levels typically assumes that once copper is bound to albumin it ends up in the liver where it can be stored or transported back to the tissues. However venous and arterial pathways from subcutaneous tissues do not automatically pass through the liver prior to recirculation. A possibility for consideration is that copper loosely bound to albumin in an area of high concentration may become unbound and deposited in tissues with a lower copper concentration without having passed through the
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liver. If this tissue is in the reproductive tract with an appropriately aged embryo in proximity then local toxicity may occur with embryonic death resulting. Higher levels of calcium copper EDTA injected result in higher peak levels of serum copper and possibly a higher risk of copper deposition in susceptible tissues. Elevations in serum/plasma copper may not be the appropriate test to determine completion of copper translocation in this scenario. Measurement of caeruloplasmin activity is a more appropriate indicator of completion of translocation.

Of particular interest was that Farms ‘X’ and ‘B’, the two farms with negatively impacted conception rates in the treatment groups, were at either end of the spectrum of reproductive performance. Farm ‘X’ arguably had the best reproductive performance in spite of the impact of treatment. The attendant veterinarian indicated this is generally a well run farm. Copper levels were regularly monitored prior to the trial as part of the farm’s preventative health plan and indicated that the herd was sitting within the reference range of 95-2000umol/kg through continued maintenance of appropriate supplementation. Average herd BCS around mating was reported to be good compared to other farms and above 4 with minimal variation. Farm ‘B’ on the other hand had the worst overall reproductive performance without considering the impact of treatment. This farm is situated on copper deficient soils with a history of high molybdenum levels in spring pasture, high sulphur levels and high levels of iron sand contamination of pasture. Farm “B” has struggled to elevate liver copper levels in spite of supplementation. Liver samples regularly indicate significant proportions of the herd sit below the reference range of 95-2000umol/kg by the early spring. The attendant veterinarian estimated the average herd BCS around mating to be poor compared to other farms at around 3.5 with minimal variation.

That there was no significant impact on submission rates goes some way to explaining why there has been a differing of opinion amongst vets and farmers over the degree of risk involved in immediate pre-mating supplementation of copper to cattle. The fact that the majority of farms, five out of the seven, showed no detrimental impact of treatment on conception rates also suggests that several vets and farmers are quite correct in saying they have not observed any problems associated with copper injection prior to mating.

Submission rate in the first round of mating is historically the most common form of reproductive assessment used on farm during the early mating period followed by the number of returns to heat. However in the middle of a busy spring an increase of returns of even up to 15% may go unnoticed, or if noticed then not investigated by the farm manager. If considered significant, by the time returns to heat are noticed management procedures prior to mating may not be deemed of importance. Additionally there has been and still is an attitude on many farms that mating is over as soon as the bulls go into the herd. Consequently returns may not be noticed, particularly in a herd with a compact A.I period.

Although this is changing, very few farms historically take the time to thoroughly evaluate their reproductive parameters at the end of the season. Consequently differences apparent between submission rates and predicted in-calf rates that throw questions on conception rates and embryonic loss rates are not identified and examined.

Conclusion

The results of this study clearly indicate that administration of 200mg calcium copper EDTA 10 days prior to mating in adult NZ dairy cattle may significantly compromise reproductive performance. The mechanism appears to be through increasing foetal loss by copper translocating to the reproductive tract from the site of injection prior to incorporation into transport proteins such as caeruloplasmin causing the death of embryos prior to implantation.

While serum and plasma copper levels return to pre-treatment levels within 10 days the exact duration of copper translocation from the site of injection to the liver is difficult to pinpoint. Copper loosely bound to albumin may be present in other parts of the body including the reproductive tract beyond this 10 day period.

Administration of parenteral copper edentate 10 days prior to the start of mating and during the mating period is not recommended. However incorporation of a reproductive analysis as part of a herd’s routine management practice is.

Acknowledgements

Thanks go to the farmers and clinical vets involved, statistical consultants and Parnell Labs Australia for sponsoring the study.
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