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## The effects of zeranol or zinc supplementation before lambing on the incidence of vaginal prolapse in twinning ewes

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### ABSTRACT

The objectives were to determine whether Zeranol (ZER) and its metabolites or pharmacological zinc levels could change the incidence of vaginal prolapse (bearings) in ewes. In the ZER experiment 100 twin pregnant ewes were controls, a further 50 ewes each received either two low (24 mg and then 12 mg) or two high (36 mg and then 24 mg) doses of zeranol (ZER) implants separated by a four-week interval with 65% of treated ewes receiving an additional 12 mg implant at set stocking. Urine samples were collected from 12 ewes, twice weekly, bulked into three samples and analysed for Zearalenone (ZEN), ZER and creatinine. Two weeks after receiving an implant, the ZER:creatinine ratio increased from 2.6 in C ewes to 21, 30 and 36 and the ZEN ratio from 3.25 to 6.7, 10.8 and 14.7 following treatment with 12, 24 and 36 mg of ZER ( $P < 0.01$ ). One month after treatment the ZER and ZEN ratios had decreased but were still higher in treated than control ewes (ZER 3.6 *vs.* 11,  $P < 0.01$ ; ZEN 1.2 *vs.* 5.2,  $P < 0.01$ ). The bearing incidence was 3%, 2% and 2% for control, low and high dose ZER respectively.

In the second experiment 100 scanned twin or triplet pregnant ewes were administered with 72 g of zinc oxide (The Time Capsule ®) as a ruminal bolus 4-6 weeks before lambing on four farms with historical high incidences of bearings. Pre-treatment and all control serum zinc concentrations were low ( $9.4 \pm 0.2$  mmol/L) but not considered deficient. Zinc supplementation increased serum zinc concentrations to 21.5 mmol/L ( $P < 0.001$ ). Bearing incidence was higher ( $P < 0.001$ ) in zinc-treated (4%) than in control ewes (1.9%). In conclusion, bearings are not caused by ingestion of ZEN in late pregnancy. The role of zinc in bearings warrants further study.

**Keywords:** vaginal prolapse, ewes, zearalenone, zeranol, zinc

### INTRODUCTION

Vaginal prolapse (bearings) is likely to have multifaceted causes associated with excessive internal vaginal pressure and/or premature birth tract relaxation, which combine to cause the unpredictable outbreaks of bearings on New Zealand farms. In order from most to least importance, vaginal internal pressure is increased by sheep lying down, by steeper slopes, by increased liveweight (*e.g.* multiple-pregnant ewes) and in ewes consuming greater quantities of feed in late pregnancy (McLean & Claxton, 1960). In an epidemiological study, ewes fed better in early pregnancy have a greater likelihood of bearings presumably due to an increase in placenta size and a subsequent greater internal vaginal pressure (Hilson *et al.*, 2002). However the metabolic and hormonal factors associated with premature birth tract relaxation are not well known.

Zearalenone (ZEN) is an oestrogenic mycotoxin produced by the saphrophytic *Fusaria* fungi that proliferate on dead matter in pastures during

autumn. Levels of urine ZEN:creatinine ratio above 10 in mating ewes reduce ewe oestrous activity, ovulation and fertilisation rates and reduce lambing percentage (Smith & Morris, 2006). We have observed mean urine ZEN:creatinine ratios of 3.7, and up to 9.7, in groups of pregnant ewes at the end of winter. Zearalenone causes vaginal prolapse in pigs (Young *et al.*, 1979, Rainey *et al.*, 1990). One of the objectives in this experiment was to determine if high levels of ZEN could trigger bearings in ewes. This hypothesis was tested by treating ewes with zeranol (® Ralgro), whose active ingredient  $\alpha$ -zearalanol, is metabolised to zearalenone and other zearalenone like metabolites (Kleinova *et al.*, 2002). Zeranol (ZER) is an anabolic steroid, with oestrogen-like activity, which is administered to male cattle to improve liveweight gain and feed efficiency (Nold *et al.*, 1992).

The second objective was to determine if zinc supplementation could impact on the incidence of bearings. Zinc interacts with a large number of hormones including thyroid hormones, steroids,

insulin, and pituitary hormones, particularly prolactin (Fitzgerald *et al.*, 1986). Prolactin concentrations tend to increase over late pregnancy and rise rapidly immediately before the onset of lambing when bearing incidence is highest. Zinc also antagonises the action of calcium necessary for the functioning of these hormones (Brandao-Neto *et al.*, 1995). Large amounts of zinc supplementation may also alter the uptake and metabolism of other cations which could be implicated in bearings.

## METHOD

### Experiment 1

Two hundred twinning Romney ewes (identified by pregnancy scanning on 17 July 2001) were randomised into three treatment groups. The objective of the trial was to induce bearings in most ewes so the small treatment numbers, given the normal low incidence of bearings and potential issues with power analysis, was considered acceptable. Treatment groups were balanced for liveweight and condition score. One hundred twin pregnant ewes were controls, a further 50 ewes each received either two (57 and 29 days lambing), low (24 mg and then 12 mg) or two, high (36 mg and then 24 mg) doses of zeranol separated by a four-week. The lower dose of the second implant offset any potential carry over effect from the first implant. Sixty five percent of treated ewes receiving an additional 12 mg implant at set stocking. Zeranol implants were placed subcutaneously on the back side of the ear using a Ralgro-gun. Ewes were set stocked two days before the expected start lambing date of 20 September 2001 though unexpectedly some ewes had lambed over the preceding week. The treatment groups were grazed together on steep hill country on Ballantrae Research station from scanning until docking. The ewes were observed daily for bearings from six weeks before lambing. When culled for age, ewes did not enter the human food chain. Administration of treatments was supervised by a veterinarian and via animal ethics proposal number 6020.

Urine samples were collected from 12 ewes, twice weekly, bulked into three samples and analysed for ZEN, ZER and creatinine. The ZEN was assayed using the ELISA test which recognises zearalenone but also has cross reactivity with  $\alpha$ -Zearalenol (220%),  $\beta$ -Zearalenol (60%),  $\alpha$ -Zearalanol (Zeranol, 110%),  $\beta$ -Zearalanol, Zearalanone, and c-Zearalenol (Garthwaite *et al.*, 1994; Miles *et al.*, 1996). The ZER was assayed using the Ridascreen Zeranol Enzyme Immunoassay, a kit by R-Biopharm, Darmstadt, Germany.

### Experiment 2

One hundred scanned twin or triplet pregnant ewes were administered with 72 g of zinc oxide (The Time Capsule®) as a ruminal bolus 4-6 weeks before lambing on four farms with high historical incidences of bearings. These ewes were grazed with equivalent group of untreated ewes.

A random blood sample was collected by venipuncture from 10 treated and 10 control ewes before treatment and from the same ewes again at set stocking 2-4 weeks after supplementation. Individual serum samples were analysed for  $\beta$ -hydroxy-butyrate (BOH; an indicator of fat mobilisation) and zinc. Samples were bulked by volume and the bulk sample was measured for whole blood selenium, serum calcium, magnesium and copper (Agriquality, Palmerston North).

Farmers recorded bearing incidence.

### Statistical analysis

In both experiments the data were analysed using general linear procedures of SAS (1990), and bearing incidence was analysed using chi-square analysis by SAS.

## RESULTS

### Experiment 1

There was no effect of treatment with ZER on ewe live weight or condition score. The initial ewe live weight and condition score at scanning were low ( $53.1 \pm 0.5$  kg, condition score 2.5) and by set stocking condition score had dropped to 2.34.

In data averaged across all treatments, two weeks after the first implantation, the ZER:creatinine ratio increased to 21, 30 and 36 and the ZEN ratio to 6.7, 10.8 and 14.7 following treatment with 12, 24 and 36 mg of zeranol ( $P < 0.01$ ) (Figure 1). One month after ZER implant, the ZER and ZEN ratios had decreased but were still higher in treated than control ewes (ZER 3.6 vs. 11,  $P < 0.01$ ; ZEN 1.2 vs. 5.2,  $P < 0.01$ ). In treated ewes the ZER ratio after one month of treatment tended ( $P > 0.1$ ) to be higher after the second month (13.1) of treatment compared to the first month (9.1) of treatment. However the ZEN ratio was lower ( $P < 0.05$ ) in both treated and Control ewes in the second month of treatment (Figure 1).

The bearing incidence for these three groups was similar at 3%, 2% and 2% for C, L and H ewes respectively.

### Experiment 2

Pre-treatment serum zinc concentrations and control levels were low ( $9.4 \pm 0.2$  mmol/L) but not deficient (Grace, 1983). Zinc supplementation increased serum zinc concentrations to 21.5



inaccurate. There is not enough statistical strength in this data to conclude that zinc has a role in bearings on farms.

Zinc could cause bearings via hormones associated with birth. High physiological doses of zinc are known to cause abnormal birth processes and increased dilation (Oner *et al.*, 2003) as well as altered progesterone/oestrogen ratios, decreased prostaglandin production (Apgar, 1985; Fitzgerald *et al.*, 1986) and abnormal prolactin metabolism (Fitzgerald *et al.*, 1986; Brandao-Neto *et al.*, 1995). Alternatively high doses of zinc could cause a direct perturbation of mineral metabolism seen in other experiment, though not this one, in the blood (Towers *et al.*, 1981; Smith *et al.*, 1984) or tissues (Lee *et al.*, 1991; Rounce *et al.*, 1998).

In an epidemiological study of vaginal prolapse in ewes, salt supplement in late pregnancy increased the chance of a bearing by 1.40 times though the authors speculated this was due to increased water intake rather than a metabolic mineral effect (Hilson *et al.*, 2002). In the same study ewes with elevated serum phosphate concentrations were 2.7 times more likely to have a bearing subsequently than ewes with low phosphate levels. But neither the feeding of anionic salts in mid-pregnancy nor the Ca or vitamin D supplements in late-pregnancy changed bearing incidence (Litherland *et al.*, 2007). Neither could bearings be induced by the *ad libitum* feeding ewes in late pregnancy on high dietary cation anion balance (620 meq/kgDM) pastures (Litherland *et al.*, 2000). So a potential role of mineral metabolism in the incidence of bearings appears unlikely.

## CONCLUSIONS

Until bearing incidence can be reliably manipulated it will be difficult to make progress on causal factors. Zinc supplementation may have increased bearing incidence but confirmation requires further studies. Bearings are not caused by ingestion of ZER in late pregnancy but this does not totally discard the hypothesis that ZEN ingestion is associated with bearings outbreaks.

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