

## Morphological changes in the reproductive tract of ewes after long-term feeding with subterranean clover (*Trifolium subterraneum* L.)

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Subterranean clover is the main annual pasture legume in southern Australia with approximately 30 million ha of the pasture legume grazed by 42 million sheep. The high level of phytoestrogens in some older highly oestrogenic subterranean clover (OC) cultivars is known to cause temporary and permanent infertility, and sometimes dystocia in ewes, as well as lamb mortality, with an estimated 10 million ewes affected per year (MLA 2002). While the impact of short-term exposure to phytoestrogens on the reproductive tract has been reported (Adams 1998), the effects of long-term exposure on reproductive outcomes has not been thoroughly investigated.

In this study, we investigated the impact of long term grazing of OC on the morphology of the reproductive tract in four groups of 5/6 year old Merinos ewes. Three groups consisted of ewes exposed to OCs for at least five years, which were grouped according to reproductive outcomes during the season: either a) ewes that did not fall pregnant (NP,  $n=10$ ), b) ewes that fell pregnant but lost their lamb within 48 h after birth (LL,  $n=13$ ), or c) raised their lambs (RL,  $n=13$ ). The control group included reproductive ewes with no exposure to phytoestrogens (CON,  $n=11$ ). After weaning, the liveweight and body condition score of each ewe was measured. The ewes were then slaughtered, and the reproductive tract was isolated and photographed. Cervix length and diameter at three points (near vagina, middle, near uterus) and uterus length and the diameter of each horn were measured from photographs using *IMAGEJ*. The data were expressed as a ratio to liveweight and analysed in SAS using ANOVA followed by multiple comparison testing using both the Dunnett and Tukey methods.

**Table 1. Morphological characteristics of the cervix and the uterus (expressed in cm x 10<sup>2</sup>/kg of liveweight, mean ± sem) of ewes never exposed to oestrogenic clover (CON,  $n=11$ ) or exposed over the last 5 years and non-pregnant (NP,  $n=10$ ), pregnant but either lost their lamb within 48 h after birth (LL,  $n=13$ ), or raised their lambs (RL,  $n=13$ ) during the last breeding season. \*: overall effect at  $P<0.05$ , \*\*: overall effect at  $P<0.01$ , a: different to CON at  $P<0.05$ , b: different from NP at  $P<0.05$**

Group	Cervix				Uterus		
	Length*	Diameter			Length**	Horn diameter	
		Near vagina**	Mid cervix**	Near uterus**		Left **	Right **
CON	13.2 ± 2.6	5.5 ± 1.0	4.9 ± 1.1	4.3 ± 0.7	14.4 ± 0.1	7.6 ± 1.3	3.6 ± 1.1
NP	10.2 ± 4.0 <sup>a</sup>	3.7 ± 1.1 <sup>a</sup>	2.5 ± 0.3 <sup>a</sup>	2.2 ± 0.3 <sup>a</sup>	7.8 ± 1.3 <sup>a</sup>	1.8 ± 0.4 <sup>a</sup>	1.7 ± 0.2 <sup>a</sup>
LL	12.4 ± 1.8	4.2 ± 1.3 <sup>a,b</sup>	3.2 ± 0.8 <sup>a,b</sup>	3.0 ± 0.7 <sup>a</sup>	9.5 ± 1.6 <sup>a,b</sup>	4.6 ± 1.1 <sup>a</sup>	2.1 ± 0.6 <sup>a</sup>
RL	13.9 ± 2.7 <sup>b</sup>	4.0 ± 1.0 <sup>a,b</sup>	3.3 ± 0.5 <sup>a,b</sup>	2.8 ± 0.4 <sup>a</sup>	9.4 ± 1.8 <sup>a</sup>	4.9 ± 0.9 <sup>a</sup>	2.2 ± 0.4 <sup>a</sup>

There was an overall effect of exposure to OC on each measurement (Table 1). The length of the cervix was shorter in the NP than in CON and RL, but similar in the LL and CON groups. Diameter of the cervix, in each of the three locations was smaller in the NP, LL, and RL than in the CON. The diameter mid cervix and near the vagina was smaller in NP than in LL and RL groups. All of the measurements of the uterus in the ewes exposed to OCs were smaller than in CON group.

Exposure to OC resulted in a reduction in size of both the cervix and the uterus, particularly in the NP ewes. We cannot conclude if a) pregnancy partially restored the morphological characteristics of the uterus and cervix to that of the CON ewes, or b) if the failure to fall pregnant was due to the different levels of shrinkage that resulted from exposure to OCs for several years prior. It is still necessary to investigate if changes in elasticity of both the uterus and the cervix might differ between individual ewes, and therefore lead to different reproductive outcomes.

### References

Meat and Livestock Australia 2002. *Sheep reproduction in Australia*. Report MS009.  
Adams NR (1998) *Pure & Applied Chemistry*. **70**, 1855-1862.

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