



Dietary supplementation of yucca (*Yucca schidigera*) affects ovine ovarian functions



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ABSTRACT

Yucca (Yucca schidigera) is a popular medicinal plant due to its many positive effects on animal and human physiology, including their reproductive systems. To examine the effect of supplemental yucca feeding on sheep reproduction, including ovarian functions and their hormonal regulators, ewes were fed (or not fed, control) yucca powder (1.5 g/head/day, 30 days). Macromorphometric indexes of the oviduct, ovary, and ovarian folliculogenesis were measured. Reproductive hormone levels in the blood were measured using a radioimmunoassay. Granulosa cells were aspirated from the ovary, and their proliferation and apoptosis were detected using immunocytochemistry. To assess secretory activity and its response to gonadotropin, ovarian fragments of treated and control ewes were cultured with and without follicle-stimulating hormone (FSH; 0, 0.1, 1, 10, or 100 IU/mL), and the release of reproductive hormones into the culture medium was evaluated. Finally, to examine the direct action of yucca on the ovary, ovarian fragments from control ewes were cultured with and without yucca extract (1, 10, or 100 µg/mL), and the release of reproductive hormones was measured. Yucca supplementation significantly decreased the size of small antral follicles (2 to <5 mm in diameter), increased accumulation of the apoptosis marker bax, and decreased serum progesterone (P4) and estradiol (E2) levels. It inhibited the release of P4 (but not other hormones), to prevent the stimulatory action of FSH on P4 output and promoted insulin-like growth factor I (IGF-I) release by fragments cultured with FSH. However, yucca supplementation did not affect the size of larger follicles and number of follicles, volume and weight of ovaries, length and weight of oviducts, caspase 3 accumulation, cell proliferation, testosterone (T) or IGF-I serum levels, or T or E2 release by cultured ovarian fragments and their response to FSH. Yucca addition to culture medium inhibited P4 and IGF-I, but not T or E2 release at the lowest (1 µg/mL) dose, and stimulated P4, but not T, E2, or IGF-I release at the highest (100 µg/mL) dose. These data suggest that yucca supplementation can reduce small antral ovarian follicle

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development possibly via the stimulation of apoptosis of their granulosa cells, suppression of ovarian P4 and E2 release, and alteration of ovarian IGF-I output and ovarian response to gonadotropin. Thus, yucca can directly affect P4 and IGF-I release by ovine ovarian cells.

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1. Introduction

Currently, hormonal preparations are mainly used to regulate reproductive processes. However, hormones are expensive, species specific, and have numerous undesirable adverse effects. Therefore, adequate natural alternatives to hormonal therapy could improve or replace currently used hormonal regulators [1,2]. *Yucca schidigera* (Agavaceae) is a medicinal plant originating from southwestern United States and Mexico [3]. It is considered as a common commercial source of steroid saponins [4]. Saponins originated from *Y. schidigera* have immunostimulatory [5], anti-inflammatory [6,7], hypocholesterolemic [8–10], hypoglycemic [9], and anticarcinogenic effects [11]. Such substances have improved feed conversion efficiency [12] and increased weight gain [13]. Recently, a positive effect of yucca on ovarian functions has been detected, and Baláži et al. [14] found a positive effect of yucca on male spermatozoa parameters. Yucca added to feed positively affected conception rate in cattle [3], and fertility rate in rabbit does [14,15] and dairy goats [10]. It promoted apoptosis and reduced proliferation in cultured porcine ovarian granulosa cells [16]. Yucca directly stimulated progesterone (P4) release by rabbit [15] and porcine [16] ovarian cells, but inhibited testosterone (T) release by cultured porcine ovarian cells [16]. Steroidal saponins are phytoestrogens [2], with similar chemical structures to mammalian estrogen, estradiol (E2), and therefore bind to estrogen receptors on various tissues and affect their function [17–19]. Besides steroids and steroid receptors, phytoestrogens can directly activate insulin-like growth factor I (IGF-I) receptors [20] and affect intracellular regulators of cell cycles and apoptosis [21]. Progesterone is a steroid hormone important for the normal functioning of ovaries that induces steroidogenesis [21,22], suppresses ovarian cell apoptosis, and promotes ovarian follicle luteinization and corpus luteum (CL) development and maintenance [23,24]. Progesterone (and other ovarian steroids) production and release by the ovary is regulated by pituitary gonadotropins and other extracellular factors, including IGF-I, an important promoter of ovarian secretory activity and granulosa cell proliferation [22,25,26]. The ability of yucca to promote P4 synthesis indicates that P4 and possibly gonadotropins and IGF-I could potentially mediate yucca stimulatory action on ovarian cell proliferation, apoptosis, and reproductive efficiency of rabbits [15] and pigs [16]. However, the hormonal mechanisms of yucca action on reproduction require further elucidation. Yucca-dependent reproductive hormones can affect ovarian folliculogenesis and fecundity. Yucca can alter progesterone and androgen release, promote direct ovarian cell proliferation (proliferating cell nuclear antigen [PCNA] expression), and suppress their apoptosis (bax expression), which is associated with improved fecundity of rabbits, pigs, and

goats. Nevertheless, theoretically and practically, the effect of yucca on endocrine and ovarian functions in other farm animal species of economic importance should be examined. To our knowledge, the influence of yucca on estrogen and peptide hormones (IGF-I) has not been studied previously. However, the effects of yucca on macromorphometric indexes of the ovary, oviduct, and ovarian folliculogenesis remain to be clarified. The aim of the present *in vivo* and *in vitro* experiments was to examine the influence of dietary yucca supplementation on the key endocrine and ovarian functions in sheep, a species that has not been studied in this respect, and to understand possible mechanisms of its effect by determining whether:

- (1) Dietary supplementation of yucca can influence macromorphometric indexes of the reproductive system (oviduct, ovary, and ovarian folliculogenesis of sheep fed and not fed (control) with yucca at a dose of 1.5 g/head/day for 30 days;
- (2) This influence could be due to changes in ovarian cell proliferation (PCNA, marker of S-phase of the cell cycle [27], and cyclin B1, marker of G2-phase of mitosis) [28] and apoptosis cytoplasmic/mitochondrial apoptosis (bax, caspase 3);
- (3) The effect of dietary supplementation of yucca on the sheep ovary can be mediated by changes in blood level of reproductive hormones (P4, T, E2, and IGF-I), and if these changes result from changes in release of these hormones by ovarian cells and their response to the upstream hormonal regulator follicle-stimulating hormone (FSH); and
- (4) Yucca supplementation can affect hormone release (P4, T, E2, and IGF-I) by sheep ovarian cells not only via the digestive tract and central regulators of reproduction, but also by directly influencing ovarian cells.

2. Materials and methods

2.1. Animals and diets

All experiments were carried out with local Ethical Committee approval (No. 3314/12–221a) and in accordance with Slovak and EU regulations concerning animal experiments. Experiments were performed under standard conditions in the accredited experimental Institute of Animal Physiology of the Slovak Academy of Science in Košice, Slovak Republic. Healthy cycling Merino ewes, 4 to 6 years of age weighing 54.23 ± 2.93 kg, were used for the

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