

# A REVIEW OF THE EFFECTS OF PLANT ESTROGENIC SUBSTANCES ON ANIMAL REPRODUCTION<sup>1</sup>

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## ABSTRACT

In the 1940's, hormone-like substances were found in plants which caused reproductive difficulties in sheep. Since that time, various substances from many plants, such as alfalfa, ladino clover, and birdsfoot trefoil, have been indicated as the cause of such reproductive problems in mammals. Estrogen is one of the main substances shown to produce such effects. Questions consistently arising concern: the amounts of estrogen in certain plants, the variation of these amounts during different seasons, the effectiveness of such hormone substances taken orally, and the effects of varying consumptions of estrogen-containing plants on growth, reproduction, and other life activities of various species of wildlife.

## INTRODUCTION

Reproductive difficulties in animals grazing certain kinds of pastures were first noticed in Australia in connection with the increased use of subterranean clover (*Trifolium subterranean*) as a sheep pasture (Bennetts et al., 1946). Moule has since (1961) reviewed all the subsequent Australian research concerning infertility in sheep associated with estrogens in forage crops.

Estrogens have been found in many plants (Cheng et al., 1953; Dohan et al., 1951; Kendall et al., 1950; Lyman et al., 1959; Ostrovsky and Kitts, 1962; Pieterse and Andrews, 1956). Allen and Kitts (1961) discussed the estrogens and/or anti-estrogens in pine needles. Later Allison and Kitts (1964) found that the effect of estradiol-17 B (a synthetic estrogen) on the uterine tissue of ovariectomized rats was inhibited by simultaneous injections of yellow-pine-needle extracts (anti-estrogens). Bickoff et al. (1958) isolated estrogens from various plants. Included in this list are genistein, biochanin A, formononetin, and most significantly coumestrol from ladino clover (*Trifolium repens*) and alfalfa (*Medicago sativa*) (Bickoff et al., 1957, 1958, 1960).

Much of the early work concerning estrogenic effects of these plants on reproduction has been done with sheep. Schinckel (1948) wrote of the "infertility in ewes grazing subterranean clover pastures." Since that time many scientists at American agriculture stations have worked with this problem. Consideration of the literature from these stations, plus other work, can give a general picture of what is happening in the field of estrogens in plants and their effects on reproduction. This review is an attempt to present this general picture, drawn from literature from many areas, with many ideas; a more detailed survey and reference list is left up to the interested reader.

## ACTIONS AND EFFECTS

The question of how plants prevent normal reproduction in animals that eat them has been asked many times. Estrogenic substances are the materials believed to cause the reproductive difficulties (Kendall et al., 1950; Leavitt and Wright, 1963; Sanger and Bell, 1961; Wright, 1960), but the possibility of anti-estrogens in plants has also been suggested (Adler, 1962; Emmens, 1962; Allison and Kitts, 1964). How certain plant substances work, and even what these substances are, is still not known with any certainty, but, regardless of the substance, reproductive difficulties have been found. Approaches to this problem have con-

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cerned the plant, biochemical isolation of estrogens, metabolism of the plant estrogens, and also what histological effects may be observed in test animals.

Such a histological approach was begun by Bell and his associates at The Ohio State University Animal Science Department approximately ten years ago. Sanger et al., (1958) began with a study of the vaginal cytology of the ewe during the estrous cycle. Using this as a basis, Bell and Sanger (1958) studied the changes found in the reproductive tract of anestrous ewes pastured on ladino clover and birdsfoot trefoil (*Lotus corniculatus*). Vaginal smears showed that cell distortion, degeneration, and clumping were more common in sheep pastured in ladino clover than in those pastured in birdsfoot trefoil, resulting in the inference that greater amounts of estrogenic substances are available from the clover. D. S. Bell (unpublished report) suggests that "the effect of the hormonal substances in these plants (subterranean clover) is, to prevent the normal implantation of the ova in the uterus." That hormones, such as estrogens, anti-estrogens, and oestrone could prevent normal implantation had already been suggested (Emmens, 1962; Greenwald, 1959; Pincus and Kirsch, 1936). Bell pastured sheep on bluegrass (*Poa pratense*), ladino clover, and birdsfoot trefoil. Significantly more ewes failed to conceive when pastured on ladino clover (19.7 per cent) than on bluegrass (8.9 per cent), even after being bred during six successive estrous cycles, while the conception rate of ewes pastured on trefoil was intermediate between the two. Estrogen content in the plants occurs in the same order, with ladino clover containing the most, birdsfoot trefoil containing less, and bluegrass containing none. Kendall et al. (1950) also noticed such trends with rabbits fed soybean hay. Sanger and Bell (1961) compared the effects of ladino clover and bluegrass pasturage on fertilization of ova in sheep. There was 59 per cent cleavage of the ova from 22 sheep pastured on ladino clover, and 75 per cent cleavage of the ova from 22 sheep pastured on bluegrass. Three possible explanations are given for this: (1) inhibition of follicle formation, (2) interference with sperm activity and travel, (3) and failure of follicles to rupture, a remote possibility.

Engle et al., (1957) also studied the effect of ladino clover, birdsfoot trefoil, and bluegrass pasturage on conception rates of ewes. Eight acres of ladino clover, eight acres of birdsfoot trefoil, and 40 acres of bluegrass with timothy were used. Those ewes pastured on bluegrass underwent estrus at an earlier date than those pastured on ladino clover and birdsfoot trefoil. Ewes pastured on ladino clover and birdsfoot trefoil conceived considerably later than ewes fed on bluegrass, 21.7 and 19.9 days later respectively. Sixty-six per cent of the ewes fed on bluegrass lambed to first service, 41 per cent of the ewes which fed on ladino clover lambed to first service, and 31 per cent of the birdsfoot-trefoil-fed ewes lambed to first service. Barrett et al., (1965) showed that there was only 25 per cent lambing of ewes grazed on red clover.

Wright (1960) used ladino clover to demonstrate infertility in domestic rabbits. Leavitt and Wright (1963) studied various influences of ladino clover on reproduction in mice. They used the mouse uterine weight method to assay for estrogens (Evans et al., 1946). This method is based on the principle that estrogens promote tissue growth, which can be seen in the weight of the uterus. Ostrovsky and Kitts (1962, 1963) induced distinct uterine responses with red clover and subterranean clover, but only minimal laboratory-rat uterine activity with birdsfoot trefoil. Negus and Pinter (1966), who also worked with mice (*Microtus montanus*), demonstrated that estrogens in wheat increased reproductive performance.

The metabolism of plant estrogens is an area of research only recently considered (Batterham et al., 1965; Cayen et al., 1965; Davies and Hill, 1966; and Nilsson, 1961). Most of these works analyze the degraded end products of estrogens, such as biochanin A, genistein, or formononetin.

Synthetic estrogens also affect the mammalian ovary directly, particularly the

follicles. Lane (1935) and Leathem and Wolf (1955) reported an increase in follicle number with estrogen injections, while Leonard et al., (1931) indicated a decrease in follicle number. Leonard et al., (1931) also showed a decreased ovarian weight with estrogen injections, and Mazer et al., (1936) and Cole, (1946) reported increased ovarian weight. These seemingly contradictory results are produced by different strengths of injections; "it is known that larger doses of estrogen can inhibit follicular growth by suppressing the secretion of pituitary gonadotrophins, whereas small doses may enhance follicular development" (Byrnes and Meyer, 1951). Smith and Bradbury (1961) also demonstrated this same mechanism. Leavitts and Wright (1965) showed that coumestrol (from ladino clover) blocked the release of gonadotrophins rather than production, since gonadotropes were plentiful.

#### EXPERIMENTAL PROBLEMS

Various factors must be considered when doing research in this field. There is a variation in the estrogenic activity of plants under different environmental conditions. Bickoff et al., (1960) studied the effects of dehydration and cutting on the estrogenic activity of plants. He showed, for example, that the estrogenic activity of dehydrated and sun-cured forages is highly variable. The estrogenic activity of spring-growth alfalfa (*Medicago sativa*) samples was only slight in comparison to midsummer samples (Bickoff et al., 1960; Pieterse, 1956). Bickoff et al., (1960, 1961) studied factors affecting estrogenic activity of white clover clones (*Trifolium* spp.) and ladino clover, and described the estrogenic content of subterranean clover and red clover (*Trifolium pratense*) cut at various stages of growth during a six-month period. The potency of the subterranean clovers reached a maximum after between three and four months of growth and then remained high. Red clover maintained a relatively high activity (although not as active as subterranean clover), with peaks at two and five months after planting. Drying did not affect the estrogenic activity of subterranean clover. Kitts et al., (1952) studied the effects of stage of maturity and frequency of cutting on estrogen activity. Uninterrupted second-year growth of alfalfa and ladino clover had high activity in spring, little activity in the summer, and intermediate activity in the fall. There was slight estrogenic activity in birdsfoot trefoil, with no increase in late season. Strain differences in plants must also be considered and may well explain some different estrogenic effects of these plants (Stob et al., 1957).

Samuel (1964), Leavitt and Wright (1963), and Pinter and Negus (1965) point out problems relating to interrelationships between nutritional factors and estrogens in plants. Samuel (1964) found a decrease in the weights of ovaries from wild rabbits fed birdsfoot trefoil and attributed this to a lack of preference for trefoil (Samuel, 1967), thus causing nutritional stress. McClure (1959) has shown that temporary nutritional stress interferes with either fertilization or maintenance of pregnancy. Meites (1949) showed that diethylstilbestrol, a synthetic estrogen, curtailed growth in albino rats by decreasing the appetite of these animals.

Bickoff et al., (1961) presents the well known, but many times forgotten, argument that the response of one animal to a biological preparation may be quite different from that of another animal. Ruminants, for example, may digestively change the plant estrogens, making them more or less potent than would show up in a uterine weight assay of mice. Thus, in order to demonstrate that plants impair reproduction, there are many factors that must be taken into consideration.

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