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Coumestrol, a potent Phytoestrogen to control male animal's fertility: A review

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Abstract
Phytoestrogens are plant substances which are produced by plants to protect themselves from grazing by herbivores. Phytoestrogens have been classified broadly into coumestanes, flavonols, isoflavonoids, lignans, mycotoxins, prenyl flavonoids and stilbens based on their chemical structure. Coumestrol is a phytoestrogen belonging to the coumestanes family which was first identified in 1957 from ladino clover. Coumestrol binding with estrogen receptor in testes can disturb spermatogenesis and can impair steroidogenic processes of testes and it has been considered as an endocrine disrupting chemical. However, the whole concept of endocrine disrupting chemicals has been challenged lately and therefore caution is warranted while reporting its effect.

Keywords: Phytoestrogen, coumestrol, male fertility, endocrine disrupting chemicals

Introduction

The word phytoestrogen consists of Greek word "phyto," meaning plant and "estrogen" due to their ability to affect estrogenic activity in the body (Sharma *et al.,* 2014) [30]. The ability of plant substances to cause estrus in animals was reported in 1926 (Bradbury and White, 1954) [8]. Phytoestrogens are plant products which have been suggested to be the defense substances of plants for decreasing their predation from herbivores which grazes on them by modulating herbivores fertility (Hughes, 1988) [10]. Phytoestrogens can act as estrogen agonist or antagonist depending on their type and amount relative to the concentration of endogenous estrogen (Adams, 1995) [1].

The adverse effects of phytoestrogens on reproduction were first reported in Australia, when ingestion of early strain of subterranean clover, Trifolium subterranean L., var. Dwalganup caused infertility, dystocia and uterus prolapse in ewes; mammary development and milk secretion in the unbred female and marked metaplastic changes in the secondary sex organs in castrated male (Bennetts *et al.,* 1946) [4]. Reproductive failure and liver disease in cheetahs kept in North American zoos were attributed to daidzein and genistein ingestion approximately @ 50 mg of these phytoestrogens (Setchell, 1987) [29].

Phytoestrogens has been under scanner of scientists recently because of their use as alternatives to estrogen replacement therapy, with reduced risk of brain function disorders, breast cancer, cardiovascular disease and obesity after their exposure (Rietjens, 2017), increase in number of people substituting animal proteins with plant-derived proteins, replacement of bone meal in animal fodder by soybean in Europe since 1995 (Izabela *et al.*, 2013) [11] and as endocrine disruptors (Patisaul and Adewale, 2009) [21]. However, coumestrol has come into limelight in the last decade due to its experimental use as a chemical for population control of male vampire bats and dogs in the Mexico (Pérez-Rivero *et al.*, 2009a & 2014) [22]. The aim of this review paper is to summarize past researches which have used coumestrol on male animals in various species to conclude its effect on male fertility.

Identification of phytoestrogens

Estrogenic activity of plants have been identified by various methods/techniques and some of them are: Allen-Doisy technique, deconvolution spectroscopy and matrix-assisted laser desorption ionization time-of-flight mass spectrometry, E-screen assay, gas chromatography coupled with a mass spectrometer, High pressure liquid chromatography, High pressure liquid chromatography coupled with a mass spectrometer, immunoassay techniques, Ishikawa cell line, receptor binding assay, reporter gene assay, reversed-phase HPLC and transient gene expression (Ososki and Kennelly, 2003) [20].

Classification of Phytoestrogens

Phytoestrogens have been classified broadly into different classes based on their chemical structure: coumestanes (11,12-dimethoxy-7-hydroxycoumestan, 3'-methoxycoumestrol, 4'-O-methylcoumestrol, coumestrol, erosnin, lucernol, medicagol, norwedelolactone, psoralidin, repensol, sativol, trifoliol and wedelolactone), flavonols (kaempferol and quercitin), isoflavonoids (biochanin a, daidzein, equol, formononetin, genistein and glycitein), lignans (enterodiol, enterolactone, isolarciresinol, lariciresinol, matairesinol, nordihydroguaiaretic acid, pinoresinol and secoisolariciresinol), mycotoxins (zearalenol), prenyl flavonoids (6-geranylnaringenin, 6-prenylnaringenin, 8-prenylnaringenin, isoxanthohumol and xanthhumol) and stilbens (resveratrol) (Bickoff *et al.*,1969; Benassayaga *et al.*, 2002; Bakker, 2004; Panche *et al.*, 2016; Rietjens, 2017) [6].

Coumestrol identification and source

Coumestrol is a phytoestrogen belonging to the coumestanes family. It was first identified by E. M. Bickoff in 1957 [5] from ladino clover (Bickoff *et al.*, 1957) [5]. Coumestrol has been found in 58 plants especially legumes, perennial Medicago, peas, soybean, limabeans, pinto beans, some clovers and strawberry clover (Reed, 2016) [25]. Clover and soybeans have the highest concentrations of coumestrol (Amin and Buratovich, 2007) [2]. The coumestrol content in plant material has been reported to vary according to climatic conditions, disease, geographic location, number of cuttings, stages of growth and varietal and genetic differences (Bickoff *et al.*, 1969) [6].

Mechanism of action of coumestrol

Coumestrol binding with estrogen receptor is its well documented action and its relative binding affinity to α-Estrogen receptor and β- Estrogen receptor has been reported to be 94 and 185 respectively in comparison to relative binding affinity of 17β-estradiol as 100 (Kuiper *et al.*, 1998) [13]. Coumestrol action as an estrogen antagonist by binding with α-Estrogen receptor which damages germinal epithelium of the seminiferous tubules and render spermatogenesis and spermiogenesis ineffective after interacting with β- Estrogen receptor has been suggested by Pérez-Rivero *et al.* (2009a) [22]. Coumestrol has been reported to prevent the release of the gonadotropic hormones from anterior pituitary gland of female mice (Leavitt and Wright, 1965) [18]. Coumestrol has been found to have an inhibitory effect on gonadotropin-releasing hormone (GnRH) mRNA expression in the study reported by Bowe *et al.* (2003) [7]. Coumestrol can inhibit 17β-Hydroxysteroid dehydrogenases enzyme which plays an essential role in production of androgens and estrogens (Krazeisen *et al.*, 2001) [12]. Coumestrol can decrease estrogen levels through inhibiting aromatase cytochrome P450 (CYP19) which converts androgen to estrogen (Hong *et al.*, 2008) [9].

Effects of Coumestrol on reproductive system in males

Dogs

Estrogen receptor-coumestrol fluorescent complexes were observed in Leydig cells, round spermatids, spermatogonia, spermatocytes and connective epidydimal tissue along with severely altered seminiferous tubules from coumestrol treated dogs @ 300 µg/kg once a week for a 4 week period (Serrano *et al.*, 2008) [28]. Coumestrol oral administration at same dose and treatment period decreased total number of ejaculated spermatozoids and also induced alterations in the olfactory behavior which decreased their smelling frequency of a container having vaginal discharges from estrus bitches from the first to the fourth week significantly after the treatment (Pérez Rivero *et al.*, 2009b) [23]. Another study by the same authors provided histopatological details of seminiferous tubules after coumestrol treatment in which no spermatozoa was distinguishable along with decrease in Sertoli cells, Leydig cells and number of ejaculated spermatozoa (Pérez-Rivero *et al.*, 2009a) [22]. Similarly, oral feeding of coumestrol @ 1.5 mg/kg body weight as a single dose affected stages of seminiferous epithelial cycle and spermiogenesis but no effects were histopathological lesions were observed on structure of spermatogonial and authors were of the view that the effects observed on spermatogenesis in the present study will be of temporary nature (Kumar *et al.*, 2017a) [14]. However, oral feeding of 300 µg coumestrol once a week for a 5 week period to the adult male dogs did not cause any adverse effects on spermatogenesis in one study (Kumar *et al.*, 2018). Similarly, coumestrol oral feeding @ 300 and 500 µg once a week for a 5 week period and 1.5 mg/kg body weight as a single dose had no adverse effects on the histology of efferent ductules in dogs (Kumar *et al.*, 2016; Kumar *et al.*, 2017b) [15, 16].

Mice

Oral administration of coumestrol @ 40 µg/kg body weight to adult male mice induced 60% loss in testis volume with 10% of abnormal spermatozoa in epididymis. Coumestrol treatment also impaired the steroidogenic process in treated animas by increasing progesterone and decreased testosterone (Serrano *et al.*, 2014) [26].

Rats

Coumestrol daily subcutaneous injection of 12.5- 400 µg for three consecutive days induced inhibition of the seminiferous epithelium phases with a clear increase of the intercellular space in adult male rats mainly in right testis. Testosterone levels showed a significant decrease with the higher doses with no changes in gonadotrophin levels (Tarragó-Castellanos *et al.*, 2006) [31]. However, in another study coumestrol diacetate feeding to weanling male rats @ 1-15mg for 21 days had no inhibitory effects on food intake or growth in either the normal or castrated animals. Testicles weights remained in the normal range after treatment and the authors summarized that coumestrol is an inactive compound and apparently nontoxic for the adult male rats (Lyman and Krueger, 1961). Similarly, no effect of coumestrol on weights of testes and accessory sex organs, sperm count and serum concentrations of testosterone, LH and FSH were observed in male rats after injection of 100 µg of coumestrol during their first 5 d of life (Awoniyi *et al.*, 1997) [3].

Vampire Bats

Pérez-Rivero *et al.* (2014) [24] observed coumestrol binding with estrogen receptors by confocal microscopy after coumestrol administration @ 5 µg per g body weight daily for 30 days along with modified testicular histoarchitecture in treated animals. Similarly, in another study coumestrol administration @ 200 µg daily for 30 days resulted in absence of mature sperm cells, leydig cells and lumen in seminiferous tubules in male wild vampire bats (Serrano *et al.*, 2007) [27].

Conclusion

Coumestrol is a complex compound and its effects may vary due to difference in anatomical design of tissue, metabolism, window of exposure and duration, dose, route of administration in various species and therefore interpretations and extrapolation of effects between species need to be made with caution and warrants further studies to conclude its effects on male reproduction.

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