Effects of Environmental Estrogens on Reproductive Parameters in Domestic Animals

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ABSTRACT

Environmental estrogens are natural products of plant (phytoestrogens) or animal origin (steroidal estrogens) that have estrogenic properties. They are a major group in a category known as endocrine disruptors. In the field of animal husbandry, the effects of these environmental estrogens are well documented. This paper discusses the effects of plant estrogens and environmental steroid hormones, such as estrogen and testosterone, as seen in animals in Israel. The areas considered are reproductive disorders, premature udder development, prolapsed oviduct, scrotal atrophy and skewed sex ratios.

Keywords: phytoestrogens, zearalenone, estradiol 17-β, testosterone, testes, oviductal prolapse, scrotum, sex ratio.

INTRODUCTION

The present review describes a number of environmentally caused reproductive disorders in domestic animals which we have observed over the last five decades. This is of interest not only for animal husbandry but these environmental hormones may responsible for human health effects as well. However effects of phytoestrogens or other naturally occuring environmental hormones in humans have been very difficult to identify (1).

Israeli scientists have been intensively interested in the effects of estrogenic substances since the early years of the State some sixty years ago (2). Mordechai Shemesh and Hans Lindner in conjunction with Natan Ayalon developed this initial interest into a major field of research to which Israel made major contributions (3,4). A major impetus to this work was Prof. Ayalon's clinical research on early embryonic death in cattle (5). First it was necessary to define the hormonal profile of estrus in cows and sheep. This was done using gas chromatography and recently developed competitive binding assays (6-10). Shemesh, Lindner and Ayalon then developed a radio-receptor assay for determining the presence of compounds with estrogenic activity in feed and forage (11).

These methods were then used to demonstrate that the phytoestrogen (principally coumestrol) content in alfalfa (*Medicago sativa*) was increased following trauma (exposure to fungus) (12) and to identify the phytoestrogens in berseem clover (13). Legumes in particular produce phytoestrogens to activate the *nod d* gene on the *Rhizobium* to produce factors which will increase nodulation in the legume plant root (14). Each legume produces its own specific phytoestrogen, e.g., soya uses genistein.

As mentioned, the principal phytoestrogen in alfalfa is coumestrol. A number of decades ago, we noticed a rise in the level of coumestrol in alfalfa plants to levels associated with reproductive disorders, unrelated to any known trauma to the plant. It was found that this rise in coumestrol was correlated with irrigation with treated sewage water. Using experimental hothouse and hydroponic experiments, it was found that the active agent in the sewage water was steroidal estrogen and that estrone was more potent than estradiol 17-β in promoting vegetative growth (14, 15).

No effects of feeding clover could be demonstrated in Israel even though clover is associated with widespread infertility in sheep in Australia (16) and resorption of equine embryos in Kentucky (Shore, unpublished observations). The major phytoestrogenic isoflavones in soya are genistein and diadzein. Both of these compounds, as is estradiol-17β itself, are biphasic, i.e., at low concentrations they can be stimulatory but at higher concentrations they can be inhibitory (17). Interestingly, genistein is a potent inhibitor of tyrosine kinase in addition to its estrogenomimetic (estrogen mimicking) properties (Figure 1) (18).

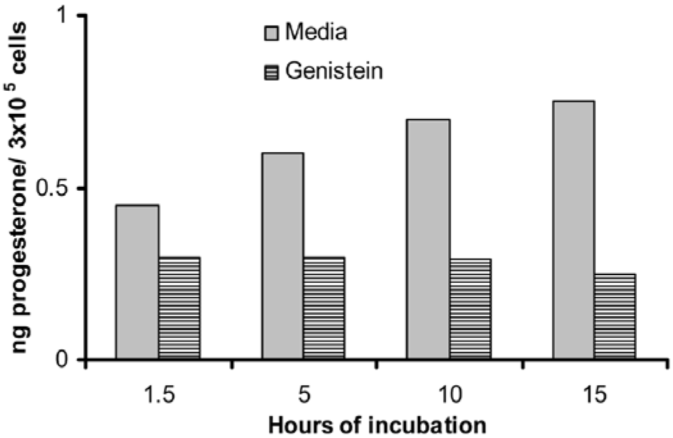

Fig. 1. *In vitro* effect of genistein on the production of progesterone by bovine placental cells. Cells ($3x10^5$) were incubated in the presence or absence of 5 μg/ml genistein in 1 ml of media. Based on data from ref. (18).

Premature udder or breast development is a cardinal sign of hyperestrogenism. In Israel, there was a period when cows were fed chicken manure with a concentration of 300 μg/kg of steroidal estrogen. Since the cows ingested 6 kg/day, this would be in the order of 1 mg/calf per day. This amount of estrogen resulted in early udder development with milk dripping in a number of calves (19). Although similar phenomenon has been reported for phytoestrogen ingestion (20), this has not been observed in Israel.

Zearalenone is a substance produced by *Fusarium spp*. and is found primarily in corn and corn products. In its more active form, zearalenol, it is widely used as growth promoter in steers in the US. It is well documented to cause vaginal prolapse in sows, especially in Canada (21). Swine prolapse has not been reported in Israel, maybe due to the insularity of the small swine husbandry community. However, we have described prolapsed oviduct in chickens associated with zearalenone (22, 23). When hens lay eggs the oviduct is externalized and then retracted. In oviductal prolapse, the oviduct fails to retract. In the average coop, this is usually results in death as the bird is attacked by its fellow hens. It was demonstrated that small amounts of estrogen (10 ng/day) could improved the condition but pharmacological amounts (1 μg/day) worsened the condition. Similarly a condition where the oviduct filled up with fluid (salpingitis) unrelated to any bacterial infection was associated with zearalenone was described (24). Recently, in an experiment done at the Volcani Institute with Uzi Moallem and Moshe Kaim, it was found that 5 mg/day of zearalenone in the food ration fed to 6 cows for three months resulted in more large follicles and lower plasma progesterone compared to controls (http://aryeh-shore.blogspot.com).

In addition to observing the effects of ingesting estrogen mimetics, we performed a number of experiments to see where the probable target sites of exposure to estrogen and testosterone in domestic and zoo animals. In Israeli bulls it was found that implants which delivered 25 mg estradiol-17β over a period of three months caused decreased tonus and testes size in bulls (Figure 2; Marcus, Shore, Shemesh, unpublished observations). However, there are no reports of declining sperm counts or decrease testes size in Israeli bulls in the last fifty years.

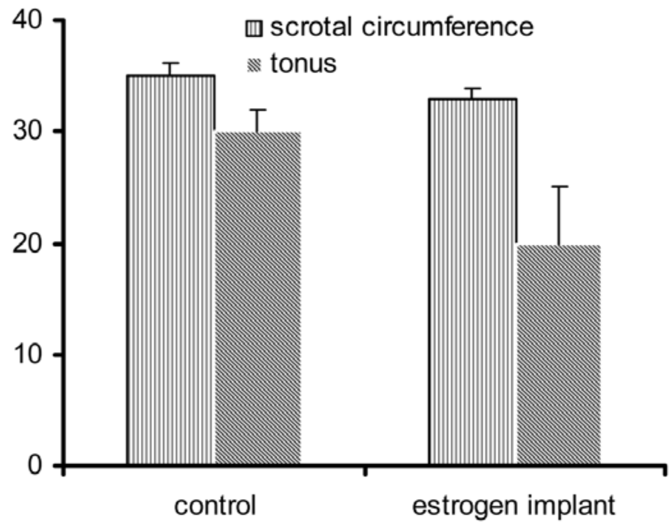

Fig. 2. Effect of estrogen implants on scrotal circumference and tonus in bull calves. Thirteen calves were implanted at 3 months of age and their testicles measured at 6 months. Fourteen other calves similarly fed and housed served as controls. The control circumference was significantly larger than the treated ($P<0.01$) and the tonus was significantly lower in treated bulls ($P<0.02$; 10=very poor; 20=poor; 30=good).

Environmental factors can influence sex at the blastocyst or implantation stage. We have observed in ibexes that the dominant female has the highest testosterone and that the level of testosterone is directly related to rank. The dominant females tended to have more male offspring (25). A similar direct effect of testosterone on bovine blastocysts has been reported (26). These effects are presumably due to variation in endogenous hormones in response to environmental stimuli but it could be a mechanism for environmental hormones to affect sex ratio (27). Recently, we reported that in soil exposed to testosterone in concentrations found in cattle manure used for fertilization of fields can affect the sex ratio of soil nematodes under field conditions, so the effects of environmental hormones on reproduction are not limited to vertebrates (28).

The steroids affect a wide variety of target organs besides the reproductive system. For example testosterone is associated with gingival hyperplasia in dogs (29) and progesterone with gingivitis in humans (30). The effects of hormones on intraocular eye pressure (IOP) are important as failure to account for the hormonal status may lead to incorrect diagnosis of glaucoma. The effects of hormones on IOP show species variation, i.e., progesterone increases IOP in lionesses (31) and estrogen increases IOP in cats (32). Both estrogen alone or combined with progesterone decreases IOP after menopause in humans (33).

SIMILARITIES BETWEEN BOVINE AND HUMAN FETAL DEVELOPMENT

Much of what we know today about sexual differentiation in humans was the result of the pioneering work of Jost who studied the freemartin in cattle (34). Cattle are unique in the case of male/female fetuses as the female twin offspring are sterile. Jost demonstrated that the sterility was the result of the exchange of blood between the fetuses and that after 60 days of gestation the exchange of blood between the twin fetuses no longer can affect the sex of the female fetus. We have shown that in both the female and male twin fetuses, there are changes in the steroidogenesis of the fetal gonads (35, 36). There are other factors involved like the mullerian inhibitory factor but the main point is that sexual differentiation in cattle and humans takes at the same time interval place at 45 to 60 days, which is not surprising as they have approximately the same length of gestation. In any event, cattle fetuses in Israel are readily available since about 5% of the spent dairy cows sent to slaughter have fetuses (personal observation). Since cattle eat the same legumes and grains, drink the same water and breathe the same air as the human population, sampling of the fetal calf blood for putative EDCs would be a cost effective way to monitor environmental endocrine disruptors.

PARALLELS IN HUMAN REPRODUCTION

Numerous attempts to use phytoestrogens as estrogens for postmenopausal syndrome have not convincingly demonstrated the clinical efficacy of the phytoestrogens (37). Similarly, soya milk supplements have long been suspected of being related to premature puberty in humans but numerous studies have not found an effect (38, 39). However, prolong phytoestrogen exposures has been associated with an incidence of uterine fibroids (40). Similarly, although human populations, especially in more Northern climes, are constantly exposed to zearalenone (41), no correlation with human reproductive health has been demonstrated.

Premature thelarche (breast development) is common in infants. Hormonal exposure may be a factor in the syndrome, and the use of diethylstilbestrol (DES) implants that were used briefly during the early 1950's was considered a possible cause. This is important, as this use of DES entered the Israeli public's consciousness that chickens receive some sort of hormonal supplementation which in turn affects childhood development (Yael Gazit, pediatrician, personal conversation). However, no poultry in Israel receives any hormonal supplementation. This is also true for cattle as the use of hormone supplementation, besides being illegal, is economically worthwhile only in steers which are not raised in Israel, as Israel only raises intact males. Nevertheless the age of thelarche associated with puberty has decreased in Israel and currently 15% of girls show breast development before 8 years of age (Itamar Gross, Min. of Health, personal communication). The etiology of this development is not known and could reflect the trend to earlier puberty due to better nutrition which began in the early 20th century (42).

SUMMARY

Many syndromes evoked by environment estrogens have been described in animals in Israel. This was possible be-cause of the uniformity of cattle and chicken husbandry in Israel throughout the country and the existence of a designated laboratory for hormone research at the Veterinary Institute. This unit was the result of the pioneering effort of Nathan Ayalon in cooperation with Hans Lindner and Mordechai Shemesh.

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