Infertility in the Southern White Rhino: Is Diet the Source of the Problem?

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As numerous species find themselves in peril due to human activity, the need to develop captive breeding strategies to save the most endangered among them has taken on an elevated sense of urgency. The Southern White Rhinoceros (*Ceratotherium simum simum*) has made a remarkable comeback in the wild but is still considered threatened due to habitat loss and poaching. Unfortunately, females born in captivity are subfertile, a phenomenon that is generating the risk of a significant population crash if the source of the problem cannot be identified. Numerous hypotheses including reproductive suppression by other females and continual close proximity to males have been purported and successively unsubstantiated. Could it come down to something as simple as diet? In this issue of *Endocrinology*, Tubbs *et al.* (26) provide evidence that it very well could. Their data suggest that the fertility of captive rhinos is being compromised, not by malnutrition but through endocrine disruption by phytoestrogens.

Zoo animals receive zoo food. The goal of a zoo diet is to recapitulate the wild diet as closely as possible, but sometimes this is not economical or practical. Cheetahs, for example, are carnivores. In the wild they chase and capture prey to survive, and the biomechanical marvel, which is a body built for speeds achievable by no other land mammal, evinces the long history of evolutionary pressures constraining them to excel at this one pivotal task. They do not graze on the sea of grass that camouflages their whereabouts from the objects of their gustatory desires, nor could they perceive it as food, even when faced with starvation. Thus, it may seem odd that a zoo would elect to rear a captive population on soy-based cheetah chow, but from a fiscal and practical standpoint, it makes a certain amount of sense. Soy is a whole protein (meaning it contains all of the essential amino acids), making it a mainstay for most vegetarians. It is also lactose free, low in cholesterol, cheap, easy to obtain, and more acceptable to distribute to a hungry carnivore in front of a zoo full of visitors than, say, a baby gazelle. But cheetahs did not evolve to consume soy and the diversity of endocrine disrupting phytochemicals it contains. They become sick. In a case strikingly similar to that of the Southern White Rhinoceros, by the mid-1980s reproductive and liver disease threatened to derail the captive breeding program for the cheetah (1). The isoflavone phytoestrogens genistein and daidzein (Table 1) were to blame.

Could a similar situation be affecting an herbivore like the Southern White Rhinoceros, a species that evolved to graze? Phytoestrogens are nonsteroidal, estrogen-like compounds produced by plants, most notably the legumes (2), and play an important role in plant defense (3) including the recruitment of nitrogen fixing bacteria (4) and conferring resistance to fungi (5). Thus, plants produce them when under stress including disease, drought, and extreme temperatures. There are several classes of phytoestrogens (Table 1), all of which structurally resemble mammalian estrogens, and many are capable of binding and activating nuclear estrogen receptors (EsR1 and EsR2) (6). The most well-known class, the isoflavones, are most abundant in soybeans and soy-based foods, whereas the coumestans are prevalent in alfalfa, clover, and other pasture legumes such as the ones rhinos and other herbivores might graze on. It is the coumestans that appear to be contributing to the reproductive impairments reported in captive born Southern White Rhinoceros.

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Abbreviation: EsR, Estrogen receptor.

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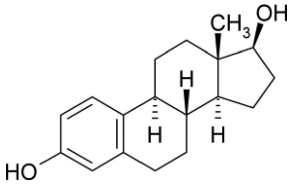
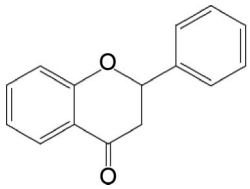
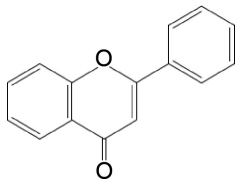
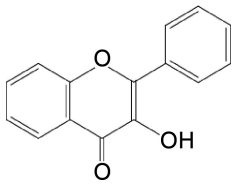
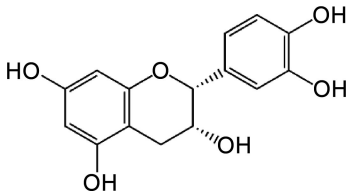
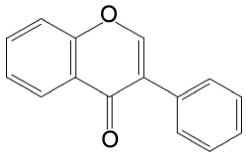
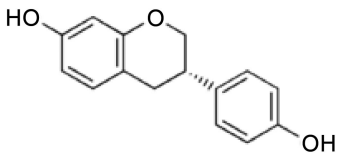
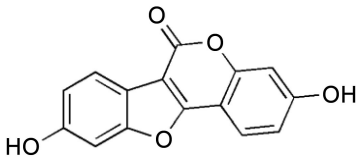
TABLE 1. Structures and common sources of phytoestrogens found in pasture grasses and other legumes

Group	Subgroup	Examples	Dietary sources	Basic structure
17β-Estradiol	Endogenous estrogen	N/A	N/A	
Flavonoids	Flavanones	Eriodictyol, hesperetin, homoeriodictyol, naringenin	Citrus fruits and juices	
	Flavones	Apigenin, luteolin, tangeritin	Parsley, celery, capsicum pepper	
	Flavonols	Fisetin, kaempferol, myricetin, pachypodol, quercetin, rhamnazin	Kale, broccoli, onions tomatoes, lettuce, apples, grapes, red wine	
	Catechins	Proanthocyanides	Chocolate, green tea, beans, apricots, cherries, berries	
Isoflavonoids	Isoflavones	Biochanin A, glycitein, daidzein, formononetin, genistein	Soy beans and other legumes	
	Isoflavans	Equol	Metabolite of daidzein produced by microflora	
	Coumestans	Coumestrol	Clover, alfalfa, spinach	

Adapted from elsewhere (7). N/A, Not applicable.

Tubbs *et al.* (26) provide evidence that the Southern White Rhinoceros is more sensitive to estrogens and phytoestrogens than their cousin, the Great One-Horned Rhinoceros. EsR1 and EsR2 were cloned from both species and transfected into human embryonic kidney 293 cells to characterize and compare their binding properties. The findings emphasize how highly conserved estrogen receptor structure is across a diverse range of species, a feature that reflects the functional importance of these transcription factors. Although appreciable differences in binding

affinity for endogenous estrogens or phytoestrogens were not found, receptor activation by coumestrol was significantly greater in the Southern White Rhinoceros than the Great One-Horned Rhinoceros. The authors surmise that this increased sensitivity may be contributing to the low fecundity in captive-born Southern White Rhinoceros females. Although more evidence is needed to affirm this conclusion, it is a reasonable presumption and not entirely remarkable because similar effects have been seen in other species including rodents (7), birds (8), cheetahs (1), and grazers like cattle and sheep (9–11).

Research spanning 6 decades has shown that when consumed, phytoestrogens can have numerous deleterious effects on female reproduction including disruption of the ovulatory cycle and subfertility (7, 12–15). Menstrual cycle irregularities have also been reported in humans consuming a soy-rich diet (16). A growing body of work has revealed that phytoestrogens may interfere with the organizational role of endogenous estrogen in the developing neuroendocrine and reproductive system. Regardless of animal model used, manipulation of estrogen during specific critical windows of development throughout the perinatal period leads to a myriad of adverse health outcomes including malformations in the ovary, uterus, mammary gland and prostate, early puberty, reduced fertility, disrupted brain organization, and reproductive tract cancers (17–24). These effects are typically subtle but devastating, often not becoming apparent until reproductive maturity and are, for the most part, permanent. In contrast, when consumed in adulthood, the effects are largely reversible once intake is reduced but not always.

Sheep raised on legume-rich pastures develop a suite of reproductive pathologies strikingly similar to those being observed in captive-born Southern White Rhinoceros, resulting in reduced conception rates and embryonic loss (9–11). Recognized since the 1940s, this syndrome is called clover disease and can be ameliorated by rearing the animals on cultivars of subterranean clover that contain fewer phytoestrogens. Prolonged exposure, however, can ultimately result in permanent infertility, even in adult ewes, because it induces the cervix to differentiate, making it more like the uterus and therefore unable to effectively store and transport spermatozoa (25). Other similar features between clover disease-stricken ewes and the subfertile Southern White Rhinoceros include estrous cycle irregularity, pyometra, endometrial hyperplasia, leiomyoma of the cervix and uterus, and cystic ovaries. In the Southern White Rhinoceros, it will be important to identify whether this syndrome results from embryonic exposure or chronic exposure across the life span.

It will also be critical to pinpoint the dietary source of the problem. On average, captive Southern White Rhinoceros are fed a diet containing 58% mixed grasses, 24% commercial pellets (which likely contain soy), and 15% alfalfa. Estrogenic pasture plants include alfalfa, annual medics, soybeans, and several varieties of clover (subterranean, red, and white). Each contains a unique mixture of phytoestrogens. Alfalfa, for example, can produce high levels of coumestans, whereas subterranean clover can contain up to 5% dry weight isoflavones including genistein (9). Sensitivity to each class of phytoestrogen is species specific. For example, infertility in cattle has been associated with alfalfa but not subterranean clover, whereas sheep are sensitive to both. It remains to be determined to which pasture legume the Southern White Rhinoceros is most sensitive, but data from Tubbs *et al.* (26) suggest that it will be those that produce high levels of coumestrol, such as alfalfa.

Phytoestrogens both expand our view of environmental substances with endocrine action and emphasize that the mammalian reproductive system has evolved the capacity to interact with them. For grazers, they may be an important signal of environmental quality because their prevalence increases when the plant is under stress. Thus, suppression of reproduction during such times may be adaptive for a species that lives in a marginal environment, like the Southern White Rhinoceros, but devastating if exposure is prolonged. Understanding these ecological relationships and recognizing the sources of endocrine disruptors in our environment are crucial to ensure the health and survival of imperiled species, such as the Southern White Rhinoceros.

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