CONSERVATION UPDATE
SAN DIEGO ZOO'S INSTITUTE FOR CONSERVATION RESEARCH SPRING 2010

# Rhinos, Reproduction, and Receptors:

## Trying to Solve a Conservation Riddle in a Test Tube

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Why do fewer than one in ten female southern white rhinoceroses born in captivity reproduce? This question has puzzled researchers for decades. At the San Diego Zoo's Institute for Conservation Research, Dr. Matthew Milnes and I are trying to answer it in a way that might surprise you. Unlike many researchers you may have read about, we don't travel to exotic locations to conduct our research. We don't chase wild rhinos through the bush and, thankfully, they don't chase us! Instead, we are answering this important question using a test tube.

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The southern white rhinoceros is one of our greatest conservation successes. Hunted to near extinction in the early 1900s, protection of the final 100 individuals allowed their numbers to reach 18,000 today. Breeding colonies were also established at zoos around the world as insurance policies against future population collapses. The San Diego Zoo's Wild Animal Park is the world's leader with more than 88 white rhino calves born since the early 1970s. But as years passed, it became clear that although wild-caught female rhinos from Africa (called the $F_0$ generation) reproduced well here and in zoos around the world, their captive-born daughters (called $F_1$) did not. Now captive populations are declining, as most of the original $F_0$ females in zoos are deceased or past reproductive age. We are at risk of having our insurance policy lapse.

Exactly what causes $F_1$ infertility remains a mystery. We know the problem is not behavioral. In fact, $F_1$ females engage in reproductive behavior just like $F_0$ females, and males are equally eager to breed with any female, regardless of her birthplace. We also know infertility is not the only problem more widespread in $F_1$ females than in $F_0$ females. Reports abound in the zoo community of malformed reproductive tracts, hormonal imbalances, and higher incidences of reproductive tumors. Interestingly, these ailments have a common thread: they can each be tied to exposure to the hormone estrogen.

Estrogen controls many processes, including reproduction. Like all hormones, estrogen is made in one part of the body and travels through the blood to the tissues it regulates. When estrogen reaches the tissue, it binds a receptor. Very similar to a key opening a lock, estrogen unlocks its receptor to elicit its effects. However, whereas estrogen production is precisely controlled, estrogen receptors are somewhat promiscuous. They can mistakenly bind to either man-made or natural chemicals, tricking tissues into thinking estrogen is present when it is not. This may prove detrimental. In other species, the reproductive problems we see described in $F_1$ rhinos are caused by fetal exposure to high levels of estrogen. Therefore, we think $F_1$ females were exposed to estrogen as fetuses, resulting in their infertility.

So where did the estrogen come from? It probably came from their mothers. $F_0$ rhinos were not fed large quantities of estrogen, but they did eat hay and commercial pellets. Reading the ingredients in pellets reveals that they are made from soy and alfalfa plants. Both are rather nutritious, but they also contain high amounts of phytoestrogens. Contrary to what their name implies (*phyto* = plant), phytoestrogens aren't actually estrogen but are plant compounds that *mimic*

## How YOU Can Help Rhinos!

The Institute depends on the generous support of individuals, corporations, and foundations to carry out our ongoing work on behalf of endangered species and habitats. Here are just two items that will immediately help our rhino reproduction research:

**High Performance Liquid Chromatography Kit – $5,000**
This kit is used to identify compounds in the diets of captive white rhinos that are likely to interfere with successful reproduction, such as those most likely to mimic estrogen. Once these chemical compounds are identified, we can make recommendations about optimal rhino diets.

**Travel for Multi-National Study – $2,000**
These travel funds will be used to continue a multi-institutional and multi-national study of the effects of phytoestrogens on rhino reproduction. The reproductive problems in captive-born white rhinos are not unique to the San Diego Zoo, so we hope to establish a collaborative program to improve rhino reproduction in zoos around the world.

estrogen. While some species can consume soy and alfalfa without any problems, other species cannot. We suspect that white rhinos may belong to the latter group.

To test our idea, we start with small pieces of rhino tissue, compliments of the Wild Animal Park's veterinary examinations, our pathology department, or even the Frozen Zoo®. We then isolate the genetic material that encodes rhino estrogen receptors and use it as a template to produce large quantities of actual receptors. We mix the receptors and phytoestrogens in a test tube and see how well they bind each other. This tells us how well the phytoestrogens act as "keys," fitting into the estrogen receptor "lock." The better they fit, the more likely they are to have harmful effects on developing rhinos.

We haven't yet solved the $F_1$ mystery, but we have made important progress. Ours were the first researchers in the world to isolate rhino estrogen receptors. Our test tube approach is the first of its kind to be applied to conservation science. It is a powerful tool that allows us to assess risks that chemicals pose to reproduction in endangered species. Better yet, we never expose an animal to chemicals, and this approach is applicable to any chemical and any species. Since hormones and receptors work the same way in most species, all we need is a receptor and a clean set of test tubes to solve our next conservation problem. That is, of course, after we finish tackling—and solving—the rhino reproduction question!

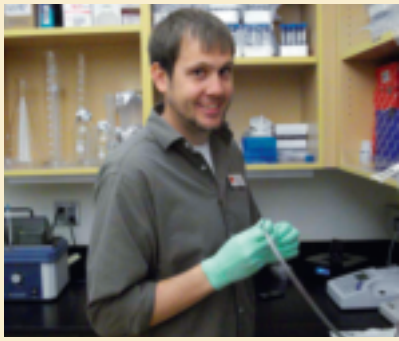
**Contact the Zoo Today**

If you are interested in supporting this program, please contact Julie Denman in the San Diego Zoo's Development Department at 760-747-8702, ext. 5762, or send an E-mail to jdenman@sandiegozoo.org.

## Meet a Conservation Researcher

## CHRISTOPHER TUBBS, Ph.D.

The great thing about going to college is meeting that life-changing professor who is so enthusiastic about their field that it just lights a fire in your belly, raises more questions than answers, and sends you on your life's journey. So it was for post-doctoral associate Dr. Christopher Tubbs as an undergraduate at the University of Florida studying the effects of chemicals on alligators and fish in Biology 101. "Being a student of Dr. Lou Guillette had a huge influence on my career," said Chris. "He is such a dynamic and accomplished professor that he really motivated me not only to pursue my own research questions but also to eventually become a professor myself. I just hope I can pass along that passion for conservation to my students!"



Growing up in Englewood, Florida, a stone's throw from the beach, it's no wonder Chris enjoys fishing, boating, surfing, and just about anything to do with water. He left the Sunshine State to pursue his graduate degree at the University of Texas–Marine Science Institute and studied fish reproduction, which led to his interest in hormone receptors (see article). Today, his work at the Institute for Conservation Research combines his interest in the effects of environmental chemicals on an animal's hormone receptors and a deep commitment to conservation.

"The great thing about working at the Institute is that we address important questions in a unique way, using the expertise from a variety of fields," said Chris. "It's a collaborative effort, and we are making progress in understanding the complexities of how hormones, receptors, and phytoestrogens interact and influence an animal's ability to reproduce. For instance, a tiny, noninvasive sample from an endangered southern white rhino allows us to make discoveries back in the lab using methods new to conservation research."

That's not to say Chris has always worked in the comfort of a laboratory. Back in his Florida days, he took his now-wife on a date to catch alligators in the swamp, a feat best tackled late at night. As the airboat purred up the channel, spotlight scanning for the red reflection of a gator's eyes, Chris was lying over the bow of the boat, poised to grab a juvenile alligator (approximately 3- to 4-feet long) with his bare hands, wrestle it into the boat (without letting go of its jaws), quickly measure it and take biological samples, then release it. Perhaps he wanted to dazzle the damsel on the boat, or just beat the hand-grab record, but Chris found himself landing a much larger alligator—6.175 feet long!—*and* breaking the hand-grab record. Did that seal the deal with his girlfriend? "Well, I don't think so…I heard the word 'stupid' a lot that night," Chris said. Yes, yet years later, they're happily married and have a daughter. That's the great thing about following your dreams.