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Stimulating Signals: Llama Mating Secrets

by Patricia Waldron

For a male animal to pass on his genes, he must create sperm. However, the fluid which carries sperm — semen — contains much more than just sperm cells. The surrounding seminal fluid is a complex mix of sugars, lipids, proteins and vitamins. Males require several accessory sex glands to create this soup of chemicals. The sheer complexity of semen and the presence of these sex glands had puzzled researchers for years. Some males use the chemical mix in seminal fluid to create a mating plug — a gooey clump that blocks the female's reproductive tract to prevent other males' sperm from gaining entry. However, male animals that do not use mating plugs still have functioning accessory glands, so they must serve a different function. In 1985, a group of Chinese researchers found that when camel seminal fluid was injected into female camels, they ovulated, even when no sexual activity had occurred. The researchers claimed that there was a chemical present in the fluid that stimulated ovulation. For 20 years their claim was ignored.

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Gregg Adams, a veterinarian at the University of Saskatchewan, and his colleagues at the Universidad Austral de Chile spent seven years looking for the mystery chemical in llama semen that triggered egg release.

In 2005, Gregg Adams, a veterinarian at the University of Saskatchewan in Saskatoon, Canada, successfully repeated the Chinese experiment in llamas. He and his colleagues at the Universidad Austral de Chile in Valdivia, Chile, then spent the next seven years trying to find the mystery chemical in semen that triggered egg release. They collected and purified semen from llamas and injected various components into females to see if they would ovulate. The protein they tracked down as the one responsible for ovulation turned out to be both surprising and familiar. The stimulatory chemical is a protein called β-nerve growth factor, or NGF, which had been known to function in the brain to keep neurons alive. NGF from semen appears to send signals to the female llama brain that result in ovulation. Though animal semen (including human semen) was known to be rich in NGF, no one had ever connected the protein to semen's stimulatory effect. (Currently it is unknown if the protein affects ovulation in humans.) The study was published in the *Proceedings of the National Academy of Sciences* August 20, 2012.

"The idea that a substance in mammalian semen has a direct effect on the female brain is a new one," said Adams in a press release. "This latest finding broadens our understanding of the mechanisms that regulate ovulation and raises some intriguing questions about fertility."

Finding the Not-So-Secret Ingredient

Llamas are useful animals for studying reproduction because they are induced ovulators. Like rabbits and camels, they release mature eggs in response to sexual activity. Humans, cows and many other species are spontaneous ovulators. They release eggs on a regular schedule regardless of sexual activity. For decades, researchers believed that the physical act of sex triggered the release of eggs in induced ovulators. When the Chinese study was published, no one believed it because the ideas it presented went against the existing theory. When Adams and his colleagues repeated the experiment decades later, they were shocked by the potent stimulatory effect of the seminal fluid.

To find the active ingredient that triggers ovulation in llamas, the researchers first collected llama semen from several males. They centrifuged the semen to separate the sperm cells from the seminal fluid. Next, by using a variety of purification processes, they narrowed down the complex mix of biological compounds contained in semen until they found the one responsible for triggering ovulation. The fluid was dripped through a tube full of a special material that separates out proteins into different sizes. The smaller proteins came out of the tube first, followed by medium-sized and then large proteins. Each size segment was injected into a different llama to test its stimulatory power. The segment that caused ovulation, as determined by a llama ultrasound, was purified further until the scientists had isolated a single protein. That protein was then compared to a database of known proteins to determine its amino acid sequence and structure.

Daniele Pellegrini/Image Bank/Getty Images

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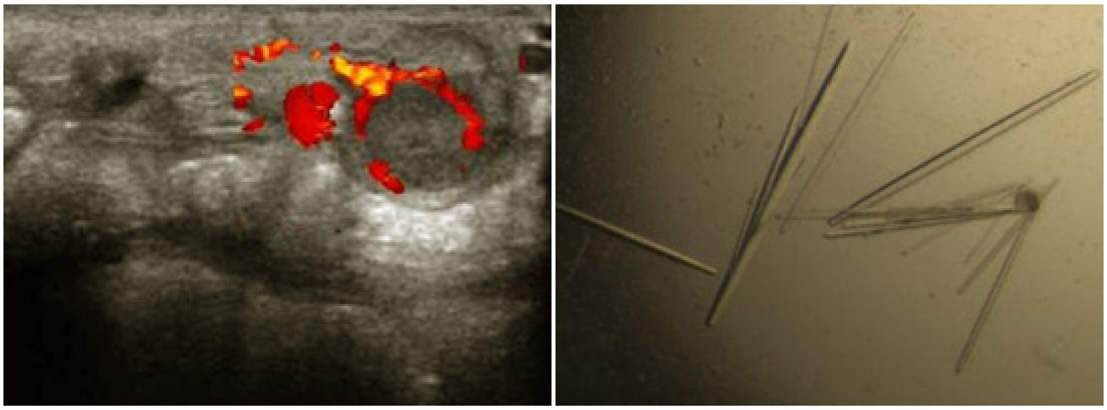

Left: Courtesy of Gregg P. Adams; Right: Yvonne LeDuc/University of Saskatchewan

To Adam's surprise, the active protein was identified as the well-known signaling molecule NGF. When they tested the protein, it acted just like NGF that had been isolated from mouse salivary glands. Proteins from both sources reacted with antibodies to NGF, and when added to petri dishes of rat neurons, they stimulated growth and caused the cells to produce the receptor protein that NGF binds to on the surface of cells in the brain. Both types of NGF also caused ovulation when injected into female llamas.

"We were looking for an unknown protein," Adams said to *ScienceNOW*, but the protein his team found is surprisingly common. "I didn't know whether to be happy or sad about that." He says that the discovery was doubly surprising since NGF is known to be abundant in mammal sperm but previously had never been linked to ovulation. NGF has been found in the sperm of all animals that have been tested so far, and the amino acid sequences of NGF are all very similar in different animals, from mice to snakes to humans. When a protein remains the same in organisms that are far apart on the evolutionary family tree, it often means that the protein has a very important function.

Surprising Signals

Though bull semen is also rich in NGF, the researchers were not able to induce ovulation in cows when injected with the protein. Cows are spontaneous ovulators that release eggs on a regular schedule. However, NGF was able to alter the timing of that schedule and caused changes to the corpus luteum — a clump of cells in the ovary that are shed from the egg after ovulation but that continue to secrete hormones that help the female maintain a pregnancy. In human females, if the corpus luteum does not pump out enough hormones, then the woman can miscarry.

NGF's stimulatory effects justify the presence of the complex male accessory organs that make seminal fluid. The discovery shows that these glands are not simply vestigial organs that have lost the ability to make mating plugs, but instead serve an important function in nudging along ovulation.

This study is the first to show that a component of semen can send signals to the female brain. NGF is a protein that is secreted by neurons and signals to nearby cells that they should stay healthy and not self-destruct. NGF from semen likely functions by traveling through the bloodstream to the brain and then acting on the female's hypothalamus and pituitary gland. These parts of the brain then secrete the hormones that start ovulation.

Sergio Ojeda, a neuroscientist at the Oregon Health and Science University in Portland, finds the discovery to be very exciting. He agrees with Adams's conclusion that NGF likely acts on females' brains. Ojeda claims that this study "is what is exciting about science. They decided to find out what the molecule was with no preconceived ideas at all. When they found it, it was extremely well done."

NGF: A New Fertility Drug?

In future experiments, Adams and his colleagues plan to examine the effects of NGF in humans. Since human females are spontaneous ovulators, it is unlikely that NGF will trigger ovulations in them, but it may have subtle effects on the timing of ovulation and could play and important role in fertility. "We're really interested to know the relationship between [NGF] and infertility," said Adams to *ScienceNOW*. "Are males with high concentrations of [NGF] in their semen more fertile?"

"If we find that NGF is also effective in women, it will obviously have huge implications for treating male infertility conditions," said reproductive biologist Raj Duggavathi of McGill University (the Sainte-Anne-de-Bellevue campus outside Montreal, Canada), to *Science News*. "It could be a big boost for couples."

If NGF can alter the timing of ovulation in humans, then it may explain the low rate of success of couples who practice the rhythm method for birth control. The rhythm method is a way for couples to reduce the chances of conceiving by charting the woman's reproductive cycle and then engaging in sexual intercourse only when the woman is unlikely to be ovulating. In about one in four couples who use this method, the woman becomes pregnant each year.

Adams also plans to explore the role of NGF in sustaining pregnancies. If NGF boosts hormone production in the corpus luteum in humans the way it does in llamas and cattle, then the protein may prevent miscarriages. After a woman becomes pregnant, a steady supply of semen may cause the release of hormones that will help her to stay pregnant.

Though the role of NGF in semen is a very new discovery, researchers hope that a better understanding of the signaling molecule will improve infertility treatments in humans and lead to more successful pregnancies. "It's still early." said Dan Bernard, a reproductive physiologist at McGill, to *Science News*. But, adds Bernard, "I think the fact that they've identified the protein will put this work on the map."

Gregg P. Adams: Advancing Reproductive Biology

Gregg P. Adams is a professor of Veterinary Biomedical Sciences at the University of Saskatchewan's Western College of Veterinary Medicine in Saskatoon, Canada. Adams earned his bachelor's degree in biology from the University of Saskatchewan in 1978 and his veterinary degree from the same university in 1982. He continued his education at the University of Wisconsin in Madison, completing a master's degree in 1987, clinical specialization in 1988, and a Ph.D. in 1991. A world-class researcher, prolific author and distinguished educator, Adams is the recipient of numerous honors.

Adams's groundbreaking research in reproductive science and medicine, and in particular on ovarian functions, has led to a number of "scientific firsts." His current research is focused on "ovulation-inducing factors in seminal plasma, factors inducing oocyte competence in cattle, and synchrotron-based biomedical imaging."

Below are Adams's September 12, 2012 responses to questions posed to him by Today's Science.

Courtesy of Gregg P. Adams

"So many advances have been made over the last 20 years, particularly in our ability to manipulate what affects the development of sperm and egg cells and our ability to influence the development of the embryo genetically and epigenetically. We thought that defining the genome was the key to understanding form and function, but now we know that environmental effects can actually be 'inherited' for

generations."

Q. When did you realize you wanted to become a scientist?

A. I wanted to become a veterinarian as far back as I can remember. Although I have always been interested in the "whys" and "hows" of things, I did not realize my passion for research until I went back to school from private veterinary practice to specialize in animal reproduction. I had a fantastic mentor for my Ph.D. at the University of Wisconsin who fanned the flames.

Q. How did you choose your field?

A. I think it was the influence of my father — a veterinarian and an academic who let us wonder and explore without pressure or any arbitrary restrictions.

Q. Are there particular scientists, whether you know them in person or not, that you find inspiring?

A. Yes, there are several people — some known as scientists and others better known in other fields. My inspirations include: early biologists and egg and sperm cell discoverers Regnier de Graaf and Antoni van Leeuwenhoek; statesman Thomas Jefferson; evolutionist Charles Darwin; physicist Albert Einstein (who was quoted as calling it "a miracle that curiosity can survive a formal education"); astronomer Carl Sagan; and storyteller Mark Twain. Lastly, and perhaps most influential, has been my Ph.D. supervisor, O.J. Ginther, who at the age of 82 is still an extremely productive and relevant scientist.

Q. What do you think is the biggest misconception about your profession?

A. That it is difficult. It is no more or less difficult than most other professions. But, one must take the initiative.

Q. Your study found that β-NGF induces ovulation in llamas; did it also rule out other proteins/elements of semen as inducing ovulation? Did you investigate whether there were any reproduction-related ends that other constituents of semen (besides β-NGF and sperm) may serve?

A. We went through a systematic process of ruling in and ruling out different fractions of seminal plasma. We treated the semen with different substances to purposefully remove (ablate) a certain fraction (by means of such methods as filtering, heating and enzyme digestion), and then tested what was left over to see if it still induced ovulation. By a process of elimination, we arrived at a protein with a singular molecular weight that, in terms of its amino acids and overall structure, was similar to β-NGF. To clinch it, we then treated animals with β-NGF and found that they ovulated!

Courtesy of Gregg P. Adams

At the Universidad Austral de Chile, Adams and his colleagues studied llamas and alpacas.

Q. Are there features shared by animals that are induced ovulators and those that are spontaneous ovulators? Which group comprises more species?

A. I've never really counted them up, but it is much easier for me to rattle off species that are spontaneous ovulators than induced ovulators, so I'd say the former are more common. The basic mechanism that results in ovulation is similar among all species; i.e., GnRH from the hypothalamus in the brain causes release of LH from the pituitary gland, which in turn causes ovulation. The difference is in what causes GnRH to be released in the first place. In discovering OIF [ovulation-inducing factor] in an induced ovulator, we were careful to determine that OIF was not just an evolutionary vestige in spontaneous ovulators. The abundance of OIF in semen of spontaneous ovulators and the effects we've seen so far, suggest that it plays an important role in both types of species. These findings indicate that a simple classification of species as induced or spontaneous ovulators is probably not as clear-cut as we once thought. The mouse is a good example — it is considered a spontaneous ovulator, but the female both requires and responds to semen to form a CL (a progesterone-producing gland in the ovary necessary to support pregnancy) after ovulation.

Q. At first glance, someone with no knowledge of the subject might be inclined to think that there might be advantages for a species to have the females be induced ovulators, as opposed to spontaneous ovulators. Did one type come first, evolutionarily speaking? Are there advantages conferred by spontaneous ovulation?

A. Yes, you're right — it seems like a good strategy to have the non-pregnant female in a "ready state," i.e., for her to be receptive and able to ovulate when chance brings a fertile male along. Induced ovulatory lineages have branched from the evolutionary tree at least 6 different times; hence, induced ovulation is not really an evolutionary precedent to spontaneous ovulation. Advantages of spontaneous ovulators? Good question. Perhaps one advantage is a distinct and short period of sexual receptivity, so that relatively more energy resources can be directed at maintenance/sustenance. I can imagine the young minds of your budding-scientist students ruminating about this!

Q. Where do you spend most of your workday? Who are the people you work with?

A. When I'm not involved with an animal experiment, most of my time is spent in my office or my laboratory at the [University of Saskatchewan's] College of Veterinary Medicine, where I teach anatomy to veterinary students and reproductive physiology to graduate students (M.Sc. and Ph.D. candidates). My colleagues are veterinarians, animal scientists, reproductive biologists, computer scientists, and physicians specializing in obstetrics and gynecology and in medical imaging.

Q. What do you find most rewarding about your job? What do you find most challenging about your job?

A. I particularly enjoy a comparative species approach. I enjoy going over new findings from different species with my graduate students, clarifying (or not) what is known and what is still not known about reproductive biology, dreaming up experiments to help answer the questions we come up with, and then submitting our findings for publication in the scientific or popular literature. I get great satisfaction when my students come to the realization that what has already appeared in writing is not necessarily correct and that they really can have an impact on the advancement of knowledge.

Most challenging — time management! There are so many opportunities and it is sometimes difficult to rein in my enthusiasm.

Q. What has been the most exciting development in your field in the last 20 years? What do you think will be the most exciting development in your field in the next 20 years?

A. This is an overwhelming question for me. So many advances have been made over the last 20 years, particularly in our ability to manipulate what affects the development of sperm and egg cells and our ability to influence the development of the embryo genetically and epigenetically. We thought that defining the genome was the key to understanding form and function, but now we know that environmental effects can actually be "inherited" for generations.

Q. How does the research in your field affect our daily lives?

A. Research in my field relates to fertility, infertility and contraception in both animals and humans. This has broad implications, influencing, among other things, breeding management of farmed livestock; recovery efforts for threatened species; population control for pets; contraceptive protocols and infertility treatments used in humans; and the health of the newborn.

Q. For young people interested in pursuing a career in science, what are some helpful things to do in school? What are some helpful things to do outside of school?

A. In school — pursue a broad-based education (resist opting out of a "tough" class). It's OK if you're not so good at physics — it's a pretty cool subject regardless. You never know where you'll end up — keep your options open.

Out of school — don't worry too much about what others think. Your thoughts and your personal space are just as relevant as anyone else's — practice not taking offense when others disagree with or criticize you.

Discussion Questions

Evolutionarily, what are some of the advantages of being an induced ovulator instead of a spontaneous ovulator?

NGF is just one component of sperm. Would you expect other chemicals found in sperm to have an effect on females? If you were a scientist conducting experiments to try to determine if NGF treatment could help reduce the risk of miscarriage, what kinds of side effects would you look out for?

Abstracts and Journal Articles

(Researchers' own descriptions of their work, summary or full-text, on scientific journal websites).

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