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SOME RECENT DEVELOPMENTS IN IMMUNOCONTRACEPTION

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This talk is not about deer biology. It is not about what we think you should do with your deer, nor is it a talk about the perfect solution to the urban deer problem. This talk is about a method that shows some promise for controlling some deer in certain sites. That method is immunocontraception.

Contraception, or fertility control is an appropriate subject for deer control. As reproductive biologists we have certain biases, and one of those is that the overpopulation of any species is not actually the problem. Overpopulation is merely a symptom of a larger problem - that being reproduction.

Contraception for deer has been around for at least 25 years. Early research involved the feeding of steroids to deer. Feeding deer natural steroids, such as estradiol or progesterone, was not a practical approach because it was necessary to feed the steroids to the deer every day. All mammals make estradiol and progesterone and the molecules are identical across species, thus the liver of the target animal "recognizes" the hormone and quickly metabolizes it. Attempts were also made at feeding deer synthetic steroid hormones. This worked a bit better, because the liver of the target animal could not recognize the steroid and it circulated throughout the animal much longer. The problem was, of course, that these synthetic steroids could pass through the food chain to scavengers and predators, including humans.

Steroid hormone implants, that are surgically placed and which release the hormones slowly were also tried in deer and these worked much better. On Angel Island, CA, this method was tried but without much success. Deer were trapped, implants placed in them, and the implanted deer became infertile. The main problem was that only about

one-third of the deer could be caught. Also the problem of passage through the food chain still existed. Finally, some of the hormones used in these experiments prevented parturition and therefore only non-pregnant deer could be treated.

More recently, Bob Warren, at the University of Georgia tested the human contraceptive implant Norplant in deer and Ed Plotka, at Marshfield Medical Foundation in Wisconsin, tested the active ingredient of Norplant, levonorgestrol, in deer and neither test was successful. Dave Jessup, with California Fish and Game administered the commercial agricultural steroid Norgestomet, packed into a biodegradable biobullet, to black-tailed deer and successfully inhibited reproduction. This particular steroid has been approved for use in cattle and may be useful for contracepting deer.

There are a number of theoretical approaches to deer contraception that do not use steroid hormones. For example, analogs of gonadotropin releasing hormone, or GnRH can be administered with the resulting complete inhibition of pituitary function and therefore gonadal function. This results in what is basically a temporary non-surgical castration. In another approach, a cellular toxin might be bound to a GnRH molecule. The GnRH would selectively seek out the pituitary cells that control reproduction and the attached cellular toxin would destroy these cells, resulting in a permanent non-surgical castration.

Our own research team got into the business of wildlife contraception not because of deer, but rather because of wild horses. In 1971 we began testing a variety of approaches and our research focused on steroid hormones and control of the stallions' ability to produce sperm. We tried a slow-release form of testosterone, which worked pharmacologically but was so difficult to administer that we finally abandoned this work.

In 1987 our research team paused to evaluate our progress, or lack of it, and in the process we also evaluated the political realities of wildlife contraception. If we only examined the biological possibilities for controlling a wildlife species, it was clear that there were many options and tools available to control wildlife populations. For example, animals could be shot, moved, poisoned, or trapped. But when we examined the legal restraints, it was clear that there were far fewer options available. Finally, the issue of publicly-acceptable methods

reduced the number of options to just a very few.

Our next step was to determine, theoretically, what the characteristics of the "perfect" wildlife fertility control agent might look like. It had to be effective - perhaps better than 90% effective. For large mammals it had to be delivered remotely, to reduce stress and eliminate handling of treated animals. It had to be safe to give to pregnant animals, and its contraceptive effects had to be reversible in order to have public acceptance, at least for wild horses. It had to be relatively inexpensive, it must not be able to pass through the food chain, and it should not cause any debilitating health side effects. Finally, the agent should have minimal effects on behavior.

The last step in our re-evaluation was to establish the necessary stages of research needed to develop a good wildlife contraceptive. These steps were built around three questions. Can the agent contracept the species? Can the agent be delivered under field conditions? Can a population effect be achieved? Of these 3 stages, number one is the easiest to carry out, but the jump between one and two, and two and three represent major steps. The public has little understanding of the conceptual jumps between step one and steps two and three.

Based on our view of the perfect contraceptive agent, we decided to throw away 15 years of research with steroid hormones, largely because of the difficulties of delivering sufficient quantities under field conditions, the potential pathogenic effects of these hormones, and finally because of the issue of passage of these hormones through the food chain. Instead, we turned our attention to immunocontraception. What led to this decision was a careful evaluation of available contraceptive technology. The history of contraception is largely steroid hormones, but it was our considered opinion that the future of contraception would be immunocontraception. Basically, immuno-contraception involves stimulating the target animals immune system to produce antibodies that interfere with some requisite reproductive event.

The next step was to evaluate available immunocontraceptive approaches and we chose the porcine zona pellucida (PZP) vaccine. In 1987 this vaccine had been around for quite a few years and had been tested extensively in non-human primates, dogs, and rabbits. The PZP vaccine fit our needs admirably. For instance, based on research with

these other species, there were no health side effects associated with its use, and being protein in nature, it could not pass through the food chain. Being a vaccine, it could probably be used in very small doses, making delivery under field conditions easier, and because it blocked fertilization rather than ovulation, it would probably not cause a lot of behavioral problems.

All mammalian eggs have a non-cellular glycoprotein membrane surrounding them. Several of these proteins function as the sperm receptor, and permit sperm to recognize the egg and to attach to it, two events necessary for fertilization. Think of the zona proteins as key-holes. All over the surface of the sperm are other proteins and I want you to think of them as keys. Only when the sperm surface proteins "fit" the zona proteins can fertilization occur.

We remove and isolate the zona proteins from pig (porcine) eggs and inject them into the target species, i.e., a horse or a deer. The target species produces antibodies against the PZP proteins because their immune system sees these proteins as foreign. The antibodies attach to the horse's (or deer's) zona pellucida and change the shape of the zona receptor proteins, so that the sperm of that species can't recognize the egg.

It is important to make a distinction between different types of PZP vaccines. We use a native PZP, which means that we simply isolate the proteins from pig eggs. Thus, our vaccine uses the entire "family" of zona proteins. It is also possible to separate individual proteins, such as ZP3. Various forms of the vaccine have also been produced with genetic engineering, but these have not yet been shown to be very effective, particularly in deer.

This approach to contraception is attractive for a variety of reasons. Because only fertilization is affected, the endocrine system should work normally and the animal should continue to have estrous cycles. This in turn means minimal effects on behavior. Breeding should even occur.

Despite extensive research with this vaccine in nonhuman primates and other small mammals, it had never been used in ungulates. My colleague Irwin Liu, at the University of California-Davis, inoculated 14 horses with the PZP vaccine and only one produced a foal, provid-

ing good evidence that PZP worked in equids.

With that background, my colleague, John Turner, from the Medical College of Ohio, and I marched off to Assateague Island National Seashore (ASIS) at the invitation of the National Park Service (NPS). The problem with horses on ASIS is that the population is growing and there is fear of damage to the island's fragile ecology. Normally the NPS has little tolerance for species that are not considered native, but the ASIS horses are considered to be a cultural and historic resource and therefore permitted to inhabit the island. Actually, the horses inhabited the island before settlers, arriving in 1670, when English settlers barged them across from the mainland to avoid the king's tax on fences.

These ASIS horses should be distinguished from the Chincoteague horses. This herd is privately owned by the town of Chincoteague, kept in fenced corrals on the Virginia portion of the island, and the foals sold at auction each summer. The ASIS horses in contrast, are free-ranging, unmanaged, and under the jurisdiction of the NPS.

In March and April of 1988 we inoculated 26 ASIS mares with the PZP vaccine. Each mare received either 2 or 3 inoculations over the 2-month period. We used PaxArms self-injecting barbless darts. The following year there were no foals born to the treated mares, regardless of whether they received 2 or 3 inoculations, while about 50% of the control mares produced foals (the normal foaling rate for ASIS mares is about 50%). Sixty percent of the treated mares were pregnant at the time of their inoculation and they foaled normally in 1988, demonstrating for the first time that the vaccine was safe to give to pregnant animals. We also kept track of the social organization of the treated horses and there were no differences between the treated bands and untreated bands. The behavioral issues involved some questions that, on the surface, sounded silly, but were actually good questions. What's a stallion going to do if his mares don't have any foals for a number of years? What will a mare do if she doesn't foal year after year? Later, after six and seven years of treatment of the ASIS horses we were able to demonstrate that there were no effects upon social organization.

The only side effect we found was that a small percentage of treated mares developed small abscesses at the injection site. These all healed

normally and there were no untoward side-effects as a result. In our estimation, green-head flies on ASIS do far greater damage to the horses. We are not sure if the adjuvant caused the abscesses or whether the problem was a result of dirt in the injection site. We washed the dart needles with alcohol but often ended up darting a mare that had 'just been rolling around in the mud. I wondered if there was really any point in washing the needles. Recently we hand-injected 70 mares in Nevada and four weeks later there were no abscesses.

The next year, in 1989, we inoculated half of the 26 mares with a single booster inoculation and left the other 12 untreated. Thirteen of the 14 inoculated mares failed to produce foals in 1990, while 5 of the 12 mares not treated produced foals, demonstrating the reversibility of the vaccine's contraceptive effects. Since 1990, we have been treating the ASIS mares with the PZP vaccine and after 105 mare-years of treatment, only 4 foals have been produced. Two of those foals were produced by the same mare.

This past March, we gave the seventh consecutive treatment to a small group of mares, and we inoculated another 60 mares for the first time. In the future these mares will be inoculated according to a very complex population model developed by Brian Underwood, of the National Biological Survey. In future years this model will determine exactly which mares should be treated each year in order to achieve specific population goals. By 1992, we changed dart systems and we now use Pneu-Dart darts and rifles, which seem to work much better with the thick viscous vaccine.

We have taken this technology to Virgin Islands National Park where we have successfully treated donkeys. In another experiment in 1992, involving a remarkable collaboration between the research team, The Humane Society of the U.S., and the Bureau of Land Management, we started treatment of large numbers of Nevada wild horses.

The next application of the PZP vaccine came as something of a surprise to us. It was zoo animals. A major problem that zoos face today is the production of "surplus" animals and the disposition of these animals in publicly acceptable and humane ways. Zoo contraception has been around for many years, particularly as applied to felids, which reproduce well in captivity. For many years melengestrol ac-

etate implants were used to contracept felids, but long-term effects of this hormone include mammary gland tumors, uterine tumors, and adrenocarcinomas. As a result, many zoos have backed away from the use of steroid contraception.

We started our work at the Cologne Zoo, not with deer but with Mongolian wild horses and banteng. We were successful in contracepting both species with the PZP vaccine and moved to the Bronx Zoo, where we began testing the vaccine in the Cervidae - the deer family. The advantages of PZP contraception in zoos immediately became apparent. The animals did not have to be handled to deliver the vaccine. Also, we could recover blood samples in certain cases, in order to measure antibody titers. This is important because it is valuable to know what the contraceptive threshold is for antibody titers. We know, for example, that the threshold is 64% of positive reference controls for horses (the positive reference standard is the average of 10 samples which show maximum titers and for which the animals did not become pregnant). We do not know that threshold for other species although we believe it is similar to horses for deer.

We have now tested the PZP vaccine on more than 60 captive exotic species, more than any other form of contraception, and have had success with more than 30 of those species (we are still waiting for the outcome of the tests with the other 30 species). We are even getting positive results with African lions.

On the strength of the positive data from zoo cervids, we moved on to white-tailed deer. First we tested the vaccine in captive deer. Of 7 deer treated, none produced a fawn a year later, while 6 of 7 control animals did produce fawns. We carried out another 3 years of tests with captive deer and demonstrated a high degree of contraceptive efficacy, reversibility of contraceptive effects, and we were even able to test antibody titers in some animals. If we gave 2 or 3 inoculations during year- I and a booster shot during year-2, the deer had no fawns in either years- 1 or 2, but 75% had fawns in year-3. If the deer received no booster shot during year-2, 28% were fertile in that second year and 75% were fertile by year-3.

At this point, we initiated our first test with free-roaming deer, at the Smithsonian Institute's Conservation and Research Center (CRC) in Front Royal, VA. We had now advanced to stage two of the three

stages in testing wildlife contraceptives, i.e., testing delivery under field conditions. The CRC is a fenced facility of about 3,500 acres, filled with endangered species and operated by the National Zoo. These deer were ear-tagged but they were very wild, and it took every possible deer-hunting trick in the book to get them treated. Ten deer received 2 shots, another 10 received a single shot of a prototype one-shot form of the vaccine, and another 10 received only adjuvant and saline.

The deer receiving 2 shots had no fawns, while 8 of the 10 control deer had fawns. By the end of July, the 10 deer receiving the one-shot form of the vaccine had no fawns and we thought we had succeeded in producing a one-shot form of the vaccine. However, by September, 7 of the 10 does had fawns.

This was a very important study for several reasons. We were able to contracept the deer which had received only a single shot, through their normal breeding season which usually ends in December, but they extended their breeding season and continued to display estrous cycles through February, until antibody titers had dropped below contraceptive levels. The two-shot animals also extended their breeding season, but did not get pregnant, because the second shot extended contraceptive antibody titers well beyond February. This response by deer had been hypothesized by critics of deer contraception, and their concern was that the continued estrous cycles would represent a dangerous energy cost to the does. However, the following summer, all the deer were trapped and weighed, and those deer that extended their breeding season and didn't get pregnant weighed an average of 20 pounds more than those animals that got pregnant during the normal breeding season. Thus, the energy cost of getting pregnant and lactating is far greater than extending the breeding season by two additional cycles.

Another question involved the bucks. If does continued cycling into February, wouldn't it be possible that bucks would use up valuable energy stores chasing estrous does'? It was a good question. One of the most fascinating aspects of the CRC study was that the older bucks did all the breeding during the normal breeding season and that the younger bucks were the ones to follow the does with the extended breeding season. There was no frenzied activity by bucks, probably because testosterone concentrations in the bucks are well on their way

down by February, and testosterone is what drives sexual behavior. So, we didn't see male deer wearing themselves out.
Last fall we initiated our second field test with white-tailed deer, on Fire Island National Seashore. This is another of those situations where a deer hunt is not possible or advisable and deer have literally overrun the island. This project is a cooperative effort involving the citizens of six communities, the New York State Department of Environmental Conservation, The Humane Society of the U. S., and the National Park Service. During September 1993 we inoculated 73 does and a month later we relocated 68 of these does and gave them a second inoculation. Each deer was identified individually on the basis of unique markings. Although reliable fawn counts cannot be made until September, in July 1994 the project looked very successful. July fawn counts are corroborated by pregnancy testing via fecal steroid analysis.

This brings up an interesting point with regard to evaluation of contraceptive results. Fawn counts alone may not be considered reliable, because of neonatal loss. In other words, neonatal deaths can confound actual results by causing an artificially low fawn count. We first learned this with wild horses, where neonatal foal mortality is very high. Thus, we rely on urinary or fecal steroid pregnancy testing. In deer, fecal estrogen conjugates (EIC), pregnanediol-3-glucuronide (PdG), and progesterone are higher in fecal samples from pregnant deer than in non-pregnant deer.

The last thing I want to talk about today is the need for a one-shot form of this vaccine. When we apply the standard 2-shot protocol, antibody titers almost always exceed 65% and contraception is achieved. By Day-215 post-inoculation, antibody titers have fallen below the contraceptive threshold. Thus, it is clear that if deer contraception is ever to reach an effective management stage, we must develop a one-shot form of the vaccine which will provide at least one full year of contraception. That is the current focus of our research with HSUS and the Department of the Interior. This research is aimed at the control of wild horses but the technology, when it is available, will be applicable to deer too. Our first attempt involved microspheres, very small particles of a non-toxic, biodegradable material in which the PZP antigen is dispersed. Upon injection, body fluids cause the particles to erode and the antigen to be continuously released. Our first attempts with the microspheres was with ASIS horses and caused infertility in 12 of

13 mares (which do not extend their breeding season if they don't get pregnant) but it did not work well in deer, and we have now moved on to microcapsules. Microcapsules are made of the same material as the microspheres, but as they erode after injection, they result in a pulsed release of the antigen instead of a continuous release. We tested this second generation of one-shot particles on Nevada horses in December 1992 and thus far none of the one-shot horses have produced foals. This research is beginning to look promising and our ultimate goal is a one-shot form of the vaccine that will provide 2 to 3 years of contraception.

Let me finish by anticipating several questions. When we first started this work, wildlife contraception was a fairly new idea. Our efforts were originally met with mockery but recently, since we have applied this technology to deer, that disdain has changed to allout opposition. I am bothered by this because this opposition is laced with hypocrisy and, in some cases, outright dishonesty. Some of the opposition attempt to link deer contraception with the issue of deer hunting. It can't be done. A rational mind cannot possibly view the crude technology I have described here as a threat to hunting. Our version of deer contraception might work for an arboretum, a small city park, or some place like Fire Island, but it can't replace hunting in places where responsible sportsmen would want to hunt deer.

The next issue is that of passage of the vaccine through the food chain. Many critics have conjured up the spector of "poisoned" meat. If this was a real threat, why would the Denver Wildlife Research Center be spending hundreds of thousands of dollars trying to figure out a way to deliver this vaccine orally if it could already go through the food chain? Proteins can't survive the digestive process; that is basic biology we learn in ninth grade, but the question keeps popping up. The fact is, it is not an intelligent question, but rather a feeble attempt to confuse issues.

Another popular attack on the PZP vaccine involves genetics. Won't unhealthy animals fail to make antibodies and ultimately keep producing fawns, while healthy animals would make antibodies and become infertile? This supposition leads to a skewing of the population toward unhealthy animals that do not respond to the vaccine. On the surface it is not a bad question, but there are no data with wildlife populations to support this contention. And, there is a growing body

of data that shows that an unhealthy animal produces antibodies at the same rate as healthy animals. If this argument had any factual basis, human vaccines would be of no use in sick people and we know that is not the case. As one immunologist put it, if an animal is so sick that it can no longer produce antibodies, it is not going to live to breed the next year. Another flaw in this argument is that no one has ever proposed mass immunizations. As I pointed out before, this technology at best will be useful only for small populations in parks and arboretums. Finally, there is great hypocrisy in this argument, because there is probably nothing that comes even close to skewing the genetics of deer populations as much as hunting. Why is the genetics argument important for immunocontraception but not for hunting? I am bothered by this argument and the hyprocrisy it reveals as both a former deer hunter and a scientist.

The issue of population modeling often comes up too. What percent of a herd has to be treated to achieve the goal? Unfortunately, the question is incomplete. What percent of the herd has to be treated to achieve what? Zero population growth? A small increase? A decrease? Modeling is useless in the absence of an articulated goal. Modeling is also almost worthless unless you use data from the site where you are going to work. Modeling must be based on site-specific data in order to answer these questions. Birth rates are not the same everywhere, nor are mortality rates, nor are age structures.

Discussion

The discussion first revolved around FDA approval of the vaccine. It was explained that the Humane Society of the U.S. had received an Investigational New Animal Drug (INAD) number for use of the vaccine with horses, deer, and zoo animals. The INAD is basically an agreement with the FDA on what kind of data will be collected and how it will be collected. It was also explained that a good deal of data is necessary before an INAD can be obtained. The CRC and Fire Island deer projects have been approved by FDA and all new deer projects will be approved or rejected by FDA on a site by site basis.

It was explained that the research team and the Humane Society of the U. S. have taken steps to make sure the vaccine cannot be patented, so that the cost of the vaccine can be kept low enough to make it practical to contracept deer.

Another research focus is the development of a marking dart, which will leave a paint spot on the deer at the same time it injects the vaccine. This will preclude the necessity of identifying individual deer on the basis of unique markings or ear-tagging.

Several speakers indicated that any studies of less than 3 years duration are useless for answering questions regarding population changes. The Fire Island project will run for no less than 5 years.

Jay McAninch was concerned that there might be a backlash against immunocontraception because people hear about it but can't use it now. It was explained that any community, park or arboretum with the manpower and commitment can apply the technology now; it is just very labor-intensive because of the need for 2 shots. In order to facilitate use of the technology, the Humane Society of the U. S. and the National Institute of Science and Technology, with financial aid from the Dodge Foundation, is developing a training program, to prepare personnel to carry out deer projects by themselves. Also, a one-shot vaccine may be ready within 18-24 months. The cost of the Fire Island project, as a research project has been about $110/deer. The cost should decrease as immunocontraception is used for management, instead of research.