Effects of Vaccination against GDF9 and BMP15 on Fertility and Ovarian Function in the White-tailed Deer

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ABSTRACT: The physiological mechanisms controlling ovarian follicular growth and ovulation involve a complex exchange of systemic signals and a localized exchange of molecules between the oocyte and surrounding somatic cells. It has been demonstrated that the oocyte itself plays an essential role in regulating these processes by secreting two key regulatory proteins: bone morphogenetic protein-15 (BMP15) and growth and differentiation factor-9 (GDF9). Natural mutations in their expression and vaccination against these growth factors have been shown to cause sterility in sheep. The aim of this 3-year study was to determine the effect of vaccination against GDF9 and BMP15 on fertility in female white-tailed deer. Does were randomly assigned to two groups (n = 10/group). Each doe received a primary vaccination followed by a booster 47 days later. After the first year, a subset of animals (n = 4-5/group) received an additional booster vaccination. Blood samples were collected at regular intervals to determine antibody titers and progesterone concentrations. Early pregnancy was assessed by ultrasound, and fawning was subsequently monitored. For the BMP15 group, over the 3 years 80%, 100%, and 75% of does fawned with fawning rates of 2.4, 3.5, and 3.3 fawns/doe, respectively. For the GDF9 group, 80%, 25%, and 25% of does fawned with fawning rates of 2.6, 1, and 2 fawns/doe, respectively. The fawning rate of untreated animals in the herd was 1.8 fawns/doe. Although all animals in the BMP15 group had high antibody titers, they were not made infertile, and they became more fecund. Most animals in the GDF9 group had high antibody titers, but fecundity was not affected the first year; however, they were made infertile in Years 2 and 3. Results from this study demonstrate that vaccination against GDF9 has potential to control fertility in deer. Further research will be required to determine the appropriate timing for administering the vaccine and the longevity of effect.

KEY WORDS: BMP15, fertility control, GDF9, growth factor, immunocontraception, *Odocoileus virginianus*, white-tailed deer

Proc. 26th Vertebr. Pest Conf. (R. M. Timm and J. M. O'Brien, Eds.)
Published at Univ. of Calif., Davis. 2014. Pp. 391-395.

INTRODUCTION

The white-tailed deer (*Odocoileus virginianus*) is commonly found throughout North America. Locally over-abundant populations are known to cause ecological damage (Cote et al. 2004) and conflicts with human activities (Conover 2002). The management of deer populations is often carried out by lethal means, which in more urban areas can be controversial. For many years, fertility control using immunocontraceptive vaccines has been investigated as a non-lethal tool to aid in these efforts, with varying results (Cowan et al. 2003). The two vaccines currently available target the reproductive hormone gonadotropin-releasing hormone (GnRH) and the zona pellucida, which is a glycoprotein coat that surrounds the egg (oocyte). Vaccination against GnRH suppresses the production of reproductive hormones, including gonadal steroids, and behavioral estrus in females, whereas vaccination against ZP prevents fertilization. One of the challenges for the use of these vaccines is maintaining an adequate immune response for the reproductive life of the animal. This requires that animals be re-vaccinated, sometimes annually, which is difficult and costly. There is clearly a need to identify new target molecules that can cause permanent sterility without requiring that the immune response be maintained for such an extended period.

The physiological mechanisms controlling ovarian follicular growth and the number of oocytes released at ovulation involve a complex exchange of systemic (e.g., endocrine) signals between various organs and the ovaries, and a local exchange of molecules between the oocyte and its surrounding somatic cells within the ovaries (Matzuk et al. 2002, Gilchrist et al. 2004, Binelli and Murphy 2010). Significant discoveries have shown that the oocyte itself produces two key regulatory growth factors, namely growth differentiation factor 9 (GDF9) and bone morphogenetic factor 15 (BMP15), which are essential for regulating follicular growth and ovulation rate (Dong et al. 1996, Galloway et al. 2000). Both GDF9 and BMP15 have been shown to be involved in early follicular growth in several species (Bodensteiner et al. 1999, Eckery et al. 2002, Juengel et al. 2004, Shimasaki et al. 200, Juengel and McNatty 2005). In sheep, it has been shown that animals that have a double copy mutation in the genes encoding either GDF9 or BMP15 are sterile but otherwise healthy. In these animals, follicles do not progress beyond the first stage of growth. Interestingly, in animals that have only a single copy mutation in either of these genes, and thus produce essentially only half the amount of protein, ovulation rate is increased (Galloway et al. 2000, McNatty et al. 2005). In mice, deletion of GDF9 leads to sterility, but if they

don't express BMP15 they are only sub-fertile and often have decreased litter size (Moore and Shimasaki 2005). Mutations in GDF9 or BMP15 have also been found in women with premature ovarian failure (Pouresmaeili and Fazeli 2014).

In a series of experiments conducted in sheep, it was shown that ewes could be made infertile after immunization against either GDF9 or BMP15 (Juengel et al. 2002, Juengle et al. 2004, McNatty et al. 2007). Moreover, specific regions of each growth factor were identified that were important for the biological activity of the respective proteins. This enabled the production of effective peptide vaccines that were specific to those regions.

The objective of this study was to determine the effects of immunization against GDF9 and BMP15 peptides on ovarian function and fertility in female white-tailed deer. Our long-term goal is to develop a vaccine that is better able to cause permanent sterility.

METHODS

Animals

White-tailed deer used in this study were maintained at the Pennsylvania State University (PSU) Deer Research Center, where a captive deer herd has been maintained since 1972. During this study, the facility encompassed 22 acres of natural woodland/forest habitat which was divided into separate paddock areas. Vegetation on the open areas consisted of a mixture of clover and orchard grasses, but most of the land was covered with dense deciduous forest that had little understory vegetation. During the non-breeding portion of the year, treated female deer were isolated from males. However, to test vaccine contraceptive efficacy each year, from the first week of November through the end of the following February, bucks of proven sire ability were introduced with the females.

Peptide Design

The peptides used to make the vaccines for this study were chosen based on results from sheep studies conducted by McNatty et al. (2007), which showed immunization against these specific peptides inhibited ovulation. The peptide sequences for both GDF9 and BMP15 are located at the N-terminal regions of the respective mature proteins. Using a basic local alignment search tool (BLAST), we found that the respective sequences in red deer are identical to those in sheep, cattle, goats, mice, and hamsters. Therefore, we assumed they would also be the same in white-tailed deer.

The peptides QAGSIASEVPGPSRGC (BMP15) and HSEYFKQFLFPQNEC (GDF9) were synthesized by Global Peptide (Fort Collins, CO). The underlined amino acids represent the native peptide sequences. For the BMP15 peptide, a glycine (G) residue was added at the C-terminus as a spacer, followed by a cysteine (C) residue to aid coupling to the carrier protein. To make the peptides immunogenic, they were coupled to a maleamide-activated carrier protein, blue protein hemocyanin (*Concholepas concholepas*; Biosonda, Santiago, Chile) as previously described (Miller et al. 2008). Each 1-ml dose of vaccine contained 1 mg of peptide-blue protein conjugate in 0.5 ml of buffer emulsified with 0.5 ml of AdjuVac adjuvant (National Wildlife Research Center, Fort Collins, CO).

Vaccination of Deer

Twenty sexually mature female white-tailed deer were divided randomly into two groups (n = 10/group), namely, BMP15 and GDF9. In July 2007, each deer received a primary vaccination (1 ml) of the assigned vaccine followed by a booster vaccination (1 ml) 47 days later. A subset of animals from each group (BMP15 n = 4; GDF9 n = 4-5) received a second booster vaccination at day 321. Vaccines were administered intramuscularly in the hindquarter.

Blood samples were collected on days 0, 47, 96, 188, 554, 754, and 918. Approximately 7-10 ml of blood was collected from the jugular vein at each time point. Following collection, blood samples were centrifuged and serum was isolated and stored at -70°C. Serum was used to determine antibody titers and progesterone concentrations.

Laboratory Testing

Progesterone

The Coat-A-Count Progesterone In-vitro Diagnostic Test Kit™ (Diagnostic Products, Los Angeles, CA) was used to determine concentrations of progesterone in serum samples according to the manufacturer's recommended procedure.

Antibody Titers

Enzyme-linked immunosorbent assay (ELISA) was used to determine anti-GDF9 and anti-BMP15 antibody titers as previously described (Miller et al. 2008) with the following modifications: plates were prepared by coating each well overnight with 100 ng of GDF9-BSA or BMP15-BSA. Titers were assigned as the highest dilution showing a significant absorbance value greater than that of the corresponding pre-bleed value.

Assessment of Fertility

The mean reproductive success of the entire PSU captive deer herd (1.8 fawns/doe) was used as a control value that was compared with that of the deer vaccinated against GDF9 and BMP15. The deer in the current study were managed at the same facility and were maintained under the same conditions. At the end of January in 2008, 2009, and 2010, ultrasound examinations were performed to determine if does were pregnant. Ultimate reproductive performance was assessed by evaluating annual fawn production.

Statistical Analysis

Data for the number of fawns produced per doe were analyzed by one-sample t-test comparing values to the mean number of fawns produced (1.8) by the PSU deer herd. Progesterone data for BMP15 animals was analyzed by ANOVA. There was insufficient data to analyze progesterone data for GDF9 animals. Statistical analyses were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA).

RESULTS

Antibody Titers

Mean antibody titers for the two groups over the course of the study are shown in Figure 1. All animals in the BMP15 group developed high titers following the first booster vaccination and maintained the high titers for the duration of the study. Animals in the GDF9 group also developed good titers, but they were more variable. One doe in GDF9 group failed to mount an immune response to the vaccine at any time during the study, and most of the variability shown in the graph was due to this one animal.

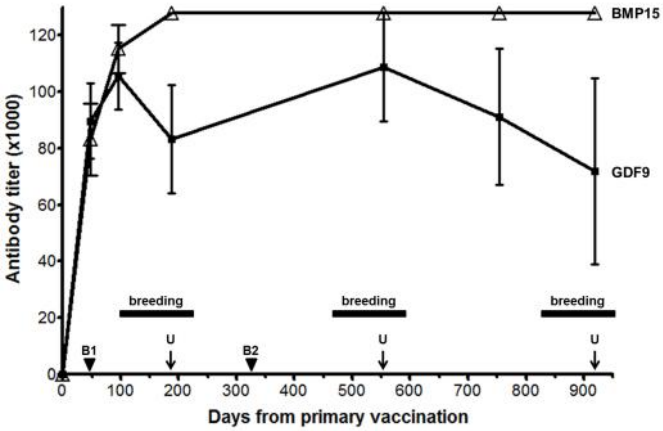

Figure 1. Mean antibody titers for BMP15 and GDF9 vaccinated animals. Days when the first and second booster vaccinations were given are shown by B1 and B2, respectively. The days when pregnancy was assessed by ultrasound and progesterone measurements were made are shown by a 'U'. The breeding period for each year is also shown.

Fertility

The results of ultrasound assessments, progesterone concentrations, and number of fawns born are shown for individual does in Tables 1 and 2. A progesterone concentration of >1 ng/ml is indicative of an active corpus luteum being present on the ovaries (Plotka et al. 1977, 1982). Mean progesterone concentrations did not differ significantly between years for BMP15 animals. Although not all does produced offspring in the first year, based on progesterone measurements nearly all animals were confirmed cycling and had active corpora lutea at the time the blood sample was taken. In Years 2 and 3, does in the BMP15 group continued to cycle and produce offspring, but fertility of does in the GDF9 group was inhibited. The doe in the GDF9 group that produced offspring was the same doe that did not mount an immune response to the vaccine. For the BMP15 group, over the 3 years 80%, 100%, and 75% of does fawned with fawning rates of 2.4, 3.5, and 3.3 fawns/doe, respectively. For the GDF9 group, 80%, 25%, and 25% of does fawned with fawning rates of 2.6, 1, and 2 fawns/doe, respectively. In Year 1, the number of fawns per doe did not differ ($P < 0.5$) for either the BMP15 or GDF9 groups compared to the control (1.8). In Years 2 and 3, does in the BMP15 group produced significantly more fawns per doe. Apart from the one doe that did not mount an immune response to GDF9, no other does in this group produced fawns in Years 2 and 3.

DISCUSSION

Both BMP15 and GDF9 have been shown to be essential for ovarian function in several species (Otsuka et al. 2011). A major finding from this study is that both BMP15 and GDF9 also have effects on ovarian function and ovulation rate in white-tailed deer. Although the antibody response to the BMP15 vaccine was very good in all treated animals, vaccination against BMP15 caused an increase in ovulation rate, resulting in an increased number fawns per doe being produced. The reason for this is unknown, but it may be that the antibodies produced were only able to partially inactivate the BMP15 protein in the ovaries, therefore causing a phenotype similar to that found in sheep that are heterozygous for a BMP15 mutation (Galloway et al. 2000). For the does vaccinated against GDF9, although the increase was not significantly different from controls, there seemed to be a trend towards an increase in the number of fawns produced per doe in the first year. Again, this may have been due to the antibodies only being able to partially inactivate the GDF9 protein. However, vaccination against GDF9 completely suppressed fertility, and no fawns were born to does that produced an antibody response in Years 2 and 3. Similar effects have been demonstrated in sheep where

Table 1. Ultrasound results, progesterone (Prog) concentrations and number of fawns born for individual animals in the BMP15 group. P = pregnant and NP = not pregnant; M = male and F = female.

BMP15	Year 1			Year 2			Year 3		
Doe	Ultrasound	Prog (ng/ml)	No. Fawns	Ultrasound	Prog (ng/ml)	No. Fawns	Ultrasound	Prog (ng/ml)	No. Fawns
1	P	5	2M	P	7.3	1M/3F	P	5.8	1M/2F
2	P	13	1M/1F	P	15.7	1M/2F	P	16.7	3M
3	P	8.9	1M/1F	P	6.6	3F	P	6	0
4	P	11	5M	P	10	2M/2F	P	5.6	3M/1F
5	P	6.7	1M/2F						
6	P	6.2	2M						
7	P	7.6	1M						
8	NP	5.7	–						
9	P	10.5	1M/1F						
10	P	8.2	0						

Table 2. Ultrasound results, progesterone (Prog) concentrations and number of fawns born for individual animals in the GDF9 group. P = pregnant and NP = not pregnant; M = male and F = female.

GDF9	Year 1			Year 2			Year 3		
Doe	Ultrasound	Prog (ng/ml)	No. Fawns	Ultrasound	Prog (ng/ml)	No. Fawns	Ultrasound	Prog (ng/ml)	No. Fawns
1	P	4.3	1M	P	5.8	1F	P	4.7	2M
2	P	4.4	2M	NP	0.4	–	NP	0.4	–
3	NP	0.2	–	NP	0.4	–	NP	0.1	–
4	P	9.2	2M/1F	NP	0.3	–	NP	0.1	–
5	P	10.7	2M	NP	0.9	–			
6	P	5.4	2M/1F						
7	P	6.4	0						
8	P	4.5	2M						
9	P	12.8	3M/2F						
10	P	9.45	3F						

immunization against BMP15 or GDF9 utilizing a 'weak' adjuvant caused an increase in ovulation rate, whereas immunization against either growth factor utilizing a 'strong' adjuvant caused a decrease in ovulation and inhibition of fertility (Juengel et al. 2004, McNatty et al. 2007).

Results from this study demonstrated that vaccination against GDF9 has potential to control fertility in deer. Further research will be required to determine the appropriate timing for administering the vaccine, improving the vaccine formulation and achieving the desired longevity of effect.

ACKNOWLEDGEMENTS

The authors thank Don Wagner and staff and students at the PSU Deer facility for care of animals used in the study and collection of data. The authors also thank Lloyd Moore (AgResearch, Wallaceville Animal Research Centre, Upper Hutt, NZ) and Sue Galloway (AgResearch, Invermay Research Centre, Mosgiel, NZ) for providing sequence data for BMP15 and GDF9 in the deer and assisting in the identification of peptide sequences for use in the vaccines.

The animal facility at the PSU Deer research Center is accredited by the Association for the Assessment of Accreditation of Laboratory Animal Care. This study was approved by the Institutional Animal Care and Use Committee of PSU.

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