

# Peptides in Veterinary Medicine

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Administration of synthetic peptides as therapeutics or diagnostics is well-established in human medicine. A few of them have also found use for comparable indications in veterinary medicine. Peptide-based drugs are especially indicated for treating animals used in food production, though regulation of fertility is their most important application. Only very small doses of these rather expensive, but highly active compounds are required. Peptides are metabolized more readily than many small molecules; this means that the risk of contamination of milk, eggs, or meat of the treated animals is greatly reduced due to the lack of unmetabolized pharmaceuticals and/or degradation products within their system.



Peptide drugs have also found use in the treatment of companion animals and riding horses, as their owners are increasingly willing to pay for expensive medication and therapies to prevent or cure health problems of their pets which can similarly suffer from most diseases of civilization that affect humans. Peptides are used in the diagnosis and management of diabetes and cancer which in recent years have become a growing problem with dogs and cats due to their increasing life expectancy in combination with obesity and lack of exercise. Additionally, vaccines based on synthetic peptides are becoming a viable alternative to vaccines obtained from viruses. Nevertheless, animal husbandry and reproduction management are currently the most important and best established areas of usage for peptide drugs in veterinary medicine. Synthetic peptide hormones and analogs are employed to stimulate and facilitate breeding as well as to prevent unwanted offspring.

*Bachem offers a choice of generic peptides for use as active ingredients in veterinary medicine, amongst them gonadorelin and a number of gonadorelin agonists and antagonists.*





## Gonadotropin-Releasing Hormone (GnRH, LHRH) and Analogs

Livestock as well as companion animals are treated with LHRH (GnRH, gonadorelin) and its agonists and antagonists to regulate fertility and reproduction as well as to treat disorders of the reproductive tract.

A number of LHRH analogs have been applied in the regulation of reproduction in animals (Alarelin, Azagly-Buserelin, Deslorelin, Fertirelin, Goserelin, Lecirelin, Leuprolide, Lutrelin, Nafarelin, Peforelin, Triptorelin)

Besides GnRH, deslorelin, leuprolide, and buserelin are probably the most often commonly prescribed synthetic peptides in veterinary medicine and livestock breeding. GnRH agonists are metabolized more slowly than the native hormone which allows for the use of much lower doses and thus reduces treatment costs. For example, the activity of buserelin was shown to be 100 to 200 times higher than the activity of GnRH, and fertirelin was 2.5 to 10 times more active than the natural peptide. In female dogs, a single dose of buserelin during heat will induce ovulation. In cows, a postinseminal application will assist the corpus luteum, and administration coinciding with insemination will delay ovulation, which alternatively can be induced by an injection of fertirelin 10 to 17 days postpartum. Repetitive injections of buserelin in mares will effect the onset of heat or ovulation, whereas in rabbits, a single injection of the GnRH analog induces ovulation and increases the conception rate. Buserelin and fertirelin have also been used in ewes. Overdosage of these highly active peptides can have a detrimental effect on ovarian response. Deslorelin is administered as an injection or implant to induce a reversible infertility in pets, especially in males. These implants can postpone the estrus of bitches for more than 2 years and have found use in the reproduction management of extensive cattle farming. Long-term release formulations of leuprolide have been shown to postpone puberty and reversibly suppressed reproductive function in male and female dogs for periods exceeding 1 year. Peforelin, which is used in the reproduction management of swine, is a naturally occurring peptide and corresponds to lamprey LHRH III. Peforelin is applied to induce the estrus of the sow after weaning. Depot formulations of triptorelin have been tested in heifers and gilts. GnRH agonists such as deslorelin and leuprolide are applied as implants for long-term reproduction management not only in domestic animals. Implants facilitate long-term administration and allow non-surgical sterilization of stray dogs and cats, population control in wildlife, and reproduction management in zoos. In dairy cattle and camelids, GnRH and GnRH analogs such as buserelin or fertirelin are indicated for the treatment of ovarian cysts. Deslorelin is commonly applied as an implant for treating hyperadrenocorticism in domestic ferrets. Gonadorelin can also be used as a diagnostic in disorders of the reproductive system, e.g. in dogs with hypogonadotropic hypogonadism to differentiate between pituitary and hypothalamic defects.

### Anti-GnRH Vaccines

As an additional tool for non-surgical spaying, anti-GnRH vaccines were recently developed. The C-terminally elongated peptide hormone is linked to a carrier protein, and the resulting conjugate is applied in combination with an adjuvant to achieve immunization against endogenous LHRH. A GnRH-keyhole limpet hemocyanin conjugate vaccine has been developed as a contraceptive to control wildlife. Effective after a single vaccination, it has been applied to bison, deer, feral pigs, and others. In male pigs, the anti-LHRH-vaccine (Improvac® (Pfizer)) is injected in two doses several weeks before slaughtering to control boar taint. Another anti-LHRH-vaccine has been registered for the treatment of benign prostatic hyperplasia in dogs.

### Ovsynch

The synchronization of ovulation in dairy cattle is the most important application of GnRH and analogs in animal husbandry. The Ovsynch protocol using gonadorelin for the controlled induction of ovulation was developed in 1995 by R. Pursley and N. Bello at Michigan State University. The program consists of an initial injection of GnRH followed 7 days later by an injection of prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ). It can be started at any stage of the estrous cycle. Two days following the  $PGF_{2\alpha}$  injection, cows receive a second GnRH injection followed by timed artificial insemination in the next 8 to 24 hours. The efficiency of the synchronization in lactating dairy cows can be further improved by pretreatment with an additional dose of  $PGF_{2\alpha}$  (8 days before starting Ovsynch) and GnRH (6 days before starting Ovsynch) ("G6G", Bello et al. 2006). Unfortunately, heifers respond only poorly to Ovsynch and artificial insemination, but suitable modifications of the protocol could be developed. Buserelin, fertirelin and other GnRH agonists have also been administered in synchronization schemes. For example, in the recently described Doublesynch scheme, lecirelin has been shown to be especially suitable for primiparous cows.

### GnRH Antagonists

Whereas the veterinarian can choose from a number of proven, highly active and efficient GnRH agonists, the situation on the antagonist side is not yet satisfactory. Third-generation antagonists such as acyline, antarelix, antide (itirelix), and cetorelix have been used in studies with domestic animals, e.g. to prevent ovulation during proestrus or terminate pregnancy.

## Oxytocin and Carbetocin

Oxytocin and its more stable synthetic carba analog carbetocin are administered to induce normal labor and facilitate parturition in livestock (cows, ewes, sows, goats) as well as in pets. In captive birds such as budgerigars or cockatiels, oxytocin and its avian analog vasotocin help induce oviposition in case of egg binding. Both oxytocin, which is produced in the hypothalamus, and synthetic carbetocin act on the smooth musculature of the female reproductive system. Oxytocin and carbetocin induce strong contractions of the estrogen-stimulated uterus, though distinctly prolonged and more frequent contractions will result during administration of the synthetic analog. Simultaneously, these hormones stimulate the lactating glands to produce milk and deblock the teats for ejection. Oxytocin and carbetocin are also applied in cases of agalactia or reduced milk flow after birth. In cows, carbetocin is administered to prevent a retarded involution of the uterus or in case of abnormal milk ejection. A single injection of carbetocin in dysgalactic sows induces milk flow post partum. Oxytocin is preferred over carbetocin due to its shorter half-life in cases where drug overdosage may cause problems. In large animals, oxytocin is administered to stimulate the expulsion of placenta after parturition in cases of prolonged retention. Oxytocin is also applied therapeutically; in mares, it is indicated for the treatment of endometritis following insemination, whereas in cows and swine it is used to treat mastitis.

## Vasopressin and Desmopressin

Vasopressin (Antidiuretic Hormone, ADH), a peptide hormone secreted by the hypothalamus, acts on the renal tubulus cells. Most mammals produce (Arg<sup>8</sup>)-Vasopressin (Arg-Vasopressin, AVP), whereas the Lys<sup>8</sup>-analog is secreted in pigs. The porcine analog shows only half of the antidiuretic activity of AVP. In higher dosage, vasopressin additionally induces vasoconstriction and stimulates the production of factor VIII and von Willebrand factor. Vasopressin is administered intravenously or subcutaneously as a diagnostic to differentiate between renal and central diabetes insipidus in dogs, cats, horses, and cattle. AVP can also be used as a therapeutic in the treatment of the central form of the disease. However, due to its short half-life, its stable synthetic analog desmopressin (DDAVP) is the preferred medication in the treatment of dogs and cats suffering from polyurea caused by diabetes insipidus centralis. Compared to vasopressin, desmopressin shows an improved antidiuretic and a reduced vasoconstrictive activity, it more effectively stimulates factor VIII and von Willebrand factor production. DDAVP is often applied conjunctivally or nasally in dogs and cats, and has even been administered orally in humans. It is applied intravenously or subcutaneously in dogs for treating moderate cases of von Willebrand's disease before surgery to avoid hemorrhagia. In animals with von Willebrand's disease, desmopressin transiently elevates von Willebrand's factor and shortens bleeding time. Therefore, it is very useful in dogs with von Willebrand's disease by permitting surgical procedures or controlling capillary bleeding. The hemostatic effect in cats has not yet been evaluated. Vasopressin is gaining attention in veterinary emergency medicine as a cardiovascular resuscitation drug in small animal patients and newborn foals.



## Glucagon, Peptides in Pancreatic Disorders

### Glucagon

Glucagon, as its antagonist insulin, is secreted by the pancreas. The peptide hormone is used as emergency medication in cases of severe hypoglycemia, as it upregulates plasma glucose. Management of bovine fatty liver disease (FLD, Steatosis hepatis) is an important application of this hormone in veterinary medicine. FLD is an accumulation of fat (especially triglycerides) in the liver occurring in cows after calving. The disease can be treated efficiently by long-term intravenous infusion of glucagon. Besides stimulating glycogenolysis, gluconeogenesis, and insulin production, the peptide hormone reduces liver triglycerides. Glucagon is also used as a diagnostic in veterinary medicine, especially in dogs (Glucagon stimulation test). Intravenously injected glucagon causes a short rise in the plasma concentration of insulin in healthy dogs. This will not happen when administering glucagon to diabetic canines due to their inability to produce the hormone. But instead of measuring the secreted insulin, determination of the amount of the concomitantly formed C-peptide is preferred, as it is not affected by insulin treatment. The measurement is performed 10 minutes after the glucagon injection.

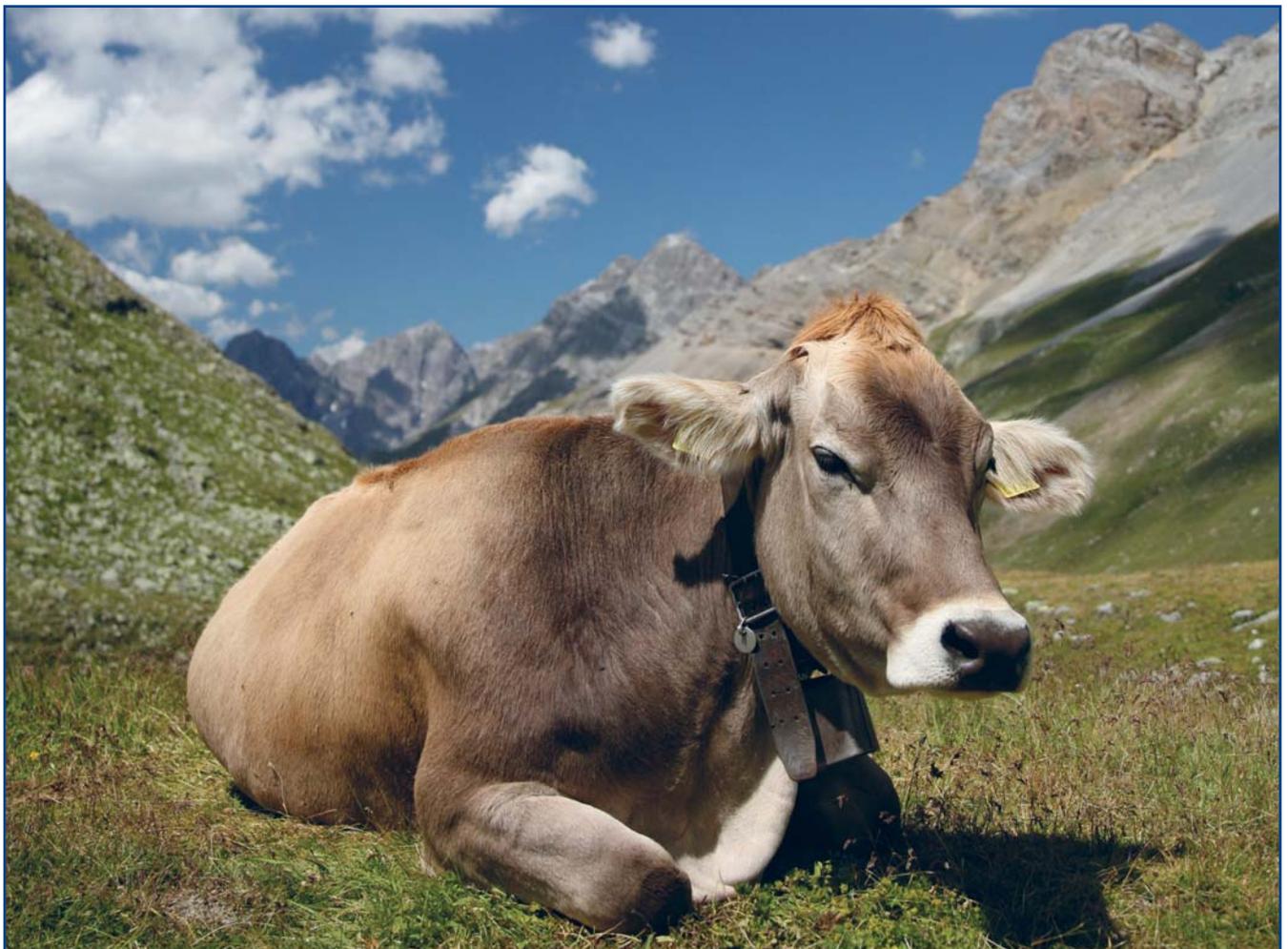
### C-Peptide

Canine C-peptide is applied as a diagnostic tool for monitoring diabetes in dogs. This peptide is released together with insulin from a precursor peptide, so its plasma concentration indicates how much insulin is being produced by the pancreas. The extent of beta-cell loss can be deduced from the result. Determining the amount of C-peptide in diabetic animals allows for therapeutic monitoring as one can differentiate between endogenous (produced by the body) and exogenous (injected into the body) insulin. Inappropriate dosage of insulin in dogs with low blood sugar levels results in a low C-peptide level. Type 1 diabetes is quite common in dogs whereas type 2 diabetes has not been observed yet.

In case of insulin resistance, the increased concentration of C-peptide results from a high activity level of the pancreas beta-cells. Abnormally high amounts of C-peptide can indicate the formation of an insulinoma which secretes insulin.

### Octreotide and Analogs

Octreotide is a synthetic somatostatin analog showing higher activity and increased half-life. The peptide was evaluated in the management of insulinomas in dogs. Octreotide is a long-acting inhibitor of pancreatic secretion, which helps in preventing complications after pancreatic surgery. Single photon emission computed tomography (SPECT) applying  $^{111}\text{In}$ -Pentetreotide allows the detection and localization of canine insulinomas.



## Diagnostics

### TRH

As in humans, protirelin (TRH) is injected or infused in pet dogs for diagnosing thyroid disorders as hypothyroidism.

TRH acts on the anterior pituitary gland. In the case of a healthy pituitary, application of the hormone stimulates the secretion of TSH, which promotes the secretion of triiodothyronine (T3) and thyroxin (T4) from the thyroid gland. T3 and T4 can be measured in plasma later on. In horses, a TRH test alone or in combination with the dexamethasone suppression test (DST) is performed to diagnose pituitary gland hyperplasia (pituitary pars intermedia dysfunction (PPID), or equine Cushing's disease (ECD)).

Bachem also offers GMP-grade pergolide mesylate which has been considered the most efficient drug in the management of ECD.

### ACTH and Cosyntropin (Tetracosactide)

Corticotropin (ACTH) and cosyntropin (tetracosactide) are used diagnostically to detect adrenal disorders, especially in dogs, cats, and horses. Both peptides stimulate the adrenal cortex (zona fasciculata) and induce the production of glucocorticoids. The use of cosyntropin is indicated in cases of allergic reactions to the natural hormone. In dogs, blood samples have to be taken preceding the intravenous or intramuscular injection of the peptide in order to obtain the basal cortisol value, and again one hour after its administration. Canine Cushing's disease (hyperadrenocorticism, rather common in dogs but occurs rarely in cats) and Addison's disease (hypoadrenocorticism, in cats and dogs) can both be diagnosed. In case of spontaneous hyperadrenocorticism, the ACTH test is also performed to monitor the effects of medication. In neonatal foals, cosyntropin stimulation is a reliable test for dysfunctions of the hypothalamic-pituitary-adrenal axis.

### Ceruletide

Ceruletide (caerulein), a cholecystokinin (CCK) analog, has been proposed as a diagnostic agent for detecting hepatic dysfunctions in dogs. Ceruletide, a more efficient secretagogue than CCK, stimulates postprandial serum bile acid. As hepatopathy can occur in dogs suffering from severe respiratory diseases, administration of ceruletide as a liver function test should be performed routinely with such patients.

## Prospective Peptide Drugs

### Peptide Drugs

Peptide drugs such as exenatide or teriparatide, which have been successfully established in human medicine, can likely also be applied to treat similar medical conditions of companion animals and horses. In the management of cancer and development of new (peptide) therapeutics, human patients can profit from the experience gained with afflicted pet dogs and vice versa, as naturally occurring cancers in these species share many features including biological behaviors and responses to conventional therapies.

### Peptide Vaccines

Peptide-based vaccines could be a safer alternative to immunizations which use the inactivated viruses. Peptide vaccines present a number of advantages as they have become easily available (also on large scale), relatively cheaper, shelf-stable, chemically well-defined compounds. A number of studies evaluating synthetic peptides (immunogenic sequences from viral proteins, used in combination with a suitable adjuvant for inoculation) as vaccines against viral diseases affecting livestock such as foot-and-mouth disease or swine fever have been published.

### Antimicrobial Peptides

An alternative to the antibiotics used in animals involved in food production may come from antimicrobial peptides, which have been described in many organisms. These peptides have a wide spectrum of action. They can kill gram negative and gram positive bacteria, enveloped viruses, yeasts, and moulds. Several peptides were recently discovered in shrimps and oysters, where they are essential elements of innate defense, in the absence of acquired immunity. These molecules could advantageously replace antibiotics, since they are less susceptible to cause resistance in the target microorganisms, due to their direct action on membranes, and to their fast degradability, which avoid the accumulation of residues. Application of antibacterial peptides is an attractive option for intensive animal husbandry such as poultry farming and, especially, aquaculture.

## Generic APIs for Veterinary Medicine offered by Bachem

### Peptidic Generic APIs

#### LHRH (GnRH) and Analogs

Buserelin Ph. Eur. DMF

Deslorelin High Acetate DMF

Fertirelin Acetate

Gonadorelin Acetate Ph. Eur.  
CEP, DMF

Gonadorelin Hydrochloride DMF

Goserelin Acetate Ph. Eur. CEP, DMF

Leuprolide Acetate Ph. Eur. CEP, DMF

Nafarelin Acetate

Triptorelin Acetate DMF

Triptorelin Pamoate DMF

#### Others

Desmopressin Acetate Ph. Eur.  
CEP, DMF

Glucagon DMF

Octreotide Acetate DMF

Protirelin Ph. Eur.  
(TRH)

pTH (1-34) (human) Acetate  
DMF

Tetracosactide DMF  
(Cosyntropin)

### Non-Peptide Generic APIs

Acetylcysteine Ph. Eur. CEP

L-Cysteine Hydrochloride  
Monohydrate Ph. Eur.

Econazole Sulfosalicylate

Etomidate Ph. Eur. CEP, DMF

Pergolide Mesylate Ph. Eur.  
CEP, DMF

Propofol Ph. Eur. CEP, DMF

Xylazine Hydrochloride Ph. Eur.  
DMF

Zonisamide DMF