

## **Allegato A Breve descrizione del Progetto formativo**

### **Title of the project**

The influence of sex hormones in dog neurological diseases.

### **Description of the scientific and training objective**

The three main classes of sex hormones are oestrogens, progesterone and androgens and perform modulation activities on neuronal excitability. Progesterone has neuro-inhibitory action while the effects of oestrogens appear to be more complex. The oestrogens action's has long been considered pro-convulsive but now clinical and experimental data show that these hormones may not have this function or even perform anticonvulsant action, related to various factors: duration of treatment and dosage (in the case of iatrogenic administration), hormonal status (whole or spayed animals), type of oestrogen, brain region involved, etc. The pro-convulsive action of oestrogens does not seem to occur at basal concentrations, while it could occur following sudden and significant increases in their concentration, as occurs during ovulation. Furthermore, oestrogens would play an important neuroprotective role on the hippocampal damage induced by the state of epileptic disease.

The role of androgens is also controversial: while their pro-convulsive action has been demonstrated in some experimental models, more recent studies suggest their protective role on the development of seizures. Testosterone is transformed into two classes of neuro-steroids, which have opposite effects on neuronal excitability: oestrogen and reduced 5- $\alpha$  androgens, structurally and functionally similar to allopregnanolone, a metabolite of progesterone which is responsible for his neuro-inhibitor action.

In some studies, in humans and dogs medicine, it is suggest a role of sex hormones in the onset of seizures, with a higher prevalence in males and in some phases of the menstrual / oestrus cycle, while in others researches it is not demonstrated.

The first reports of urinary incontinence in neutered bitches is dated half a century ago and an increase probably as a result of the increasing spread of neutering to control reproduction in dogs. Incontinence after castration occurs more frequently in female dogs and there are relationships between the pathology and some factors, such as the breed, the age at the time of castration, the weight and the type of surgery undergone (oophorectomy or ovariohysterectomy). The mechanism of the development of urinary incontinence following castration remains unknown and it is not yet fully understood how sterilization increases the risk of developing urethral sphincter incompetence. The incompetence of the urethral sphincter in the female does not seem to be attributable to a single cause but to a set of factors such as

hypoestrogenism, the increase in gonadotropins, the position of the bladder neck, the urethral conformation and its length, which represent the result of metabolic and morphological changes induced by castration. After sterilization, in fact, the animal has a predisposition to deposit more perivisceral adipose tissue and to modify the body structure: the displacement of the bladder neck and the consequent variation in length of the urethra is favored, thus altering the activity of the urethral sphincter. The lack of oestrogens seems to play a role in the incontinence of spayed bitches, as occurs in post-menopausal women, however it is not sufficient to explain all the pathophysiological aspects of the condition. In the bitch, the cyclic stimulation operated by oestrogens during the reproductive cycle keeps the urethral sphincter healthy, thanks also to the sensitization action operated by oestrogen on the alpha-agonist receptors of the urethra and bladder neck, with trophic action on the muscle striate fibers and on the urethral epithelium. In addition to lacking the cyclic hormonal stimulation typical of the estrous cycle, in castrated bitches, lower oestrogen levels are found than in bitches in anaestrus: muscle hypotrophy and of the urethral epithelium are consequences, which reduce the urethral closing pressure and the effect of "seal" given by the epithelium.

The concentration of gonadotropins seems to have a direct or indirect relationship with urinary incontinence due to sterilization. The gonadotropins, in fact, together with oestrogens, can be involved in the regulation of bladder tone, in the maintenance of the thickness of the urethral wall and in the reflex of urination. Gonadectomy alters hormonal homeostasis, with a reduction in sex steroids and an increase in LH and FSH in both males and females. Receptors for LH and FSH are present in the urinary bladder and urethra of healthy male and female dogs. Differences between whole and sterilized dogs in the expression of receptors for LH and FSH and in the proportion of collagen and muscle fibers, as well as in the composition of glycosaminoglycans in the bladder and urethra seem to confirm the role of gonadotropins in the development of the disease. In fact, in spayed bitches, a reduction in receptors for gonadotropins was found, which would be responsible for a reduction in bladder tone and a negative influence on the urination reflex. The increase in collagen fibers and the reduction in glycosaminoglycans observed in the lower urinary tract of sterilized subjects can compromise the structural and functional integrity of the lower urinary tract and appear to be involved in sterilization incontinence.

The incompetence of the urethral sphincter after castration in males is less frequent than in females. While a normal-sized prostate exerts traction on the urethra, keeping the bladder neck more cranial than the pubic margin, after castration, the reduction in volume of the prostate causes a caudal displacement of the bladder neck, which will

assume an intrapelvic position. Prostatic hypertrophy also induces less compression on the prostatic urethra, thus reducing urethral resistance to urine flow.

Urinary incontinence involves a series of problems that involve both the health of the animal, in terms of predisposition to urinary and skin infections, and considerable inconvenience for the owner, so much so that it can become a stressful situation.

The scientific and training objective of the project is to provide a contribution to the knowledge of these aspects, through the evaluation of the influence of sex and the different phases of the oestrus cycle on dog epilepsy, in order to further clarify the role that sex hormones play in epileptogenesis and any benefits that could derive from castration. In fact, it still remains to be clarified whether the practice of castration can always represent a benefit for the animal, since in females the proconvulsant action of oestrogen would be eliminated but also the protective action of progesterone and in males the action of testosterone would be less. Whose role is controversial, being transformed into both pro and anti-convulsant neuro-steroids.

The aim of the project is also to explore the causes and risk factors of the association between urinary incontinence and sterilization in order to obtain knowledge to be used in the decision process whether or not to sterilize an animal. Another objective of the project is to use the dog as a model for pathologies also found in the human species, within the concept of "One Health". To this end, the project aims to use the databases of the clinics belonging to the "Vet Partners Group", responding to the "open science" approach, an approach to the scientific process based on cooperation and on new ways of disseminating knowledge, improving the accessibility and reusability of research results through the use of digital technologies and new collaboration tools and respecting the principles of the "FAIR Data Principles" guidelines and best practices to ensure that research data is Findable, Accessible, Interoperable and Re-usable, in compliance with ethical constraints, commercial and confidentiality and the principle "as open as possible and closed only when necessary".

The project is in line with the research lines of the PNRR: Mission 4, Component 2 "From research to business", topic 12 (Neuroscience and neuropharmacology).

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### **Methods of carrying out the training and research activities**

The training activity will begin with a bibliographic study on the topics covered by the research, completed by the attendance of seminars or conferences. The research activity will take place in Italy at the Veterinary Clinic "Camagna" and abroad

Universitat Autònoma de Barcelona and will use the databases of the structures and their networks in order to obtain a large amount of data useful for achieving the objectives research itself. The research results will be the subject of scientific publications and will be completed by the preparation of the thesis.

**Effects and expected results with particular emphasis on promoting economic development and the production system**

Pet medicine is an important sector in the National economy. The Veterinary Clinic “Camagna” (Vet Partners Group) belongs to a large network of national and international veterinary clinics at the forefront of the hospital activity for pets. Being a network, it makes it possible to use a large amount of clinical data, which can be used to improve performance in the field of pet medicine.

The Veterinary Clinic “Camagna” (Vet Partners Group) will host the PhD student beneficiary of the scholarship funded on the resources of Ministerial Decree 352/2022 for 18 months during the course of the PhD program.

The period abroad, lasting 6 months, will be carried out at the following institution  
Universitat Autònoma de Barcelona