Non-surgical sterilisation *iSpay*

Will pet-spaying operations soon be a thing of the past?

Dr CheMyong Jay Ko is the founder of Epivara and a professor in the College of Veterinary Medicine at the University of Illinois Urbana-Champaign, USA, working on reproductive endocrinology. His team has recently achieved a breakthrough in the search for a non-surgical method of permanently sterilising female companion animals such as dogs and cats. Here, we present their proof-of-concept study showing that a single dose of estrogen delivered at the prepubertal stage of life via a slow-release formulation prevents the development of reproductive organs. This novel technology, named iSpay, opens up a possibility for a practical alternative to many millions of spaying operations currently used to prevent unwanted pet pregnancies.

The life of a well-cared-for pet is idyllic: a warm home, food served up daily, and perhaps a cosy lap to sit on. In stark contrast, dogs and cats that can't find a nurturing home will often end up leading a painful and tough life. Domesticated animals that are left to fend for themselves on the streets are vulnerable to abuse, starvation, disease, and early death. Some animals do get rescued by charities that run animal shelters and rehousing schemes, but their resources are limited and not all stories have a happy ending.

Many people love and enjoy their pets but don't necessarily want to share their home with a constant stream of extra kittens or puppies. To avoid unwanted litters, many pet owners and animal charities decide to have female animals spayed.

WHAT IS SPAYING?

Spaying is an operation where both the ovaries and the uterus are commonly removed. Such surgeries are mostly successful; as well as eliminating unwanted sexual behaviour and pregnancies, they can lead to a reduction in the risk of some cancers. Unfortunately, they also have downsides. Health risks to the spayed animal include obesity, urinary incontinence, and unwanted behavioural changes.

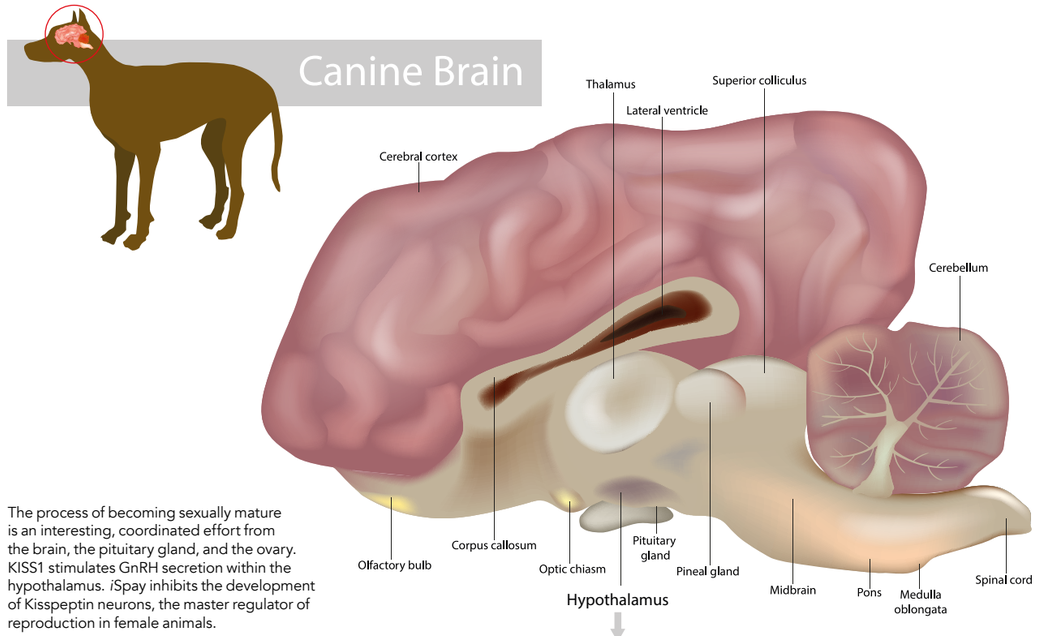
Furthermore, surgical procedures need highly skilled vets, anaesthetics, and properly equipped operating theatres and recovery spaces. There are plenty of countries that do not have many of these facilities. Some countries may have the resources but have made the cultural decision that performing surgery on pets for the purpose of birth control is unnecessarily invasive. Many Scandinavian countries rely on very strict anti-roaming laws to prevent unwanted pregnancies instead of spaying.

A simpler, non-surgical method of sterilising pets could greatly reduce the population of unwanted animals, eliminate the dangers of surgery, and save a lot of time and money. The Alliance for Contraception in Cats and Dogs (ACC&D) is an organisation that has joined forces with animal advocates and researchers to foster the efforts of developing easier and less invasive ways to control feline and canine reproduction than surgery.

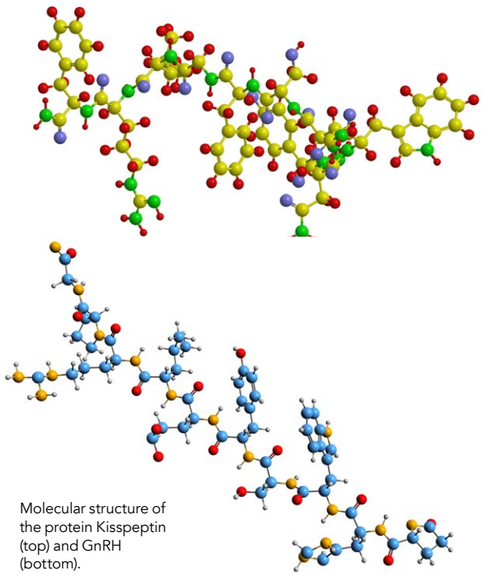
HOW DO MAMMALS BECOME SEXUALLY MATURE?

Mammals are not able to have babies immediately after birth. The process of becoming sexually mature is an interesting, coordinated effort from the brain, the pituitary gland, and the gonads. We are just beginning to understand how exactly this intriguing and complicated system works, and it seems all three of these areas of the body are needed for success.

The brain's role happens in the hypothalamus, an area known to control the maturation and function of reproductive organs. When allowed to develop normally, specialised nerve cells in this area called Kisspeptin neurons produce a hormone called KISS1, which then locks onto a second type of neurons called GnRH (gonadotropin releasing hormone) neurons. GnRH neurons are then triggered to start rhythmically producing GnRH. As the name suggests, this hormone then goes on to tell the pituitary to make and release gonadotropins – namely luteinising hormone (LH) and follicle stimulating hormone (FSH) – which in turn stimulate the ovary to synthesise sex steroid hormones and release eggs. If the Kisspeptin neurons never develop in the first place, then the cascade of neuronal and pituitary hormone releases can't be activated, and the animal therefore can't release eggs and become pregnant.



The process of becoming sexually mature is an interesting, coordinated effort from the brain, the pituitary gland, and the ovary. KISS1 stimulates GnRH secretion within the hypothalamus. *i*Spay inhibits the development of Kisspeptin neurons, the master regulator of reproduction in female animals.



Molecular structure of the protein Kisspeptin (top) and GnRH (bottom).

A simpler, non-surgical method of sterilising pets could greatly reduce the population of unwanted animals.

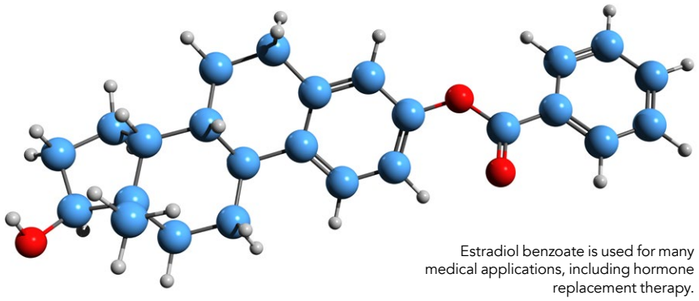
THE SEARCH FOR AN 'OFF SWITCH'

Dr CheMyong Jay Ko is a professor in the College of Veterinary Medicine at the University of Illinois at Urbana-Champaign, USA working on reproductive endocrinology. In 2016, he founded Epivara Inc. to develop a practical non-surgical sterilant.

We might predict there to be many potential molecules or compounds that could disrupt this complicated chain reaction. Indeed, some are well-known and used as contraceptives both in humans and occasionally for pets. Contraceptives are useful in situations where the aim is only to temporarily prevent pregnancy. For permanent sterilisation, they are relatively impractical since they require repeated dosing, which is time-consuming and prone to mistakes.

In the search for new medications, existing drugs are often found to have previously unknown properties. Estradiol benzoate (EB) is such a drug. EB was already a well-known form of the reproductive hormone estrogen. It's used for many medical applications, including hormone replacement therapy to reduce menopausal symptoms, as part of treatment against prostate cancer, and as a growth stimulant in the cattle industry.

Previous studies had found that repeated daily dosing of newborn female rats with EB for 11 days disrupted the development of their reproductive organs by targeting Kisspeptin neurons



Estradiol benzoate is used for many medical applications, including hormone replacement therapy.

(Minabe et al, 2017), so Ko's team (Drs Chanjin Park, Rex Hess, Po-Ching Lin, Sherry Zhou, Sandra Soto, Lindsey Reinacher, and Katie Chai) decided to take these findings further and develop a practical method to create a sterilant.

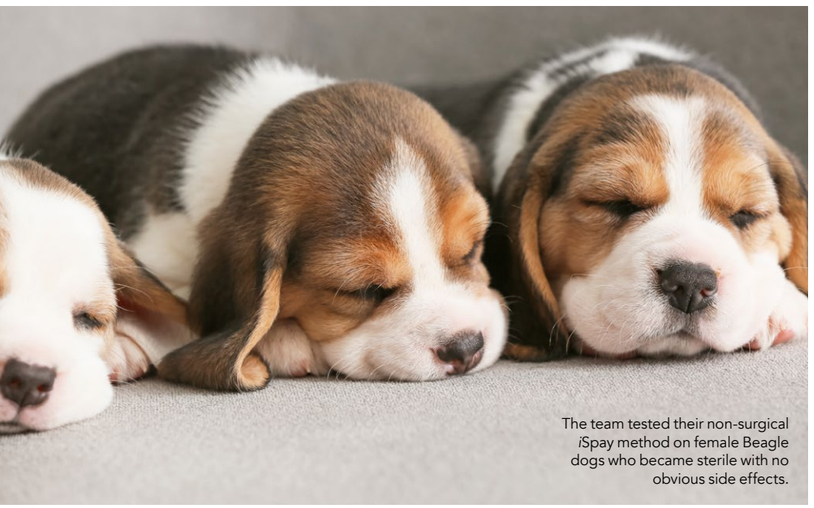
GETTING THE RIGHT METHOD OF DOSING

The team first tested if a silicone implant that released a significantly smaller amount of EB over 2-3 weeks than the total amount of EB injected daily for the 11 days in the previous study would sterilise the female rats. However, they found that making EB-containing silicone implants and inserting them into small animals was inconvenient. Instead, it would be preferable to deliver the drug using an injection method.

Ko and his collaborators developed a new method to deliver the EB using biodegradable microspheres. The microspheres used are less than 10μm in diameter and contain many tiny droplets of EB. The spheres are way too small for the human eye to see individually and appear as a powder that is then diluted before being injected with a routinely used syringe and needle. The researchers hoped that the extra time needed to break down the microspheres in this new formulation would deliver the EB droplets over an extended time, therefore smoothing out the EB dose enough for it to be equivalent to the 11 daily doses or the silicone implants.

The team found that a single dose of EB in a microsphere formulation was indeed enough to limit the development of both ovaries and the womb and permanently sterilise young female rats. Encouragingly, no significant side effects have been detected for the animal's overall health and other hormone functioning. Recently, they also developed a pellet form of EB formulation which showed the same effectiveness and safety in a trial as the microsphere form.

This non-surgical method of sterilisation for companion animals could replace hundreds of thousands of spaying operations each year.



The team tested their non-surgical *i*Spay method on female Beagle dogs who became sterile with no obvious side effects.

THE FUTURE POSSIBILITIES

Ko hopes this new technique of single injection using a biodegradable formulation of EB will now provide an affordable, effective, practical, and humane method to prevent unwanted pregnancy in domestic pets. The team tested this non-surgical method – called *i*Spay – on female Beagle dogs and found that they also became sterile with no obvious side effects. The dogs that participated in the experiment are currently known to be healthy and over 3 years old. It is also likely that a sustained dose of EB will sterilise young cats and other mammals that are currently spayed by surgery.

This non-surgical method of sterilisation for companion animals could replace hundreds of thousands of spaying operations each year and greatly decrease the suffering and damage caused by unwanted free-roaming dogs and cats.

Unlike previous methods of reproductive control, *i*Spay will prevent the development of the reproductive system before it fully matures. Therefore, it will lead to permanent infertility, a feat that has not been accomplished before.

As we gain a better understanding of the interesting biology of reproduction, it is likely that more and more options for birth control will be discovered. Perhaps the greater challenge will then be how to navigate the ethics of these new powers. With this in mind, ACC&D has brought together experts in this field to focus on improving ethical decision-making tools for researchers working in this area.

Behind the Research

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Research Objectives

Dr CheMyong Jay Ko and his team developed *i*Spay, a non-surgical method for the permanent sterilisation of female mammals.

Detail

Bio

Dr CheMyong Jay Ko is a professor in the College of Veterinary Medicine at the University of Illinois Urbana-Champaign. His primary research focuses on reproductive endocrinology. He received a PhD degree from Seoul National University and postdoctoral training from the University of Kentucky. Before moving to Illinois in 2011, he worked as a professor in the Medical Center of the University of Kentucky for 10 years. In 2016, Dr Ko founded Epivara at the University of Illinois' Research Park.

Funding

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Collaborators

The researchers are grateful to Dr Ozawa (Nippon Medical School, Tokyo, Japan) for providing the anti-kisspeptin polyclonal antibody (RRID: AB_2910199). They also thank Dr C L Wright, Dr C J Fields, Dr J Drnevich, and Dr M Tseng for their assistance in performing single-cell RNA sequencing and analysing data.

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'*i*Spayed' adult Beagles remain infertile at the age of 3 years and are very playful.

References

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Personal Response

What inspired you to conduct this study?

"My work at the university includes not only teaching veterinary students but also counselling them on their vision and challenges. I have found that many students dream of becoming veterinarians to help and treat sick or suffering animals, but in reality, many veterinarians spend their careers performing spay and neuter surgeries. My vision primarily focuses on enabling the students I teach to lead more meaningful lives as veterinarians, and I take great joy and a sense of purpose in the belief that such work can also make people and animals happier."

Do you think there may be applications for this new technique in nature conservation, perhaps in the control of invasive mammals?

"The current developed technology does not work in animals beyond achieving sexual maturity. Therefore, there are many limitations to its use in controlling the population of wild animals. However, this technology can be used for animal species where easy and efficient access to newly born individuals is possible."

When will your first product become available on the market?

"Our *i*Spay technology is protected by patents in the USA and EU countries. The team is seeking FDA approval of their first product, which may take approximately 3–5 years. Immediately after receiving the FDA approval, the product will become available on the market."