A full-page photograph of a jaguar in its natural habitat. The jaguar is captured in mid-leap, emerging from a dense green bush. Its body is arched, and its tail is extended upwards and backwards. The jaguar's fur is a mix of orange, black, and white, with a prominent spotted pattern. The background is filled with lush green foliage, and the ground in the foreground is a light-colored, sandy or dusty path.

Derek Andrew Rosenfield

**Wildlife population control
comprehensive and critical literature review**

**on contraceptive methods in wildlife -
mammals**

**São Paulo
2016**

DEREK ANDREW ROSENFELD

Wildlife population control
comprehensive and critical literature review
on contraceptive methods in wildlife - mammals

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Pós-Graduação em Reprodução Animal da
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Reprodução Animal

Área de concentração:

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Orientador:

Prof. Dra. Cristiane Schilbach Pizzutto

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A Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, no cumprimento das suas atribuições, **ANALISOU** e **APROVOU** a Alteração do cadastro (versão de 25/fevereiro/2016) do protocolo de estudo acima referenciado.

Resumo apresentado pelo pesquisador: "Esta revisão sistemática, consolida e discute todas as vantagens e desvantagens de cada método contraceptivo, organizada por espécies de mamíferos, com ênfase em imun contracepção reversível, obtidos em literatura científica internacional. O objetivo é aprofundar os conhecimentos e elucidar soluções adequadas para o grande problema mundial do controle das populações de animais silvestres. Além disto, pode servir como pré-projeto para a próxima fase de desenvolvimento de um método contraceptivo economicamente viável, com melhores atributos, alta eficácia da ação, técnicas de aplicação melhores e mais seguras, e mais importante, garantir saúde geral e genética das populações. Finalizando, esta revisão oferecer de forma breve e concisa, uma atualização sobre o conhecimento de métodos contraceptivos reversíveis, organizada por métodos, táxon, fármacos, e riscos associados."

Comentários da CEUA: "".

Profa. Dra. Denise Tabacchi Fantoni
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade
de São Paulo

Roseli da Costa Gomes
Secretaria Executiva da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade
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Prof. Dr. _____

Instituição: _____ Julgamento: _____

Prof. Dr. _____

Instituição: _____ Julgamento: _____

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*With profound gratitude, I dedicate this work to the memory
of my friend and mentor*

Prof. Dr. Marcelo Alcindo de Barros Vaz Guimarães

&

To the wellbeing and survival of all of God's creatures!

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*"When I hear of the destruction of a species,
I feel just as if all the works of some great writer have perished."*

– U.S. President Theodore Roosevelt

RESUMO

ROSENFELD, D. A. **Controle das populações de animais silvestres revisão bibliográfica compreensiva e crítica sobre os métodos de contracepção em animais silvestres - mamíferos.** [Wildlife population control comprehensive and critical literature review on contraceptive methods in wildlife - mammals]. 2016. 219 f. Dissertação (Mestrado em Ciências) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2016.

Esta revisão sistemática, consolida e discute todas as vantagens e desvantagens de cada método contraceptivo, organizada por espécies de mamíferos, com ênfase em imun contracepção reversível, obtidos em literatura científica internacional. O objetivo é aprofundar os conhecimentos e elucidar soluções adequadas para o grande problema mundial do controle das populações de animais silvestres. Além disso, pode servir como pré-projeto para a próxima fase de desenvolvimento de um método contraceptivo economicamente viável, com melhores atributos, alta eficácia da ação, técnicas de aplicação melhores e mais seguras, e mais importante, garantir saúde geral e genética das populações. Finalizando, esta revisão oferecer de forma breve e concisa, uma atualização sobre o conhecimento de métodos contraceptivos reversíveis, organizada por métodos, táxon, fármacos, e riscos associados.

Palavras-chave: Animais Silvestres. Mamíferos. Contraceptivos reversíveis.

Imun contracepção. Antifertilidade.

ABSTRACT

ROSENFELD, D. A. **Wildlife population control comprehensive and critical literature review on contraceptive methods in wildlife - mammals.** [Controle das populações de animais silvestres - revisão bibliográfica abrangente e crítica sobre os métodos de contracepção em animais silvestres - mamíferos]. 2016. 219 f. Dissertação (Mestrado em Ciências) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2016.

This systematic review consolidates and discusses all the advantages and disadvantages of each contraceptive method, organized by mammalian species, with emphasis on reversible immune-contraception, obtained from the international scientific literature. The objective is to deepen the knowledge and elucidate adequate solutions to a serious global problem of wildlife population control. Furthermore, serving as pre-project to the next stage of development of a contraceptive method, economically viable, with better attributes, high effectiveness of the action, better and safer techniques of application, and more importantly, ensure overall health and population genetics. Finalizing this review by offering in a brief and concise manner, an updated understanding of reversible contraceptive methods, organized by methods, taxon, drugs, and associated risks.

Keywords: Wildlife. Mammals. Reversible contraceptives. Immunocontraceptives. Antifertility

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LISTA DE ABREVIATURAS

LIST OF ABBREVIATIONS

APHIS	Animal and Plant Health Inspection Service
AZA	Association of Zoos and Aquariums
CBEE	Centro Brasileiro De Estudos Em Ecologia De Estradas
CRAS	Centro De Recuperação De Animais Silvestres
DNA	Deoxyribonucleic acid
E ₂	Estrogen
EAZA	European Zoos and Aquariums
EC	Emergency contraception
EE	Ethinyl estradiol
ELISA	Enzyme-linked immunosorbent assay
ER	Estrogen receptor
FSH	Follicular stimulating hormone
GnRH	Gonadotropin release hormone
GnRH Vaccine	Gonadotropin Release Hormone - Vaccine
HDL	High density lipoprotein cholesterol
HPG	Hypothalamic-pituitary-gonadal axis
Ig	Immunoglobulin
LDL	Low density lipoprotein cholesterol
LH	Luteinizing hormone
MGA	Melengestrol acetate
MI	Myocardial infarction
NWRC	National Wildlife Research Center
OC	Oral contraceptives
P ₄	Progesterone
PAMPs	Pathogen-associated molecular patterns, or PAMPs, are molecules associated with groups of pathogens, that are recognized by cells of the innate immune system.
PCR	Polymerase chain reaction
PGF _{2α}	Prostaglandin
pZP	Porcine Zona Pellucida
pZP	Porcine Zona Pellucida
RNA	Ribonucleic acid
T	Testosterone
USDA	United States Department of Agriculture
VTE	Venous thrombo-embolism

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1 INTRODUCTION

It is the year 2015, and instead of having achieved major environmental improvements, including better survival chances for wildlife species worldwide, things are becoming drastically worse. Thinking about the efforts put forth, in researching, analyzing and discovering better ways of wildlife protection, trying to improve their well-being, while attempting to manage their population. It reminds a little of the Cervantes Saavedra's story of Don Quixote de la Mancha, the ingenious Gentleman, fighting to undo wrongs and bring justice to the world by attacking windmills, which he perceives as ferocious giants in his imaginary world. However, in the real world fights to protect and save wildlife species from the brink of extinction, these ferocious giants are humans. With human populations reaching larger numbers ever, crossing the 7 billion mark (WORLDOMETERS, 2016), it appears as if we are fighting unsuccessfully, perhaps, even senseless battles, just like Don Quixote.

The rate of today's wildlife species extinction might only be matched by past catastrophic events, leading to mass extinction, such as the one at the end of the Cretaceous Period, believed to be responsible for the death of almost all vertebrate animals at this time (COWEN, 2000). The culprit for these events was either an asteroid or meteor and/or some combination of volcanic activities. Nevertheless, nature was to blame, or with other words, these events could not have been avoided. Very different from today's *raison d'être* of extinction, where with the right education, culture, attitude, and better choices, we could avoid destruction of wildlife habitat and species, in fact, even evade our very own self-destruction. However, with our the massive appetite for more and more agriculture soils and living space, uncontrolled "*raubbau*" of the world's resources, along with its linked consequences of forest destruction, air, land and water pollutions, we are continuing with ever increasing velocities. Killing animals in the thousands as they choke on plastics are contaminated with heavy metals, agro-toxins, and hormones that find their way into ground waters provoking mutations and cancer. Last but not least, the direct human behavior towards wildlife, like "sport" hunting, exotic animal trafficking, or killings for food, out of fear or ignorance, does the rest, in driving these species into extinction.

Moreover, yet, with all its complexity of challenges that humankind and its progress must conquer, giving up is not an option, so we move forward with (semi)

blind optimism, believing in the cause, and that one day, it will all change for the better. At least, I do.

Human-wildlife conflict (DISTEFANO, 2005), a term used to describe situations where wildlife interferes with any human interest, yet these are provoked by the dynamics of human impacts, such as the initial invasion of humans into wildlife space. Resulting in a rapidly shrinking wildlife habitat, and creating fragmented habitats, and in consequence, the re-invasion of wild animals into agricultural and urban areas. Driven by the need to search for food and water, or just plain curiosity, looking for new means of shelter. Moreover, some are even able to go through an adaptive metropolitan evolution, becoming syntopic, in once human-only habitation.

Now, this new living together comes, more often than not, with consequences, such as damage to crops (FERRAZ et al., 2003) and traffic accidents, among others (DISTEFANO, 2005). Which, by the way, in Brazil alone, 15 wild animals are killed every second by vehicles., leaving Brazil 475,000,000 animals poorer each year (Source: Statistics of the Brazilian Center for Road Ecology Studies, and *Sistema Urubu, Brasil*, 2015). Some high-proliferative species, their robust nature to adverse conditions, and the vanishing of their natural predators thrive well in their newly found habitats within the human colonies, so much so, that they turn into wildlife "pests." Depending on the region, they are South American (neotropical) primates species, such as common marmosets, or wild pigs, capybaras, and bats. In North American Mustangs, armadillos, snakes, beavers, raccoons, skunks, bears, coyotes, and deer. In Europe foxes, or squirrels, rats, mice, and pigeons (CBEE, 2015).

Above all, one serious concern, very high on any Government's priority list, is the war on disease spreading species. Many vertebrates are considered potential hosts for zoonotic infections, such as Malaria, Rocky Mountain Spotted Fever, Leptospirosis, in Brazil, namely possums, capybara, feral pigs, rats, as well as some avian species (GRECA; LANGONI; SOUZA, 2008).

That leads to the utmost importance of population control in wildlife species, in situ or captivity, representing an ever-growing challenge for wildlife management around the globe, especially when trying to employ practices of animal well-fare and overall health, physiological as well as psychological, of the individual and the social integrity of the group.

In the past culling (hunting), and poisoning was the answer to controlling wildlife populations around the world. Moreover, even still today, for many local

Governments, culling seems the panic-like response to an acute problem (RANSOM et al., 2014; MONTY, 2015; SKINNER, 2015). However, because of growing public awareness and the dedicated fight of wildlife enthusiasts, over the last four decades, a clear movement away from lethal towards non-lethal population management methods could be observed, moving toward the development of contraceptive, or antifertility methods. Although a step in the right direction, these first and second generations of population control methods and anti-fertility agents were still far from perfect, provoking unwanted side-effects (DELIGDISCH, 2000; BHATTI et al., 2007; CURTIS et al., 2007; MCALOOSE; MUNSON; NAYDAN, 2007). However, thanks to the continuous quest for product improvement by countless scientists, antifertility agents, and species depending administration strategies, contraceptive methods are becoming more and more safe in their application. Nevertheless, research and development for better methods are a continuous responsibility, contributing to wildlife conservation and animal well-being.

Historically, contraceptive attempts in animals date back hundreds of years, like the anecdotal report of Bedouin's contraceptive practices in Camels, by placing heated stones into the female camel's uterus to prevent pregnancy during long marches on desert caravans. Medically speaking, this might not even be so unrealistic, as provoked irritation of the uterus lining might impede the attachment of a fertilized egg cell. However, as mentioned at the museum for Contraception and Abortion (MUVS) WEB-site; it is somewhat unlikely that such contraception ever had been employed with camels. When Arab gynecologists hear this story at European conferences, they normally respond with a short question: "Have you ever tried to put a stone in a camel's uterus?".

Also, still, as of 2015, no anti-fertility method can be considered safe, especially when considering their application in wildlife species. Although, science has come a long way and many products available are indeed more efficient, with longer-lasting contraceptive effects, reversible and much safer as their predecessors (ASA; PORTON, 2005; ASA; BOUTELLE; BAUMAN, 2012).

Needless to say, markets for anti-fertility drugs for wildlife on a commercial scale is small, and associated costs for drug research, approval and launch are astronomical, making procurement of suitable products an added challenge. And, inadvertently, leads to chose the "next best thing", which are contraceptive drugs

developed for human use, potentially, bringing with them all the undesirable effects (ASA; PORTON, 2005).

It is the purpose of this extensive review to summarize all employed wildlife population control methods, and their infertility or contraceptive agents used. Starting in the year 2000 until 2015, investigating their principles of action and physiological mechanism, potential adverse effects, if any while evaluating which methods are mostly employed, in which species, and why. Highlighting if these methods are being used under field conditions or in a captivity setting. Furthermore, providing an overview of the trends and future developments, emphasizing on methods that are currently considered the most reliable, reversible, safe and economically feasible, in the application in wildlife *in situ*.

A point of discussion that, repeatedly throughout this work, will be brought to attention, is to call for the "truly best" population control method.

Let's be candid for a moment, the "best" population control is the one where humans are not involved and only nature is in control, meaning predators taking care of prey. But for this to occur, nature has to be in equilibrium, a state that is becoming increasingly a rare reality. Leading from human caused to human controlled. And for this to be applied, the question is, what would be the best method

1.1 CHAPTER PRESENTATION

This dissertation is organized into 8 chapters, are in the format of scientific articles for further publication in International Scientific Journals.

Chapter 1 consists of a general introduction, mentioning the importance of population control, some facets on Human-Wildlife Conflicts, while at the same time, trying to encourage to maintain a critical thought on ethics and morals involved in this ecological challenge.

Chapter 2 goes back in times, visiting the history of contraception.

Chapter 3 provides a brief overview of the anatomical, physiological, and endocrinological aspects of mammalian reproduction, serving as a foundation that allows for a better interpretation of subsequent chapters.

Chapter 4 features the principal research focus, providing a comprehensive insight and compilation on all contraceptive methods currently employed in wildlife population control, their known pro & contra, with quick view figures.

Chapter 5 due to the overwhelming results of the review analysis, immunocontraception was identified as the leading research focus during the last decade. Therefore, the author believed to add this chapter, with an in-depth view on immunocontraception, with aspects of the mechanisms and dynamics involved.

Chapter 6 offers an opinion piece on the issues of Human-wildlife conflicts, pest species and Wildlife population control in Brazil.

Chapter 7 provides information on material and methods employed, and

Chapter 8 concludes this literature review by presenting the results of the data extraction, organized by numbers and types of studies, year published, authors, contraceptive method, species, genders, separated in studies conducted in free-range wildlife or in captivity, offering statistics on efficiencies, reversibility, side effects, and behavioral impacts, and interpretation, and discussion.

In the **appendix**, Presentation of the Data base (Excel) used data extraction;

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2 A BRIEF HISTORY OF CONTRACEPTION IN ANIMALS

Abstract

Wildlife "population control" did exist in ancient times as well, not so much for noble ecological reasons, other than for efforts to minimize a constant threat of animal attacks on settlements, livestock, or crops, or for the simple pleasure of killing. Today's wildlife population challenges have similar reasons but different causes, its inevitability is 100% due to the explosion of the human population, and as a consequence, rapidly shrinking habitats, provoking a ubiquitous imbalance that leads to situations known as human-wildlife conflicts. Methods of animal population control have come a long way, reflecting on killing or castration only to reversible immunocontraceptives of the 21 century. With the invention of the microscope, in the 1700's, a new momentum in science occurred, that brought an end to some of the ill doctrines from the past. Compared to the last 3000 years, the next 300 would bring major progress to the study of biology and medicine. With the creation of professional conduct in the veterinary field, a remarkable knowledge gain on reproductive anatomy, physiology, the creation of endocrinology as a new discipline developed. With the discovery of sex hormones, the understanding of their role in reproduction, the first concepts for contraception methods by using gland tissues, believed to contain these sex hormones, and finally the development of synthetic hormones, all lay the foundation and made way for future means of population control, human and animal alike, as well as becoming the stepping stone for, what we know today as biotechnology.

Keywords: Animal population control. Antifertility. Contraception. Immunocontraception.

2.1 INTRODUCTION

One might be curious to find out what reasons for controlling animal population during ancient times could have been, and the answers provided by historical data might have surprised, realizing that the reasons back then weren't that much different than the ones today.

For instance, when taking out the most obvious reason, which is space limitation, one, somewhat unexpected motive, considering the level of knowledge of reproductive physiology at that time, was the attempt to avoid inbreeding. A clear indication that already then a certain genetic comprehension existed, recognizing that consanguinity was a threat to desired animal attributes, but without an understanding of the mechanisms involved.

Another reason to maintain population under control was based on the observation, that when many animals were crowded into small spaces, aggression would increase, animals could get injured, and crowd control was more difficult (ROBERTSON, 1921).

Wildlife "population control" did exist in ancient times as well, not so much for noble ecological reasons, other than for efforts to minimize a constant threat of animal attacks on settlements, livestock, or crops, besides for the simple pleasure of killing. Which essentially translates into the attempts to eradicate predators, done so in the cruelest ways possible. There are numerous examples of such practices depicted in the book "Endangered Species Handbook", written by Greta Nilson (2005), that gives insight on species culled (hunting/killing). Depending on the continent, entailed wolves, foxes, bears, and bobcats, wild dogs, as well as many other, non-predator mammals.

Today's wildlife population challenge (and I use the term "challenge" instead of "problem" as deliberately as possible, as the problem lies within humankind and not with wildlife) is unique to modern times. They predominantly become a problem due to the explosion of the human population, with the consequence of rapid shrinking habitats for most species around the globe. This ubiquitous imbalance has created situations that are known as human-wildlife conflicts, referring to consequences of the interaction between wild animals and people and the resultant negative impact on people or their resources, or wild animals or their habitat (WWF, 2005).

One of these major conflicts is the fear of wildlife "invading" human populated areas, giving rise to epidemiological concern, destruction of crops, and a threat to domestic livestock provoking traffic accidents, and even the direct risk of death for humans (DISTEFANO, 2005).

By looking back at history, how animal populations were managed, how knowledge and understanding of reproductive physiology evolved, and the quest for contraceptive methods developed over time, driven by the experience of practical application, will help to improve future methods on animal population control, including Wildlife and feral animals. But it must be done in a responsible manner, where animal health and well-being is the driving force while continuing to evolve the ethical and moral "School of Thoughts", with the absolute conscience that wildlife survival means human survival.

It is the intent of this chapter to provide a brief historical introduction to animal population control, and the development of contraceptive methods over time, with the emphasis on wildlife. Understanding from where we were coming and to where we are going allows for an interpretation of trends and future concepts of contraceptive methods. However, this review does not claim to be complete on all the events and methods that might have occurred, but a sincere attempt to highlight historical key moments on this subject.

2.2 THE BEGINNINGS OF ANIMALS POPULATION CONTROL

2.2.1 Ancient Times (Classic Antiquity, anything BC - 7th Century AD) From Physical Separation, Castration, and the first Contraceptives

Historical evidence of animal population control can be found scattered throughout all human civilization epochs. These records are available on cave walls, canvas paintings, or written on papyrus and books.

The necessity to control animal population, specifically wildlife and feral populations are numerous, from limited space availability to crowd control, protection of genetic information, minimizing the spread of zoonotic diseases, avoid damage to

crops and livestock, and the protection of human lives, by preventing traffic accidents and direct attacks, are all too obvious.

The most effective form of population control, practiced since the first efforts of animal domestication, is by physical separation. But for many reasons, this practice was not always possible, or feasible, and the only other 100% effective form of avoiding pregnancy in animals was by castration, documented throughout different ancient cultures, and as early as 4000 - 6000 years BC, without much mentioning on technical specifics (PURSEWELL, 2010).

Although, there is one anecdote, about Bedouins in North Africa, said to have placed small pebbles into the reproductive tract of female camels, for the purpose of preventing pregnancies during their Sarah journeys, perhaps analog to a modern time inter-uterine device. Not that this tale is based on any evidence of historical value, besides, one would wonder how this invention came about, but it serves well as a somewhat amusing story on ancient contraceptive methods.

Not much evidence of animal population control, from the historical timeline between the Ancient History (Bronze Age and Early Iron Age) the Classical Antiquity and the Middle Ages, is available. Very different when dealing with human contraceptive methods, which mentions countless treatments for women. Things that today we would judge as obscure, bizarre, and plain ridicules. Techniques from holding the breath right after coitus and jumping up and down seven times, to applying a paste of crocodile dung, and other herbal extracts, to be used as a pessary, other past-like substances included honey, salt and gum-like extracts that would be applied intra-vaginal (ROBERTSON, 1921).

Material that indeed had some spermicidal properties, either by being moderate to strongly acidic or alkaline, besides the physical barrier aspect that hindered sperm-motility. Sponges soaked in lemon juice and then pushed into the vaginal cavity was one such acidic measure. Mercury, back then, was believed to have medicinal properties and in ancient China, women might have used it to prevent pregnancy. Considering it's toxic properties in provoking multiple organ failures, it most likely did offer some contraceptive capabilities. Human male contraception was rather straight forward and less creative, castration, or animal parts used as condoms (ROBERTSON, 1921).

2.2.2 Early Modern Times (16th - 19th Century) Microscope and Reproductive Anatomy and Scientific Revolution

In Europe, throughout the 1600 and 1700s, separation, castration, and culling remained the principal methods of animal population control.

During the later part of the 1800s, however, things were about to change. Castration procedures in animals started to become organized and controlled, such as regulations, requesting to be licensed, in order to perform procedures like castrations, and even fees for such rendered service would need to be approved by authorities. Furthermore, the first professional developments in veterinary services started to take shape, when in 1792, the first veterinary school opened its doors in Lyon, France. Nevertheless, back then, animal welfare, other than disease control, wasn't much of a big concern, and when it comes to castration, procedures remained still rather rudimentary, mainly regarding pain,- and infection control. Practices that continued well into the later part of the 19th century, when new thoughts on animal well-being transpired and those begun to manifest into professional conduct. For instance, the first written declaration in 1975, by the British Small Animal Veterinary Society, stating that: "anyone older than 18 years of age was legally entitled to perform castration of a cat or a dog at any time until it is 6 months of age, provided that adequate anesthetic was administered."

Nonetheless, it wasn't until 2007, when in the UK law finally declared that only veterinarians could provide the service of castration of dogs and cats (PURSEWELL, 2010).

And from then on, the dark ages of population control were left behind, and things continued to become better, particularly, with the formation of special laws for the protection of domestic animals and wildlife. Of course, intensity and variations in their interpretation, control, and execution are as plentiful as the cultures that created them, including rules, regulation, and conduct of this developing veterinary profession. Parallel to the legislative improvements, enhancements on more adequate and humane means of animal population control were being studied and developed.

The biggest change, of course, was when the dogmatic belief in castration, separation, and/or culling, as the only true means of population control, turned its

attention to alternatives, not just to prevent reproduction, but to do so with reversibility in mind.

Strangely enough, it is evident that some basic notion of reproductive physiology and fertility existed for millenniums, thus, the association that by removing testis would result in infertile males. Yet, clusters of the scientific community, during modern ages (starting in the 1600s), were still in doubt about what organs were responsible for the formation of life, even preferred to believe in "spontaneous life" as a valid explanation. A moment where "believing" in something, instead of evidence-based conclusions (facts) seemed so "unscientific."

With the invention of the microscope, in the 1700's, with great controversies, throughout the literature, on who deserves the credit as the inventor, naming de Vinci, Janssen, Galileo, Hook, Amici, Malpighi, Swammerdam, and Newton. However, the one making the biggest impact at this time must be attributed to Antoni van Leeuwenhoek, when things became, literally, more clear and detailed, due to his improvements he made. Permitting him the identification of the red blood cells, and contributing to another very important observation, done by Hamm (Leeuwenhoek's student), who identified for the first time human spermatozoa (ROBERTSON, 1921).

2.2.3 The loss of the " Theory of Spontaneous Generation" Doctrine & Fecundation

Lazzaro Spallanzani, a priest and scientist, made it his mission in 1768, to disprove the theory of spontaneous generation. Doing so by using flasks that were sealed tight by flaming, proving that there could not be any microorganism growth afterward. Another, and, even more, important contribution by Spallanzani was the observation that there is no fecundation without the presence of semen. In his experiment, he placed a piece of linen over a male frog's genital, confirming that no conception took place after copulation. Noteworthy, Spallanzani is also being credited as the father of in-vitro fertilization and artificial insemination in dogs, even though, it appears that Arabs were practicing artificial insemination in horses since the 1300s, (CAPANNA, 1999).

Nevertheless, at this time, the human egg was not yet identified. Only the existence of an ovary's follicle had been confirmed by de Graaf, hence the "Graafian follicles." It was only many years later, when Carl Ernest von Baer, in 1827, discovered that the mammalian follicle contained the ovum, complementing the bigger picture of the reproductive process, and Edgar Allen discovered the human ovum in 1928 by microscopic examination of the ovarian follicle contents of a dog.

Robertson (1921, p.57), in his book 'An Illustrated History of Contraception' also wrote about the first observation of the fusion of sperm and ovum, made by Martin Barry in 1843, and Oscar Hertwig, in 1875, had observed a sperm actually entering the ovum. The conclusion was then drawn, that the resulting organism must contain the genetic properties of both parents.

In his experiment, Hertwig used sea-urchin eggs, which were considerably more transparent than those of the frog (CAPANNA, 1999).

Before any manipulation of fertility could even be thought of as possible, scientists came along way, from once believing in spontaneous life, to the concept of sperm and egg fertilization. Understanding reproductive physiology today seems obvious and logic to the modern mind, but just a few hundred years ago, it was everything but. Of course, with the understanding the anatomy, and to some degree the process of fertilization, and the all important invention of the microscope, the pathways were cleared for further studies on reproduction with great discoveries to follow.

The next rational question that needed to be answered might have been; what was the stimulus that puts the whole reproductive physiology into motion, and what controls these events?

2.2.4 Late Modern Times (20th Century) Endocrinology - A New Discipline & When Science brought Contraceptives to the Market

2.2.4.1 Endocrinology

During the late 19th century, experimental studies conducted by Berthold, Germany, and Bernard, from France, led to the hypothesis that there must be a chemical communication between different organs. Which was later answered in human medicine, when some diseases were treated with extracts from animal endocrine glands, like the pancreas, thyroid, or adrenal gland, resulting in positive responses, postulating that these disorders were due to hormonal deficiencies. The discovery of substances that act as chemical messengers was now called "hormones." A term defined by Professor Starling in 1905 from the Greek word (*impetus*), meaning "to arouse", or "to excite." Starling referred to its function as: "...the chemical messengers which speeding from cell to cell along the blood stream, may coordinate the activities and growth of different parts of the body". A few years later, the study of hormones and its functions became part of a new discipline, "Endocrinology", from the Greek words *endon*, "within"; *krino*, "to separate"; and *logia*, "study of", coined by the Italian physician Nicole Pende, in 1909. This discipline became the foundation for all future advances in the development of contraceptive methods (TATA, 2005).

2.2.4.2 The father of hormonal contraception (sterilization)

Dr. Ludwig Haberlandt in 1919 hypothesized that by hormonal manipulation could potentially provoke temporary infertility in an animal. Keyword: "Temporary". In his writings, Haberlandt explained his idea analog to parking a car in an occupied space: "If you don't want to let a vehicle park in a space, then block this place, pretending it is occupied." Similarly, this should work physiologically in the body of a woman, where the contraceptive mimics a pregnant state. Hormones will block the

way to the uterus so egg and sperm cannot meet, thus, the way to the uterus is blocked. In this way, contraception is a pretended pregnancy by hormones”.

By 1921, together with his colleagues, Haberlandt proved his hypothesis of temporary contraception in rabbit and guinea pig females, by transplantation of ovaries of pregnant, into fertile females, resulting in suppression of ovarian activity.

During the following 10 years of his research, Haberlandt recognized that within the *Corpus Luteum* must be the responsible factor, which, in 1932, he related in his book; "The Hormonal Sterilization of the Female Organism." He also described results of experiments of oral administration done on mice, would be the method of choice, further explaining that periodic withdrawals from the oral contraceptive are necessary to allow menses to occur. Later, it was understood that the responsible hormone was progesterone (HABERLANDT, 2009; DJERASSI, 2011; MUVS, 2016).

2.2.4.3 The "Marker Degradation" and The Decade of Sex Hormones

One cannot talk about oral hormone contraceptives without mentioning the first developments of synthetically produced sex hormones. The 1930s, also known by steroid chemists as "The Decade of the Sex Hormones," because for the first time, the molecular structures of the androgen hormone testosterone, as well as the female steroid sex hormones estrone, estradiol, and the pregnancy hormone progesterone could be determined and were incorporated in medical drugs. In 1938, a chemistry professor at then Penn State College, could isolate a plant steroid, called sarsapogenin, from sarsaparilla, and discovered that, because two oxygen atoms that were bound to the same carbon atom of the molecule's side chain, the molecules proved to be chemically reactive. Based on this reactivity, Marker invented a chemical reaction sequence, which would remove most of the side chain atoms (called the degradation process), leaving a duplicated side chain of progesterone, hence, the "Marker Degradation." However, to create pure progesterone, some more modification to the initially produced progesterone-ring were necessary, which started to be produced and sold by Syntex, S.A., a Mexican-based company, co-founded by Marker, in 1944, but the association was short-lived, and Marker left. Although Marker was no longer part of the Company, in 1945, under the guidance of

Rosenkranz, Marker's replacement, the company went on to extend the production to other synthetic steroid hormones (GORTLER, 1999).

2.2.4.4 Contraceptives for Domestic Animals

During the 1950s, when the first concepts of hormone-based contraceptive methods were developed, the target market and end-user have mainly been directed towards humans. Nevertheless, most tests, before human trials were conducted at first on laboratory animals, such as rats, rodents, rabbits, cats, dogs, and primates (Pursewell, 2010).

Unsurprisingly, these study results on animals would sooner or later serve as a basis for contraceptive methods in the veterinary sector. When in the 1960s the first products finally became available for the human market, they soon started the era of studies of application in animals, not just for population control of domestic or feral animals, but also for wild animals. Moreover, the livestock science was about to make a leap in breeding efficiency, based on these inventions.

2.2.4.5 The Intrauterine Device (IUD)

1909, was the first introduction of the IUD concept, by Dr. Richard Richter, however, never reach further development, and application as a birth control.

In the 1920s, the German gynecologist Ernst Gräfenberg thought of as the forerunner for the development of the modern time intrauterine device. He fabricated a ring made of silk and wrapped with silver wire, and once placed into the *Cavum uteri*, showed promising results as a contraceptive method. The silver wire available in the 20s had a high content of copper, which some 40 years later, was recognized for the spermicidal efficiency. Not very relevant for animal population control, perhaps just curiosity, Ernst Gräfenberg, is also credited with the discovery of the G-spot in human females, ergo, the "G-Spott", an abbreviation for "*Gräfenberg Zone*", (MACHO, 2014).

IUDs, continued to be developed, with different shapes, different metals, and in 1976, the third generation, and first IUD treated with progesterone. 1996 was the year of the fourth generation IUD, in fact, of its special design, now called an Intrauterine Contraceptive Implant (IUCI), sold under the brand name GyneFix®. As the latest product for contraception, it also offers the potential for additional applications, such as the delivery of other pharmacological agents into the uterine cavity (THIERY, 1997).

2.2.4.6 The "Pill", first oral contraceptive

With the gained understanding that progesterone inhibits ovulation, and now having synthetic progesterone limitless available, all research focused on the development of an oral contraceptive, as it would be the perfect anti-baby method, comparing to injection, or the cumbersome application of an IUD.

Carl Djerassi, a Vienna-born, American Immigrant, who earned his Ph.D. from the University of Wisconsin. His thesis was on the transformation of testosterone to estradiol, a sex hormone produced by the ovaries. Djerassi was responsible for the research efforts at Syntex, Mexico, and in 1951, he and his team filed the first patent the production of norethindrone, (19-nor-17a-ethynyltestosterone), being much more potent than the natural progesterone. The pre-fix "nore" indicates the absence of a methyl group, allowing the compound to enter the bloodstream unaltered, different to progesterone, which would be broken down in the stomach. Norethindrone became the first active ingredient for the production of oral contraceptives. However, the first release of the famous contraceptive "Pill", was done by the Searle Company in 1959 (POTTS; CAMPBELL, 2009; ZARE, 2015).

2.2.4.7 Hormone Employment in Animal Breeding Programs

The only available products for reversible contraceptive came from the human market, and eventually found their way to the veterinary sector, starting out in animal science for breeding programs of livestock. As with everything in economics and the free markets, it is all about maximizing profits and to stay competitive. Once the concept of being able to control estrus cycle in livestock by employing hormonal agents, allowing, therefore, to synchronize females for more efficient insemination procedures, lots of research efforts were put into this science to fine-tune breeding programs, and successful their results drove the meat and dairy industries to new heights.

In 1948, Cristian and Casida were the first to administer daily injections of progesterone in cattle, proving that estrous cycle could be synchronized. These hormone based agents became a very successful and viable tool in reproductive biotechnology for breeding programs, p. 159 (BENNETT; VALLANCE; VICKERY, 1968)

To enter the historical realm of breeding programs in livestock would go far beyond this chapter, but it is important to recognize that "material & methods, based on thousands of fields studies of hormonal biotechnologies, lend itself as a starting point for wildlife population control programs, and might continue to serve as a reference.

2.2.5 Wildlife Population Control - from the 20th to 21st Century

For thousands of years, the only practiced method of wildlife population control was by hunting and killing indiscriminately. Not so much to control population, but mainly to protect livestock, crops, and human lives, in addition to hunting as a "sport".

2.2.5.1 Menagerie

In ancient times, the first zoos were referred to as a "Menagerie", recorded to have existed in Egypt approximately 3500 BC (ROSE, 2010). However, managing wildlife population in zoos, for the purpose of genetic preservation, and considering animal well-being as a top priority, began only in the 20th century, mainly owing it to the change of moral and ethical attitudes and the newly gained consciousness for ecological issues. It was around that time when contraceptive alternatives first were employed in zoos for population control.

For many, Dr. Ulysses S. Seal, a psychologist and biochemist by trade is seen as the father of contraception in captive wildlife. In 1976, he was the first to conduct studies on captive wildlife with hormone based contraceptive agents. Initial tests included large feline species, with the objective to compare two steroid hormones progestins, medroxyprogesterone acetate (MPA), and melengestrol acetate (MGA), in their effectiveness (ASA, 1993).

2.2.5.2 Improvement of Hormonal Contraceptives and Alternatives

Throughout the later part of the 20th-century studies started to investigate pathological impacts provoked by modern contraceptives. The number of wildlife species tested increased, and first long-term impacts of hormonal contraception treatments could be gathered. Unfortunately, there were many serious side effects to be discovered, in humans and animals alike. The severity of adverse effects reported depended largely on the species involved (ASA; PORTON, 2005; GRAY; CAMERON, 2010).

Over the next decades, enormous research efforts were undertaken to develop safer, more efficient, and of course, less expensive alternatives. Hormone-based contraceptives were improved, principally in regards to the concentration of the active ingredient, therefore, safety for the animal's health. Other alternative product concepts included the combination of different sex hormones, believed to

offer certain advantages of synergic actions, tests with androgen hormones, non-steroid (peptide) hormones, and their synthetic analogs.

Another such alternative that gained large interest were the actions of GnRH agonists, discovered in 1971. GnRH agonists are unique in their actions, leading to the exciting application in many medical specialties, including reproduction. GnRH's acts in a pulsatile manner on the hypothalamic-pituitary-gonadal axis and does so through distinct mechanisms. On one hand, it is responsible for the stimulation of synthesis and secretion of gonadotrophin hormones (LH and FSH), which in turn, controls gametogenesis and steroid hormone synthesis, driving all physiological reproductive events. On the other hand, when pituitary GnRH receptors are under constant influence of long-acting GnRH agonists implant, a paradoxical desensitization occurs, also called down-regulation, inciting a suppression of all subsequent events of the hypothalamic-pituitary-gonadal axis, with the effect of a biochemical castration, except fully reversible (YEN, 1975; P. MICHAEL CONN; WILLIAM F. CROWLEY, 1994).

As of 2015, GnRH agonists are routinely used, and continuously being studied in many wildlife species (ASA; PORTON, 2005; COHN; F. KIRKPATRICK, 2015).

2.2.6 The New Millennium's Contraceptives, "Immunocontraception", the next best thing to the perfect method?

Because of the bad rap of undesirable characteristics resulting from the treatments with hormonal contraceptives, serious efforts were undertaken to search for better alternatives, and eventually to move away from hormone preparations, not just because of potential side effects, but also to offer better, more adequate options for Wildlife field application.

A new concept that generated quite some interest was called "Immunocontraception", and as the name already implies, involves the animals own immune system.

Contrary to most research done on contraception, which focuses almost exclusively on female fertility control, earliest immunocontraception studies entailed the male reproductive organs, when first results on infertility effects by use of

antigens, were published in the 1950s. In fact, the first discovery of antigenicity of spermatozoa, using immunization into a foreign species, happened as early as 1899 by Karl Landsteiner, an Austrian/American immunologist and pathologist (SHULMAN, 1971). Not related to contraception, but noteworthy, Landsteiner received the Nobel Prize in 1930 for his discovery of different major blood groups in different individuals.

In 1908, doctors Emil Savini and Tereza Savini-Castano, conducted their initial studies on fertility control by immunization, done on female mice, rabbits, and guinea pigs, and first published their paper in 1911. But their work was about to be heavily criticized and found not to be in agreement with some other authors, based on work done, some 30 years later, p 183 (BELL, 1969).

It took until the late sixties, for the concept of fertility control by immunization to be a proven and functional contraceptive, and with intensified research, in the early 1970s, immunocontraception got close for human application (JOSHI, 1973). In fact, this period is considered by some as the cornerstone for the change in research interest from hormonal contraceptives to Immunocontraceptives (COHN; F. KIRKPATRICK, 2015).

Studies conducted in the late 1970s (LINCOLN; FRASER, 1979) with GnRH vaccine in ram, showed similar effects to a GnRH analogue implant, by inhibiting the synthesis and secretion of a Gonadotrophin Releasing Hormone (GnRH), subsequently slowing down the activities of the reproductive endocrinological cascade, resulting in the inhibition of Gametogenesis (BALET et al., 2014). Naturally, this vaccine would be effective in both sexes.

Another possible immunocontraceptive action is by inhibition of sperm-binding to the ovum's zona pellucida receptors, hindering the diffusion of any sperm into the ovum, therefore, avoiding the formation of a zygote (DUNBAR; SHIVERS, 1976; TYNDALE BISCOE, 1991).

Late 1980 and throughout the 1990s, Dr. Jay Kirkpatrick, director of the nonprofit science and conservation center at Zoo Montana in Billings, conducted successfully extensive long-term research with pZP vaccines (porcine zona pellucida), with an emphasis on Wild Horses, and North American deer. Kirkpatrick, in his 1996 article, confirmed that further research was performed on 74 species of captive zoo animals, with documented success in 27 species, including members of the orders Perissodactyla (Equidae), Artiodactyla (Cervidae, Capridae, Giraffidae, Bovidae), and Carnivora (Ursidae, Mustelidae, Felidae), (KIRKPATRICK et al., 1996).

2.3 CONCLUSION

As we can see, the need to control animal population is as old as the first rudimentary husbandries, and since then, animal/wildlife population control and contraceptive methods have come a long way. Many very important discoveries and inventions have been made, which without, we would not have moved beyond castration technique as the only method.

This review identified a clear trend in contraceptive studies for wildlife species, from hormonal contraceptive methods to immunocontraceptive methods, during the last three decades, as well as indication for a movement from predominantly female contraceptives to fertility control to males.

Now, in the 21st century, the quest to develop the best contraceptive method, one that would offer all the benefits of the wish-list, research for alternative concepts continues with great efforts, and many advances have been made. An expansion of choices of contraceptive products, for greater variety of species and a wider spectrum of application needs, as well as a more satisfactory means when it comes to being more species-specific, an applicable for both genders, and as always, the most important factor, a lesser degree of potential side effects, all wish-features combine, are becoming a reality.

Nevertheless, specialized research in wildlife, in reproductive anatomy, physiology and endocrinology has most certainly become an important scientific player in its own right. With great progress and important discovery, for example, in the field of non-invasive hormone analysis, and the development of very effective, safe, reversible and long-acting contraceptive methods, and will continue to do so during the technological advances of the 21st century.

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3 REPRODUCTIVE PHYSIOLOGY AND ENDOCRINOLOGY FOR CONTRACEPTIVE APPLICATION IN WILDLIFE

Abstract

Endocrinology studies hormones, their secreting organs, tissues, down to the cellular level, location of synthesis, how they are traveling throughout the body, how do they find and bind to their target cells. What biological action hormones initiate, as well as pathological aspect, like what happens when there is a hormone imbalance. Reproductive hormones, follow a very precise hierarchy, or pathway, and depending on their starting point, for example: the Hypothalamus, than the pituitary gland, and from there to the gonadal (hypothalamic-pituitary-gonadal axis, or HPG-Axis), function like a cascade, which does not imply that it is a one-way street only. Hormones do travel also in opposite direction, referred to as a positive or negative feedback loop, responsible for controlling hormone synthesis and secretion. This review focuses on the understanding of the dynamics and interactions of mammalian reproductive physiology, and endocrinology, as it applies to contraceptive methods. Also, their desired and adverse impacts, with emphasis on actions of endogenous reproductive hormones, as they represent the mechanism and dynamics of contraceptive agents, either by provoking inhibition or mimicking intrinsic hormonal effects.

3.1 INTRODUCTION

The biggest challenge in wildlife animal reproductive physiology and endocrinology comprehension is the vast variety of wildlife species. Just in the mammalian class alone, there are more than 5500 known species (IUCN 2016), every single one with its anatomic and physiologic particularities. Any campaign to manipulate a particular species population, demands and deserves a dedicated understanding of its biology to warrant the overall safety, health and well-being, of the individual animal as well as of its community, besides, to assuring successful treatment and effective population management. Let's keep in mind, that most contraceptive products were developed and optimized for the human use, and almost exclusively, for women. Although approved by regulatory authorities, and tested for safety and efficiency, there is still a large number of reported adverse effects in women, let alone, the even higher potential for side effects in little to never tested animal species.

The understanding of the exact mechanism of contraceptive agents on biological processes is therefore extremely critical, allowing to make an appropriate choice of an adequate method of fertility control. While considering the species, the individual's gender, age, physiology, the environmental situation, are the animals free living, or in captivity, if so, single, paired, or in a group, and if a female, her parity history (nullipara, primipara, or multipara).

As most contraceptive agents are designed for female fertility control, it is also crucial to understand the species-specific estrus cycle. Exactly in which cyclic phase the female is, and consequently, which reproductive hormone is prevalent in this moment. Allowing to prevent potential adverse effects from contraceptive treatments, for instance, from some progestins, potent synthetic progesterones.

For example, as reported in carnivore species, during the luteal phase, indicated by an increase in serum progesterone levels, and, simultaneously, contraceptive treatments with exogenous synthetic progestin, might result in synergic effects and the potential to provoke uterine pathologies (MORESCO; MUNSON; GARDNER, 2009; ASA et al., 2014).

As we will see throughout this chapter, modern contraceptive methods can manipulate biological processes at any point in the reproductive process. From the

cellular level, with DNA transcription and hormone synthesis, or receptor expression, to inhibition of gametogenesis, prevent spermatozoa capacitation, block sperm motility, retard folliculogenesis, block the mechanism of sperm-ovum diffusion, hinder implantation of the zygote, or even provoke abortion of a fetus, to name a few.

(DELVES; LUND; ROITT, 2002; ASA; PORTON, 2005; COOPER; LARSEN, 2006; PICKARD; HOLT, 2007; KIRKPATRICK; LYDA; FRANK, 2011; KAUR et al., 2014; KOGAN; WALD, 2014).

Mammalian life, in particular, is driven by obligatory sexual reproduction, the dominant mechanism to propagate, and to pass on information of adaptation to any given environmental reality. A biological process that brings into being a genetically more adapted individual not just morphologically and physiologically but also in behavioral characteristics, all necessary means for the species survival.

This review focuses on the understanding of the dynamics and interactions of mammalian reproductive physiology, and endocrinology, as it applies to contraceptive methods, their mechanics and desired, as well as adverse impacts, with emphasis on actions of endogenous reproductive hormones, as they represent the mechanism and dynamics of contraceptive agents, either by provoking inhibition or mimicking intrinsic hormonal effects.

3.2 ENDOCRINOLOGY

Communication between cells in a living organism is done in two forms, by way of electrical signals, travelling along the nervous system, and considered the faster one, the other form, is by means of messengers, called hormones, from Latin word *impetus*, meaning "an attack", or "assault", and is part of the endocrine system. Using the circulatory system for transportation, the velocity, compared to a nerve impulse, is much slower. Even though they are considered systems in themselves, they do depend on one another and work together.

Endocrinology studies hormones, their secreting organs, tissues, down to the cellular level, where they are synthesized, how they are traveling throughout the body, how do they find and bind to their target cells, what biological action hormones

initiate, as well as pathological aspect, like what happens when there is a hormone imbalance.

The endocrine system is made up of a large number of different kinds of hormones which essentially are participating in all biological processes of a living organism.

This chapter focuses on the reproductive aspect of endocrinology, as a review of the entire scope of endocrinology would go far beyond the realm of this work. However, general principals of actions are they same for all hormones.

Reproductive hormones, follow a very precise hierarchy, or pathway, and depending on their starting point, for example at the Hypothalamus, then the pituitary gland, and from there to the gonadal (hypothalamic-pituitary-gonadal axis, or HPG-Axis), function like a cascade, which does not imply that it is a one-way street only. Hormones do travel also in opposite direction, referred to as a positive or negative feedback loop, responsible for controlling hormone synthesis and secretion. Although, they are considered reproductive hormones, they do execute many other different functions.

Reproductive hormones, ones secreted by endocrine glands, and/or gender specific gonads, initiate processes of controlling, influencing, acting on, and directing all reproductive functions throughout the entire body. Depending on where they are secreted, and where they are about to perform their biological actions, the hormone release is called either, the endocrine (within), exocrine (outward), paracrine (into extracellular space), neurocrine (hormonal-neurological interaction) autocrine (auto-stimulation), or hemocrine (into the vascular system) secretion (MCDONALD, 2003; JOHNSON, 2013).

3.2.1 Hormone Characteristics

Although the endocrinal system in this context is of principal interest, with its hormones in the role as a messenger, as before mentioned, we cannot ignore the nervous system as an integral part of the communications network, mediating information, captured physically, by initiating and propagating electrical signals between the peripheral and central nervous system, and likewise, between organs bi-

directional. The nervous system, in many instances, is the first to convey sexual stimuli, exciting the reproductive physiology. Sensorial examples are sight, olfactory stimuli, and tactile perceptions, provoking consequential hormonal and physical responses, leading eventually to mating, fecundation, and finally birth.

Hormones can broadly be classified into three chemical groups, which affects the way that they are transported to their target tissue, to what kind of receptor they bind to, and how to act as a ligand.

- Amines, polypeptide hormones, and protein hormones, derived from tyrosine and tryptophan, chains of < 100 amino acids (aa), and chains of > than 100 aa in length, respectively. Examples: Amines: NE, Epi, T₄; Polypeptides: ADH; Protein Hormones: Growth hormone. They are mostly not lipid soluble (lipophobic), or polar, meaning H₂O soluble, consequently, cannot diffuse through a cell's phospholipid-bilayer membrane. Therefore, they bind to receptors on the cell's surface.
- Lipid hormones, derived from cholesterol, are lipid soluble (lipophilic), or non-polar, meaning H₂O insoluble, and can diffuse through the cell wall, and bind either to receptors in the cytoplasm or to receptors on the cell nucleus where they would initiate their biological response. Examples: Steroid hormones Estradiol & Progesterone; Testosterone; and Cortisol.
- Glycoprotein hormones, (GPH) family is made up of gonadotropins, the luteinizing hormone (LH), the follicle-stimulating hormone (FSH) and chorionic gonadotropin (CG) and a non-gonadotropin, the thyroid-stimulating hormone (TSH). And together with the steroid hormones, are the most important reproductive hormones. Glycoprotein hormones are two noncovalently bound polypeptide subunits, termed alpha (α) and beta (β), the last being chiefly responsible for the biological activity. GPH are lipophobic and bind to cell surface receptors. (MCDONALD, 2003; JIANG; DIAS; HE, 2014).

3.2.2 Hormone Synthesis

Prohormone, Preprohormone, and Prehormone

Before hormones are being secreted it has to pass through a number of chemical processes, such as cutting and splicing together, and depending on their process step are referred to as prohormones, preprohormones, or prehormones.

Prohormone: precursor are long-chained polypeptides, example proinsulin.

Preprohormone: derived from the larger precursor prohormone, example preinsulin.

Prehormone: are inactive hormones until modification (activation) at the target cell, examples are thyroid hormones T_4 being converted into T_3 .

Hormones are being synthesized by starting the cell nucleus the DNA transcription, producing a messenger RNA (mRNA), responsible for the encoding of a prohormone in the rough endoplasmic reticulum, and after a number of processes, are carried to the Golgi complex, where they are going through their final processes and packaging into vesicles. Ones receiving the right extracellular stimulus, the hormone filled vesicles, migrate to the cell's plasma membrane, transfuses, and releases (secreting) the hormones into the extracellular space, or vascular system (MCDONALD, 2003; SQUIRES, 2013). The biological properties of different hormones are based on their chemical structure, and determines its ligand fit and its effectiveness on a the target receptor, a characteristic that all hormones have in common.

3.2.3 Hormone Secretion

Hormone levels in the blood can change from one moment to the other, as it is illustrated in figure. 3.1, some hormones are present just for a few minutes while others may remain for hours, or days, but vary in concentration. Some remain steady (tonic) in their secretion, like the liberation of GnRH, or are released in a pulsatile pattern like glucocorticoids, others increase slowly, reach their peak and then suddenly drop, and after a certain interval, repeat their pattern. Whereas some just being released in a burst-like fashion, as it is the case with LH, for instance, leading to ovulation.

Figure 3.1 - Hormone tonic secretion pattern

Source: Rosenfield (2016)

- Tonic secretion pattern means: consistent secretions
- Phasic: hormone presented in phases or bursts
- Pulsatile: secreted in a burst-like or episodic manner rather than constantly
- Surge: brief, but massive output

In the graphic example of GnRH, in figure 3.2, it is shown that there are two different centers, each responsible for their specific hormone release pattern.

Contraceptive methods release their agents in simulated pattern, for example, a GnRH agonist as part of implants, would liberate deslorelin acetate in a tonic manner (ORTMANN; WEISS; DIEDRICH, 2002; MCDONALD, 2003; GAN; QUINTON, 2010).

Figure 3.2 - Hormone release pattern: Surge vs. tonic release

Source: Senger (2003)

3.2.4 Hormone Transport

Depending on the hormones chemical class, once secreted in a hemocrine fashion, they are either enter the circulatory system as a water-soluble hormone, being hydrophilic in nature, allows them to dissolve into the bloodstream, and do not require a carrier protein for transport, or as a fat-soluble hormone, which makes them hydrophobic, and require a carrier protein to reach their target tissues, as shown in figure 3.3.

Figure 3.3 - Protein hormone transport and steroid hormones bind to specific sex hormone binding globulin (SHBG) and plasma albumin carriers for transport.

Source: Pearson Endocrinology. Adopted Rosenfield (2016)

3.2.5 Hypothalamic–pituitary–gonadal axis (HPG axis)

Even though the hypothalamus, the pituitary, and the gonadal glands are distinct entities, in their execution of controlling the reproductive function, they work together and also depend on one-another.

The Hypothalamus is the organ that links the outside world via stimuli; transformed into electrical signals by the nervous system, to the endocrine system. This perception could be the sight of a female in heat or the intensity of the light (favorable season for reproduction) captured by the retina's, and deep-brain photoreceptors, that also involves the pineal gland effects; smell (pheromones), recognizes by chemical receptors of the olfactory system; taste, detected by chemoreceptor, located on the tongue and oral cavity, as well as touch, perceived by

tactile receptors, positioned throughout the skin (MCDONALD, 2003; JOHNSON, 2013).

Taking into consideration contraceptive/breeding methods, alone in this cerebral region, we have a number of possibilities to manipulate fertility. Controlling the intensity and duration to light, is one of the most employed strategies to control food consumption, growth and reproduction for breeding purposes, such as the famous dark house in the poultry industry (BRENNAN; JAN; LYONS, 2006).

Different seasonal light conditions are the driving force behind the reproductive cycles in many different species. Another example is the impact of light on the pineal gland or epiphysis, that, depending on the exposure time to light, synthesizes and secretes the hormone melatonin (not to confuse with the pigment melanin), responsible for controlling the circadian rhythm and the regulation of reproductive hormones. Melatonin does this by inhibiting the secretion of the two gonadotropins, the luteinizing hormone (LH), and the follicle stimulating hormone (FSH), from the anterior pituitary gland, responsible for controlling gonadal functions in both sexes. And *voilà*, another possibility for a contraceptive method.

The pituitary gland also called the hypophysis, that is divided into two independent glands, the anterior lobe, with its three subdivisions (*pars distalis*, *tuberalis*, and *intermedia*); the posterior lobe (*pars nervosa*), or adenohypophysis and neurohypophysis, respectively. The hypothalamus and the anterior pituitary gland are connected by the hypophyseal portal system, which supplies the blood and passageway for the hormones (MCDONALD, 2003; CUNNINGHAM; KLEIN, 2007).

Hormones synthesized and release from the Hypothalamus, the GnRH, and the gonadotrophin hormones from the adenohypophysis are basically the same in male and female, with the same general function of the gonads, as shown in figure 3.4, which is stimulating gametogenesis, and the synthesis and secretion of sex hormones, although, not their only function.

Figure 3.4 - Schematic representation of the hypothalamic–pituitary–gonadal (HPG) axis.

Source: Rosenfield (2016)

3.2.6 Endocrinological Feedback Control

In endocrinology, feedback control is the homeostatic mechanism of regulating synthesis and secretion of many hormones, mediated by the concentration of circulating hormones (the same or other hormones), that can be either a "negative feedback" with inhibitory, or a decreased production of hormones, or a "positive feedback", with exactly opposite effects.

As illustrated in figure 3.5, the negative feedback occurs on two levels:

1. Once the plasma concentration of prior targeted gland hormones reaches its saturation point for a given process, it acts on the hypothalamus and inhibits the secretion of releasing hormones, in turn, inhibiting the secretion of pituitary tropic hormones, leading to inhibition of hormones secretion or suppression of physiological activity at the target tissue.
2. Target gland hormones act on the anterior pituitary, inhibiting the response of releasing hormones, which in turn suppresses any following process.

Figure 3.5 - Simplified representation, male reproductive feedback loops

Source: Pearson Endocrinology. Adopted Rosenfield (2016)

- **Positive Feedback:** GnRH stimulates the release of gonadotropins. LH and FSH stimulate spermatogenesis and testosterone synthesis and secretion by the testis.
- **Negative Feedback:** Increased plasma concentration of inhibin, secreted by the Sertoli cells, and testosterone, secreted by the Leydig cells, lead to the inhibition of GnRH secretion by the hypothalamus, as well as the inhibition of LH and FSH secretion by the anterior pituitary gland.

In female reproduction, feedback signaling is somewhat more complex, see figure 3.6, as they depend on the phase during the menstrual cycle. These phases are not separate entities, but depend on one another, leading in subsequent order from the follicular phase, to ovulation, to the luteal phase, only interrupting this repetitive cycle by pregnancy, or the cessation of the ovarian activity, either due to menopause, or suppression by of contraceptive methods.

Figure 3.6 = Simplified representation, female reproductive feedback loops.

Source: Rosenfield (2016)

Female Reproductive Feedback Loops

During follicular phase

Positive feedback: GnRH stimulates the secretion of gonadotropins LH and FSH from the anterior pituitary gland, which in turn, drive the ovarian follicle growth.

Negative feedback: The ovarian follicles synthesis and secrete low concentration of estradiol, eventually provoking an inhibitory effect on the release of GnRH from the hypothalamus, consequently maintaining the secretion of LH and FSH to a minimum. Also, estradiol reaching the uterus will cause arteries within the endometrium to constrict, inciting the shedding of the endometrial lining.

During ovulation

Positive feedback - only: Continuation of GnRH release and the persistent liberation of LH and FSH, causing more ovarian follicles to grow. Once these follicles reach a certain size, they start to produce higher concentrations of estradiol, which continues to promote the release of GnRH, increasing the plasma concentrations of LH and FSH, allowing eventually a dominant follicle to mature, and consequently, leading to the liberation of an oocyte. In preparation for a potential implantation of a fertilized egg, the estradiol secreted from the follicles also promotes a thickening and vascularization of the endometrium.

During luteal phase

Positive feedback: After ovulation, the follicle tissue, under the influence of LH, transforms into a corpus luteum (CL), and together with FSH, maintains the release of estradiol.

Negative feedback: Also, the corpus luteum starts to synthesize and secrete the hormone progesterone. Increased plasma levels of progesterone and estradiol will act on the hypothalamus, as well as on the anterior pituitary, causing an inhibition of the secretion of GnRH, LH, and FSH, driving down any activity of ovarian follicles while continuing the development of the endometrial lining, preparing the uterus for a potential pregnancy (NUSSEY; WHITEHEAD, 2001; MCDONALD, 2003; FELDMAN, 2004; NORRIS; CARR, 2013; SQUIRES, 2013).

3.2.7 Hormonal Interactions

An individual hormone that initiates a physiological response ones linked to a receptor is called an agonist. But in many instances hormones do not just execute individual biological processes, but do work synergistically, meaning they work together to produce a biological result. That can either be additively or complementary, produce permissive or antagonistic effects.

Additive: Although, each hormone individually produces a response, two hormones, in with combined concentration, execute an even greater biological effect. Example: NE and Epi.

Complementary: In order to initiate or maintain a certain biological process, hormones executes their specific role during different steps of the process, but are dependent on the action of another hormone. Example FSH and testosterone.

Permissive effect: The action might also be termed as "priming", meaning preparation or upregulating, whereby a the "first" hormone provokes a target cell's responsiveness to a "second" hormone. Permitting, or enhancing the second hormone's biological action. Example: Estrogen effects on the progesterone receptor formation in the uterus.

Antagonistic effect: which signifies opposition. An effect where a hormone binds to the same target receptor of another hormone, but without provoking its activation, thereby inhibiting (antagonizing) the primary biological effect of the "antagonized" hormone. Endogenous example: Insulin Glucagon. And an example of a contraceptive agent would be Cetrorelix acetate, a synthetic decapeptide, that acts as an antagonist of GnRH receptors (ZAFEIRIOU; LOUTRADIS; MICHALAS, 2000; NUSSEY; WHITEHEAD, 2001; MCDONALD, 2003).

There are three principal factors that drive a target cell's response to a hormone:

1. Plasma concentration of the hormone
2. The number of receptors on, or within the target cell
3. Specificity and affinity of the hormone for the receptor and vice versa

As before mentioned, depending on the chemical makeup of the hormone, either being polar, or nonpolar in nature, either bind to a cell surface receptor, to a cytoplasm receptor, or a nuclear receptor, which they would activate the same, initiating the process of transcription in the cell nucleus.

3.2.8 Cell Receptors, Ligand, General Cell Signal Transduction and Response

Target cells have high-affinity receptors, which can be located on the cell's external surface, or inside the cell, in the cytoplasm, or the cell's nucleus. Although target cells have the capability of binding to corresponding hormones, they need to have the receptors adequately expressed (present in sufficient quantities to have a biological effect), which depends on the physiological necessity at the time. In some processes, the cell must undergo first a "priming", or upregulating, which describes the event of amplifying the expression of receptors.

On the other hand, there is "downregulation" of receptors. Classic contraceptive example: Deslorelin, a synthetic GnRH analog, that due to downregulation suppresses ovarian activities as long as the agent remains its concentrations to be biologically active (ROSENFELD, 2012).

3.2.8.1 Downregulation, or Desensibilization

GnRH-stimulated gonadotropin secretion might be blocked by the administration of GnRH antagonists (see figure 3.7) or by mimicking agonists (GnRH analogs) due to constant stimulation which provokes a desensitization, both ligand eventually resulting in reduced gonadal steroid levels. The initial response to prolonged exposure to a GnRH analog is to provoke a "flare effect" or an increase in plasma LH and FSH levels, followed by a paradoxical effect, of a sharp decrease of these gonadotropins. This desensibilization and/or downregulation of GnRH receptor (GnRHR) can either occur by a decrease in the overall number of expressed surface receptors, or by blocking the mechanism of the G-Protein Coupled Receptor. (FINCH et al., 2009).

Figure 3.7 GnRH G-Protein Coupled Receptor Block - Desensibilization

Source: Rosenfield (2016)

3.2.8.2 Ligand & Receptors

Hormones, neurotransmitters, and pheromones are considered ligand, because of their binding action with specific receptor protein, which is either located on the cell's surface, in the cell's cytoplasm, or at the cell's nucleus. Specific means that a ligand molecule most "fit" the receptor like a key in a lock, and if they do, causes the receptor to a conformational change, or better, a change of the macromolecule shape, necessary to initiate the first step of a cell's signaling cascade, leading a specific cell response.

Once a ligand has fulfilled its purpose of transmitting a signal, is either being modified and degraded by the target cell, terminating its action. Intrinsic signal molecules have often a much lesser half-life than their synthetic analogs, in many cases the desired effect.

As before mentioned, receptors are highly specialized structures, with the purpose to only respond to very specific molecules, thereby, ignoring all the others. That is why there are so many different types of receptors. The big challenge of pharmacology is trying to design a perfect agent to function and mimicking the natural corresponding signal molecule as precise as possible. Another important consideration is that most contraceptive methods are developed for the human markets.

And herein lies the problem in veterinary medicine, and wildlife population control. Unfortunately, there are not many species-specific developed contraceptives available. So in practice, it is being used what is obtainable, and, to a larger degree, through dedicated studies and accessible Information from central databanks, the lesser-evil can be chosen. Nevertheless, depending on the species, abnormal activation of non-target receptors by hormone based contraceptive agents often provoke cancerous implications, and/or any of the other undesirable side-effects.

A classical example of adverse effects in carnivore species, provoked by the administration of synthetic androgens by mimicking the actions of progestins due to potent interactions with progesterone receptors. Potential consequences, for instance, are modulated functions of the endometrium, causing uterine pathologies, or synthetic progestins, inducing hypersecretion of growth hormones (GH), potentially developing into cystic endometrial hyperplasia (LODISH, 2000; BHATTI et al., 2007; GREGOIRE, 2013).

3.2.8.3 Schematic presentation of ligand & receptors

As shown in the schematic of figure 3.8, depending on the messenger's chemical make, like protein hormones, being lipophobic, or non-polar, they bind to protein receptors on the cell's surface, for example to a G-Protein Coupled Receptor (GPCR). Cholesterol derived hormones are lipophilic, or with polar characteristics, which allows the diffusion through the cell's membrane and binding to its specific receptor within the cytoplasm or the cell nucleus.

Figure 3.8 - Ligand & Receptors, Lipophilic transmembranal (TM) diffusion; 7TM GPCR (G-Protein Coupled Receptor), Ion channel; TM transport; Enzyme-linked signaling

Source: Rosenfield (2106)

3.2.8.4 Cellular Signaling and Transduction

Once a messenger reached its target cell, the first action is the binding to the hormone's specific receptor, called the signal reception. Depending on the chemical class of the hormone, can be on the cell surface, the cytoplasm, or direct at the nucleus. In sequence, the newly formed unit of a ligand and the receptor, called now a *dimer*, (di = two + mer = parts), referring to the formation that consists of two structurally similar monomers, will suffer a structural change, necessary to initiate the 2nd step, the signal transduction, a cascade of chemical processes, making up the pathway of signal relays, until reaching its final target at the cell nucleus, where it will execute the specific cellular response.

Some hormone concentration are secreted in very minute quantities, or have a very short half-life, for these kinds of hormones to take effect, the cell transduction mechanism is capable of a sophisticated way to amplify and fast relaying the signal to the nucleus. As shown in figure 3.9, this requires the help of 2nd messengers, which are small water-soluble molecules (ions), the 1st messenger being the initial signal on the cell surface receptor. Examples of 2nd messengers are cyclic AMP (cAMP) and calcium ions (NUSSEY; WHITEHEAD, 2001; MCDONALD, 2003; JOHNSON, 2013; NORRIS; CARR, 2013).

Figure 3.9 - Three stage cell signaling

Source: Rosenfield (2016)

3.2.8.5 Schematic example of a cell nuclear response to a testosterone ligand

In figure 3.10, a testosterone ligand is executing a steroid signal pathway by directly diffusing through the plasma membrane, binding to an Androgen Receptor (AR) within the cytoplasm; the formed AR then translocates to the nucleus, binding to a gene promoter region, called the Androgen Response Elements (AREs), and, together with a co-regulator, is controlling gene transcription, shown in figure 3.11 (WALKER, 2011).

Figure 3.10 - Schematic example of a cell's nuclear response to a testosterone ligand

Source: Rosenfield (2016)

Figure 3.11 Transcription & Translation

Source: Rosenfield (2016)

3.2.9 Overview Reproductive Hormones, Synthetics, and their Biological Action

Tables 3.1 to 3.9 provide an overview on all reproductive related hormones

Table 3.1 - Reproductive Hormones, synthetic analogs, and their biological actions

Hormone M = male F = female	Chemical class	Gland origin producing cell	Regulator	Target	Biological Action / release pattern	Synthetic forms
Hypothalamic hormones						
Gonadotropin-releasing hormone (GnRH) 16 different forms GnRH, I, II, III in mammals* M/F	Neuropeptide (Neuro-hormone) Decapeptide hydrophilic	Hypothalamic Neurons of the preoptic area (POA) Surge and tonic centers Pulsatile secretion The median eminence, in mammals, contains the greatest amount of GnRH, stored in neuronal terminals prior to release into hypophyseal portal blood The placenta, during pregnancy	Kisspeptin Testosterone Estradiol (E ₂) Progesterone Melatonin Stress Nutrition	Anterior pituitary gland Gonadotrope cells	Stimulating the release of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH)	Buserelin Deslorelin Degarelix Goserelin acetate Histrelin acetate Leuprolide acetate Leuprolide acetate Nafarelin acetate Triptorelin pamoate
Kisspeptin (KP) M/F	Peptides hydrophilic	Hypothalamus Hippocampal dentate gyrus Adrenal gland Pancreas	Example: Leptin	multi-target	Upstream regulator that integrate central and peripheral signals with GnRH release. Stimulates secretion of aldosterone, insulin and believed influence LH and FSH. Sexual development	

Table 3.2 - Reproductive Hormones, synthetic analogs, and their biological actions

Hormone M = male F = female	Chemical class	Gland origin producing cell	Regulator	Target	Biological Action / release pattern	Synthetic forms
Hypothalamic hormones						
Melatonin M/F	Idolamine (monoamine) family of neuro- transmitter Dual character: Lipophilic/ hydrophilic	Pineal gland (epiphysis) Pinealocytes Serotonin derivative Highest synthesis and secretion during darkness	Nor- epinephrine Intensity and duration of light	Supra- chiasmatic nucleus Target tissues throughout the body	Principal function, regulation of seasonal rhythms (photoperiod breeding cycles) by inhibition of GnRH- induced gonadotropin release free radical scavenger and an indirect antioxidant in reproductive processes	
Adeno- hypophysis						
Luteinizing hormone (LH)	Glyco- proteins hydrophilic	Anterior pituitary Gonadotropin cells Pulsatile secretion	GnRH Kisspeptin E ₂ P ₄	Ovary: follicle Testis: Leydig cells	Regulates gonadal functions Stimulate steroid hormone synthesis and secretion	
Follicular stimulating hormone (FSH)	Glyco- proteins hydrophilic	Anterior pituitary Gonadotropin cells	GnRH Kisspeptin E ₂ P ₄	Ovary: Granulosa cells Testis: Sertoli cells	Maturation of germ cells in testes and ovaries Male: stimulates the secretion of inhibin, ABP (Androgen Binding Protein) follicular development estradiol synthesis	

Table 3.3 - Reproductive Hormones, synthetic analogs, and their biological actions

Hormone M = male F = female	Chemical class	Gland origin producing cell	Regulator	Target	Biological Action / release pattern	Synthetic forms
Hypothalamic hormones						
Prolactin (PRL)	Protein hormone hydrophilic	anterior pituitary lactotroph cells	various neuro-hormones most important one: dopamine, or prolactin inhibitory hormone, (PRL-IH) Estradiol	Ovaries Leydig cells	Stimulatory effect on milk synthesis in mammary glands. Prolactin synth. increases when suckling reflex present. Reduces the secretion of dopamine, increases the prolactin releasing hormone (PRL-RH) Some degree of gonadal function in some domestic species and rodents Influence on brooding behavior in birds Inhibitory effect on androgen secretion in the ovary.	exist
Activin	Glycoprotein hydrophilic	Ovary: Granulosa cell Testis: Sertoli cells		Female: anterior pituitary Gonadotropin cells Male: epididymis	stimulation of FSH synthesis and increases FSH secretion increases estradiol synthesis enhances activation of LH on the ovary enhances spermatogenesis and effect of LH on the testis cell proliferation, cell differentiation, apoptosis, and homeostasis	

Table 3.4 - Reproductive Hormones, synthetic analogs, and their biological actions

Hormone M = male F = female	Chemical class	Gland origin producing cell	Regulator	Target	Biological Action / release pattern	Synthetic forms
Gonadal hormones						
Estrogen, estradiol (E2)	Steroid Cholesterol derivative lipophilic	Ovarian granulosa / theca interna cells of the follicles placenta (pregnancy) zona reticularis of the adrenal cortex mesenchymal cells of the adipose tissue and skin Testis: Sertoli cells Leydig cell germ cells in various stages of differentiation	FSH LH	ER α and β receptor At reproductive organs: Vagina Cervix Teats Uterus Blood vessels CNS Fallopian tubes Mammary gland Corpus luteum Bone tissue Cardio- vascular tissue 2nd sex character.	follicular growth estrous behavior growth and development of mammary tissue prep uterus for parturition Vagina: slight mucous secretion, hyperemia, edema Cervix: relaxation, liquefy. of mucous plug Uterus: uterine gland development, sensitization of the endometrium to oxytocin, immune activation (local), leukocyte infiltration, secretion PGF2a/ PGE2 Fallopian tube: increased motility and cilia activity Mammary gland: stimulates mammary duct development Corpus luteum: Luteolytic (bovine / ovine) luteo-trophic (equine / porcine) Influencing spermatogenesis	Conjugated equine estrogens (CEE) pregnant mares' urine Estradiol acetate Estradiol hemihydrate Ethinyl estradiol Mestranol Tibolone Nonsteroidal: Di-ethinyl stilbestrol Hexestrol Dienestrol

Table 3.5 - Reproductive Hormones, synthetic analogs, and their biological actions

Hormone M = male F = female	Chemical class	Gland origin producing cell	Regulator	Target	Biological Action / release pattern	Synthetic forms
Gonadal hormones						
Testosterone (T) Or 5 α - dihydrotestosterone (DHT) More potent, by 5 α -reductase	Steroid (androgen) lipophilic	Ovary theca cells Placenta Adrenal zona reticularis Skin Testis: Leydig cell	LH Gonadocrinin In synergy with FSH and prolactin Estradiol	Leydig Cells AR throughout the body (muscles, bones,	Spermatogenesis Anabolic growth Promotion of secretion from the accessory sex glands Influences sexual behavior and aggression Causes an increase in the level of pheromones, secreted by glands in the skin, evokes sexual behavior in females. Glands use in scent marking are activated by testosterone In certain species, tusks, antlers, and horns are also stimulated to develop Testosterone is ineffective orally (inactivated by the liver), and is usually given as i.m. injections of testosterone esters.	Methyl- testosterone (17 α methyl- testosteron) Fluoxymesterone Propionate Enanthate

Table 3.6 - Reproductive Hormones, synthetic analogs, and their biological actions

Hormone M = male F = female	Chemical class	Gland origin producing cell	Regulator	Target	Biological Action / release pattern	Synthetic forms
Gonadal hormones						
Progesterone (P4)	Steroid	<p>Ovaries: corpus luteum follicular cells</p> <p>Uterus: Placenta and fetoplacental unit</p> <p>Zona reticularis of the adrenal cortex</p> <p>Male gonads Leydig cells</p>	estradiol	<p>steroid- sensitive neurons in the central nerves system</p> <p>Progesterone receptor through the body</p>	<p>Modulates behavior</p> <p>Pregnancy: inhibits secretion of FSH and LH (negative feedback by inhibiting GnRH), preventing ovulation.</p> <p>Prepares the uterus for pregnancy by endometrium growth and proliferation to secretion.</p> <p>In the absence of pregnancy leads to organized shedding (menstruation).</p> <p>After fertilization: Organizes the vasculature of the endometrium, prepares for implantation</p> <p>Inhibits contractions of the uterine myometrium and counteracts the effects of oxytocin on contractility</p> <p>Promotes lobuloalveolar growth to prepare for lactation</p> <p>Males: Influences spermiogenesis, sperm capacitation/acrosome reaction and testosterone biosynthesis in the Leydig cells. Modulator of male sexual response / behavior.</p>	<p>Medroxypro- gesterone acetate (MPA)</p> <p>Micronized progesterone USP</p> <p>Norgestrel</p> <p>Norethindrone acetate</p> <p>CEE MPA</p> <p>Ethinyl estradiol</p> <p>norethindrone acetate</p> <p>17 beta- estradiol</p> <p>norethindrone acetate</p>

Table 3.7 - Reproductive Hormones, synthetic analogs, and their biological actions

Hormone M = male F = female	Chemical class	Gland origin producing cell	Regulator	Target	Biological Action / release pattern	Synthetic forms
Gonadal hormones						
Inhibin M/F	Glycoprotein hydrophilic	Ovary follicles Granulosa cells Believed, also present in the placenta Testis: Seminiferous tubes Sertoli cells	Androgens GnRH Insulin-like growth factor (IGF-1)	Adenohypophysis (Pituitary) Gonadotroph. cells	Part of a negative feedback loop within the pituitary-gonadal axis to regulate (inhibit) synthesis of FSH Male: results in decreased spermatogenesis Females: inhibits FSH secretion Doesn't effect on the secretion of LH.	
Uterus						
Prostaglandin F2 α (PG) luteolysin (dinoprost)	Eicosanoid Fatty acid *Amphipathic	Uterine endometrium Vesicular glands	Oxytocin from CL E ₂ positive P ₂ negative stimulation molecules secreted by the developing embryo	Female: CL, uterine myometrium, Ovarian follicles Kidney Spleen Heart	In female PGF2 α cause luteolysis, forming a corpus Albicans, stopping the production of P ₄ Cause the induction of tone and contractions within the uterus (labor inducer). Vasoconstriction Bronchoconstriction Breeding: Estrous synchronization In medicine: induces labor and as an abortifacient, besides other therapeutic application	Bimatoprost Carboprost Luprostiol Latanoprost Travoprost

Table 3.8 - Reproductive Hormones, synthetic analogs, and their biological actions

Hormone M = male F = female	Chemical class	Gland origin producing cell	Regulator	Target	Biological Action / release pattern	Synthetic forms
Gonadal hormones						
Prostaglandin E2 (PGE2)	Eicosanoid Fatty acid *Amphi- pathic	Ovary Uterus Embryonic membranes Kidney Spleen Heart		Cervix potent cervical dilator corpus luteum oviduct, induces ovulation	Vasodilation smooth muscle relaxation Inhibition of the release of noradrenaline from sympathetic nerve terminals. Aids secretion of progesterone from the corpus luteum during labor softening of the cervix aids stimulation of uterine contraction. Preparation of the tract for parturition.	
Relaxin	Protein hydrophilic	Ovary Corpus luteum Placenta		Cervix Vagina Pubic symphysis	Pregnancy: prevents the initiation of uterine contractions Responsible for the softening and relaxation of connective tissue prior to parturition. Estradiol priming is required for this. Relaxin performs in conjunction with prostaglandin.	
Gonadocrinin	GnRH-like peptide	Gonads		Pituitary	Stimulates secretion of gonadotropins	

Table 3.9 - Reproductive Hormones, synthetic analogs, and their biological actions

Hormone M = male F = female	Chemical class	Gland origin producing cell	Regulator	Target	Biological Action / release pattern	Synthetic forms
Placenta						
Human Chorionic Gonadotrophin (hCG)	Glyco-protein Hydrophilic	produced by embryonic cells trophoblast cells of a blastocyst also present in male seminal vesicle fluid Prostate?		primary target: ovaries Postulated: as a component of seminal fluid, might exert its functions in the female repro-tract	Promotes progesterone synthesis stimulating synthesis of progesterone and estrogens Facilitate the formation of the accessory corpora lutea and ensure that progesterone production is maintained. In males, increases the growth of the fetal testes. Presence of this hormone within maternal blood can be used for pregnancy confirmation	
Equine Chorionic Gonadotrophin (eCG)	Glyco-protein Hydrophilic	chorion of pregnant mares. eCG in fetal endocrine cells		stimulate follicular growth and ovulation in the horse	Chorionic tissues in horses, as well as primates, also form hormones. eCG is also thought to stimulate follicular growth and ovulation in the horse.	
Placental lactogen (PL), or Chorionic Somatomammotropin (CS)	Protein Hydrophilic	Placenta		Similar to a GH Mammary glands	Stimulate the growth of alveoli	

Source Rosenfield (2016)

Source: (SIMPSON et al., 1999; MCDONALD, 2003; CLARKE; POMPOLO, 2005; CUNNINGHAM; KLEIN, 2007; MEAD et al., 2007; REITER et al., 2009; ZOUBOULIS, 2009; TASSIGNY; COLLEDGE, 2010; ROTSTEIN, 2011).

Obs. It is not intended to be a recommendation of products nor represents a complete listing of all products available.

3.2.10 Reproductive Steroidogenesis Pathways

Figure 3.12 shows an overview of the sex steroid hormone biosynthetic pathway, with associated nuclear receptors, progesterone receptor (PR); Androgen receptor (AR); Estrogen receptor (ER), and the necessary process enzymes P450ssc, P450-linked side chain cleaving enzyme; CYP17, cytochrome P450 17; 3 β -HSD, 3 β -hydroxysteroid dehydrogenase; 17 β -HSD, 17 β -hydroxysteroid dehydrogenase (CAREY et al., 2007).

Figure 3.12 - Reproductive Steroid Hormone Pathways

Source: Carey, 2007; Adopted Rosenfield (2016)

Estrogens are produced in the female follicle, by the interaction of the theca interna and the granulosa cells, controlled by the positive feedback of LH and FSH and the negative feedback of inhibin. Initiated by the stimulus of LH, androgen is being synthesized from cholesterol, and subsequently diffuses into the granulosa cell, where, under the regulation of FSH, it is being aromatized into estrogen.

Testosterone is produced, under the influence of LH, in the testicular Leydig cells, where free cholesterol passes through testosterone pathway, until transformed into testosterone. Sertoli cells may uptake testosterone where it would be transformed into estradiol 17 β , regulated by FSH (MCDONALD, 2003).

3.2.11 Male Specific Steroidogenic Function On The Reproductive System

In males, the primary reproductive organ is the male gonads, or testis and responsible for steroidogenesis (production of Androgens) and gametogenesis (production of spermatozoa). Their activity is regulated by gonadotropins, the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH), synthesized and secreted, in a pulsatile pattern, from the anterior pituitary gland, also called the adenohypophysis or pars anterior, which are under the positive feedback control of the gonadotropin-releasing hormone (GnRH), produced in the hypothalamus, and the negative-feedback control by testosterone, acting on the hypothalamus and pituitary. In addition to external stimuli that either promote or inhibit GnRH secretion.

Testosterone, the chief testicular androgen hormone, is the product of biosynthesis from cholesterol, within the Leydig cells of the testis. Noteworthy, androgen synthesis also occurs in other organs, such as in the adrenal cortices, ovaries, placenta, and even the skin.

Testosterone has two principle functions, first, its androgenic effects, and secondly, its anabolic effects.

Androgenic effects already take place during the stages of sexual differentiation in the developing fetus, include maturation of the male sex organs during puberty and adulthood, plus the development and maintenance of secondary sexual characteristics, physical and behavioral. Examples of male adornments (visual masculine characteristics) are the lions mane, the elk or deer antlers, or the birds plumage, as well as pheromone glands, the skeletal and muscular appearance, as the most noticeable. The later due to anabolic effects. In addition to intratesticular androgenic effects, describing spermatogenesis, which requires the largest amount of testosterone

As before mentioned, anabolic effects include the growth of bone density and muscle mass, serving as an attraction, for combat (territorial disputes, fights over females, mating rights), and hunting.

Estrogen (testicular estrogen), although thought of as a female sex hormone, is being synthesized in the Sertoli cells, controlled by FSH, from testosterone and believed to play a role in the regulation of the pituitary-gonadal axis, as well as to inhibit the secretion of testosterone from the Leydig cell.

Other hormones that are produced in the testis are estrogen, cytokines inhibin, activin, MIH (Müllerian-inhibitor hormone), oxytocin, InsI3 (insulin-like hormone) and gonadocrinin, a GnRH-like factor, participate at the intratesticular regulation of testosterone secretion by co-regulating gonadotropin secretion. Also, testicular Sertoli cells secrete a transport protein, called Androgen-binding protein (ABP), important in its role to concentrate testosterone in the seminiferous tubules and to carry it throughout the body (SIMPSON et al., 1999; MCDONALD, 2003; CLARKE; POMPOLO, 2005; BERGER et al., 2007; CUNNINGHAM; KLEIN, 2007; MEAD et al., 2007; REITER et al., 2009; ZOUBOULIS, 2009; TASSIGNY; COLLEDGE, 2010).

3.2.12 Female Specific Steroidogenic Function On The Reproductive System

Female Endocrinology is by far more complex due to the purpose of conception, embryo development, birth-giving and the nourishment of the fetus during the first periods of upbringing.

The two major female gonadal steroid hormones are estradiol-17 β , also known as the most potent form of the female sex hormone estrogens (or oestrogens) and progesterone, and like males, are under the influence the hormone cascade of the hypothalamic-pituitary-gonadal axis. Estrogens are synthesized in the ovary's theca interna cells and the granulosa cells, controlled by the positive feedback mechanism of LH and FSH and the negative feedback of inhibin, which in turn, are under the influence of organs that are responsive to ambient conditions, such as light, food availability, olfactory visual and tactile stimulus, relaying these external conditions via specific hormones messengers to the central nervous system, with either promoting or inhibiting effects on the GnRH release.

Depending on the species-specific reproductive characteristics, such as seasonal or non-seasonal breeders, mono,- or polyestrous, spontaneous, or induced ovulation, which drives their unique endocrinological physiology. Females also have the most dramatic physical changes, chiefly organs like the vagina, vulva, uterus, vasculatory system and mammary glands.

Analogue to the males testosterone, estrogen is responsible for secondary sex characteristics, the development and maintenance of the functional structure of the

female reproductive organs, including sexual behavior (receptiveness for mating), also referred to as behavioral estrus.

Other hormones produced by the ovary are oocyte maturation inhibitor (OMI), responsible for the preservation of the oocyte during the arrest stage. Inhibin, like in males, blocks the secretion of FSH from the hypothalamus pituitary. Gonadocrinin (GnRH-like peptide) influences the steroidogenesis by the theca cells. Activin, postulated to have an impact on the activity of the ovaries and relaxin, a polypeptide important in the preparation of parturition (SIMPSON et al., 1999; MCDONALD, 2003; CLARKE; POMPOLO, 2005; BERGER et al., 2007; CUNNINGHAM; KLEIN, 2007; MEAD et al., 2007; REITER et al., 2009; ZOUBOULIS, 2009; TASSIGNY; COLLEDGE, 2010; ROTSTEIN, 2011).

3.2.13 Estrus cycle

Most female mammals demonstrate very similar estrus cycles, which can be divided into three principal phases, although, sometimes difficult to distinguish where one starts, and the other one ends, see figure 3.13.

1) The follicular phase, or proestrus, during which the ovarian follicle undergo a rapid growth, synchronously increasing estrogen levels while progressively declining progesterone levels, as the secreting Corpus luteum, from the previous cycle, regresses.

2) Ovulation phase, estrus, or the female being in "heat", representing the period of sexual receptivity, during which ovulation occurs, possible mating and fertilization of the ovum, and post-ovulation, the formation of the Corpus luteum (CL), entering the third phase,

3) Luteum phase, the transition from metestrus to diestrus, where the CL is fully developed, and the female is under the dominant influence of progesterone.

The duration of each phase depends on the unique reproductive characteristics of each species, as well as ambient, and corporal conditions. Periods of sexual quiescence is called anestrus (MCDONALD, 2003; SQUIRES, 2013).

Figure 3.13 - Changes of hormone levels during estrus cycle

3.2.14 Estrus Cycle under the influence of contraceptives

GnRH Analogue based contraception: Following (figure 3.14), an example of endocrinological dynamics with contraception. For example with the administration of a GnRH analog implant, which would result in a first flare response of LH and FSH secretion, and subsequently sharp drop, until an inhibitory effect on gonadotropin secretion as long as the GnRH implant maintains its bioactivity (FINCH et al., 2009).

Figure 3.14 - Estrus Cycle under the GnRH Analogue Contraceptive Method Influence

Source: Rosenfield (2016)

Steroid-Based Contraception: Administration of a combination contraceptive of estrogen and progesterone functions by basically tricking the body into believing it is already pregnant. Plasma levels of estrogen and progesterone remain constant throughout the treatment, see figure 3.15. And results in failure to produce an estrogen and LH peak, consequently no ovulation occurs, with an added effect of preventing fertilization mechanically, through a thickening of the cervical mucus, interfering with sperm motility after ejaculation (MARTIN, 2016).

Figure 3.15 - Estrus Cycle under Steroid Hormone Contraceptive Influence

Source: Rosenfield (2016)

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4 CONTRACEPTIVE METHODS IN WILDLIFE POPULATION CONTROL

Abstract

It is well established, why wildlife population control is so important, and why it is even more important to do it without killing the animals, while attending to one of the greatest challenges and demands of modern life, trying to manage, what is termed the "Human-Wildlife Conflict" (HWC). The idea behind this review was to provide a basic understanding of the mechanisms and dynamics involved in antifertility treatments and the *status quo* on contraceptive methods as it applies to wildlife population control today. The results of this investigation can confirm the excel of a very successful contraceptive method, purely developed and constantly improved for wildlife population control, based on immunocontraceptive technology. Either by impeding a sperm from fertilizing the ovum, or inhibiting gametogenesis and steroidogenesis by blocking the release of gonadotropins, and that as a single-shot, long-lasting, remotely deliverable antifertility vaccine.

Keywords: Wildlife population control; Contraceptive methods; Antifertility; Immunocontraception;

4.1 INTRODUCTION

To open the debate on why wildlife population control is important, and why it is even more important to do it without killing the animals, following a brief review on how intensive human population growth, with all its oppressive impacts on flora and fauna, brings about one of the greatest challenges of modern life, trying to manage, what is termed the "Human-Wildlife Conflict" (HWC). Characterized by situations whereby wildlife (including feral animals) incite negative impacts on human civilization, and its individual citizen, either physically, psychologically, and most often economically, (DISTEFANO, 2005; MENDONÇA et al., 2012; BARUA; BHAGWAT; JADHAV, 2013; MASSEI; COWAN, 2014). Although, and philosophically speaking, it would be more accurate to call it the "detrimental impact on fauna by humans."

On the other hand, there is a certain irony involved in this "Human-Wildlife Conflict" image, being that, urban area architectures, as well as non-urban manmade structure, are serving as the perfect habitats for highly adaptable species, thriving on the unwilling availability of thousands of tons of thrown away but still comestible foods, providing the perfect nourishments for survival and upbringing of new synanthropic generations. And while on one side, major efforts are put forward to control and minimize such populations, antagonistically, citizens deliberately feed or even offer shelter to the new wildlife neighbors, until realizing that the first so cute and helpless looking fellows, are turning into pest-like creature, a nuisance, and disease carrier, that needs to be get rid of (SOULSBURY; WHITE, 2016).

The traditional means to minimize or avoid these conflicts was done by lethal control programs, even until today (SKINNER, 2015). In many instances, they are not legal, nor represents an adequate choice, lacks safety, and the quickly growing disapproval by the public; thus, alternative approaches are necessary.

Contraceptive Methods can chiefly be organized into general methods, that further divided into sub-categories, as shown later in the chapter, like their pharmacological actions, chemical makeup, or their "generation" (indicating when developed), applications, route of administration, gender specific, and in veterinary medicine, of course, application by species, in the field, or in captivity, with short, or long term effects, even based on species-specific know adverse effects.

For example, the well-known sensitivity of carnivore species to some hormone contraceptives agents, in comparison to primates. Of course, the main reason being, many products for the human markets have been tested on primates first, as they are the closest relatives, and if no significant side effects were identified, the product made it one step closer for approval. Meaning, at the same time, and to a certain degree, could be seen as a primate-specific agent, conversely to applications in felids or canids.

As before mentioned, earlier contraceptive methods lacked mainly species-specificity, but intensive research with a great number of wildlife species, and constantly improved, or novel methods, have lead to an extensive database, made available by some institutions around the globe. One of the best known, and perhaps the most comprehensive one, can be found at AZA Wildlife Contraception Center (WCC) at the Saint Louis Zoo, dedicated since 1999, in collecting, and continuously monitoring results of contraceptive studies, shared by zoos and wildlife experts from around the world, reporting on efficiency, health effects, and making available, species based recommendations on contraceptive methods (WCC, 2016).

While, mass population control of vertebrate "pest" species are an important part of wildlife conservation, referring to rodents, lagomorphs, marsupial and avian species, this research does not enter into great detail on these methods.

The review is organized in a general overview of the history of contraceptive methods, the mammalian reproductive physiology as it applies to contraception, the chemical classification of contraceptive agents, their biological actions, their pro and contra, and adverse effects. Including a more in-depth view on the concept of immunocontraception. Continuing with an opinion piece on the Brazilian challenge of Wildlife and pest species population control. Material and Methods, and a representation of the results. Figure 4.16 depicting feral pigs.

Figure 4.16 - Feral pig

Source: Fotosearch (2016)

4.2 CONTRACEPTIVE METHODS IN WILDLIFE POPULATION CONTROL

When it comes to choosing the right wildlife population method, the most important features must be. "health & reversible", for the animal's well-being and the simple purpose of securing the individual's genetic information, which is contributing to the species overall genetic diversity and survival. Once all other conservation, ethical and moral issues are resolved, special consideration must be given to the species specificities in reproductive and social behavior, breeding cycle, and their habitat, to guarantee the success of the chosen management program.

Also, when planning for the most appropriate method, other questions to consider are, is the individual, or group, free-ranging, or in captivity. If free-living, contraceptive methods, that are "one-shot" applications, would be an adequate choice, as finding future access to the treated animal might be rather difficult, and delaying the second treatment may hinder, or nullify the success of the entire management operation.

Based on the animals specific characteristics, such as, are they prey species, or predators, what are the behavior patterns, feeding habits, are they nocturnal or diurnal, far-roaming, or do they maintain in a certain locality, all these information will assist in choosing the right method, and administration techniques. For illustration, contraceptive methods may target either an individual, or a number of group members, or even to an entire population, like small vertebrate pest populations, such as rodents (rats), marsupials (opossum), certain avian groups (pigeon), or Lagomorpha (rabbits). Furthermore, population control techniques can be directed toward females or males, depending on their social makeup and mating system, for instance, monogamy, or polygamy, (polyandry or polygyny = one female mates with two or more males, and one male mates with two or more females, respectively). Looking at the polygyny mating system, one strategy, for example, could be to apply an antifertility drug to the dominant male, instead of treating two or more females. Of course, this can only work if the alpha male maintains its dominance, and no opportunistic second-ranked males are available for takeover. Figure 4.17 depicting an Australian Bilby.

Figure 4.17 - Bilby (*Macrotis lagotis*)

Source Jaian, R. (2016)

On a global aspect, population control methods currently applied, depending if in free-ranging, or captive animals are culling, physical separation, surgical and chemical castration, steroid hormone and non-steroid hormone based contraceptive agents, and immunocontraceptive technologies.

When focusing on pharmaceutical contraceptives, the following sections will confer, to a more elaborate extent, the types, actions and potential side effects of employed antifertility agents in male and females.

4.2.1 Overview Contraceptive Methods and their principal targets

Contraceptive methods employed today may act at any given point of the reproductive process in males and females. They might interfere with the synthesis of reproductive hormones from the hypothalamus, the pituitary, and from the gonads; hinder gametogenesis; interfere with follicular development and ovulation; block sperm fecundation at the ovum's zona pellucida; impede the transport of the ovum, sperm, or zygote; prevent implantation of the ovum; or obstruct sperm motility, among others (MCDONALD, 2003)

As a generalized concept on pharmacokinetics, synthetic hormones are, most often, more potent, longer acting, and much slower metabolized and inactivated than endogenous hormones. Furthermore, regarding contraception, blood hormone concentrations are determined by the rate of hormone agent absorption and their metabolism, as well as the capacity to bind to plasma (lipophilic steroids transport) proteins. The concentration of free, or unbound hormones is what determines the drug's capacity for biological actions, who's effectiveness is proportional to the receptor affinity, once they steroid hormones diffused through the cell membrane, and a ligand-receptor bond is established within the organ's target cell, such as at the hypothalamus, pituitary, gonads and in females, the uterus and mammary glands (CHATTERTON, JR, 2009).

Now, ligand-receptor binding affinity is not the only mechanism that determines the biological efficiency of a hormone, equally important are the receptor's responses, like it's transactivation to initiate the DNA machinery. A ligand-

receptor binding without any further actions would describe an antagonistic effect (LIU et al., 2002).

4.2.1.1 Progestins Progestational, Androgenic, Estrogenic Effects

Depending on the type of progestin, they will have either a higher or a lower affinity (effect, or selectivity) for progesterone, estrogen, or androgen receptors, resulting in various desirable, or undesirable effects.

Progestational Effects: executed on progesterone receptors (PR), and are the contraceptive targets of synthetic progestins.

Androgenic Effects: refers to the effect on adrenergic receptors (ARs) by progestins, potentially leading to undesirable effects (side, or adverse effects).

Estrogenic Effects: refers to the effects of estrogen α and β receptors (ER), potentially leading to undesirable effects (side, or adverse effects). (MCDONALD, 2003; DAVTYAN, 2012)

4.2.1.2 Generations of Progestins

A number of new steroid hormones have been synthesized over the last two decades, designed to have next to none androgenic or estrogenic effects while very closely mimicking the activity of physiological hormones. Where they mainly differ are in their potency, type, and severity of side effects (SITRUK-WARE, 2006).

Hormone products are also divided into monophasic (administration of the same dose each day), and multiphasic (dose intake varies daily).

Progestin is the key active agent in most contraceptive products, there are about 19 progestins, and most of these synthetic progestins, are derivatives of testosterone, also termed 19-nortestosterone derivatives, except fourth generation progestin drospirenone, which is derived from 17 α -spiro lactone.

As an indicator of a hormone contraceptive's "technology" age, mainly applied to the dosage and/or molecular makeup of a progestin, with, or without the addition of an ethinyl estradiol, they are classified into "generations", the higher the generation number the newer the technology of the agent(s):

- First generation (estrane family) containing progestins like noretynodrel, norethisterone, norethisterone acetate, or etynodiol acetate (and $\geq 50 \mu\text{g}$ ethinyl estradiol).
- Second generation (gonane family) would identify the progestin ingredients as norgestrel, levonorgestrel, norethisterone, norethisterone acetate, etynodiol acetate, and $< 50 \mu\text{g}$ ethinylestradiol.
- Third generation (also gonane family) would have either desogestrel, gestodene, or norgestimate, and $\leq 20\mu\text{g}$ Ethinyl estradiol.
- Fourth generation may contain drospirenone, dienogest, or nomegestrol acetate (17 α -spiro lactone derived) and $\leq 20\mu\text{g}$ Ethinyl estradiol

Figure 4.18 showing a condom package as a metaphor for wildlife conception.

Figure 4.18 - Wildlife Contraception

Source: Douglas Fox (2008)

4.2.2 Overview of Contraceptive Methods used in Wildlife Population Control

In summary

Contraceptive agent's primary action is to interfere at any given level of the reproductive process. For instance, by either inhibiting hormone secretion, disrupting follicular development and gametogenesis, blocking sperm motility, or sperm-binding at the ovum's surface, hinder the implantation of the zygote, or by mechanically obstructing the passageway for the sperms toward the uterus and oviducts, by increasing the mucous secretion and endometrial swelling. The same mechanism that provokes these desired effects, simultaneously, might cause undesirable, secondary (pathological) effects.

Figure 4.19 showing thousands of rabbits crossing open areas during the 1997 "Rabbit plague" in Australia.

Figure 4.19 - Rabbit plague, Australia 1997

Source: Unknown photographer; <http://www.dailytelegraph.com.au/news/bunnies-by-the-billions-thanks-to-one-gunloving-englishman/news-story/5faae61840b34527e255bce656c4bd60>

Table 4.10 - Contraceptive Methods Overview, Biological actions, Pro, Contra, & side effects, Authors

Method/Type	Technique/Agents	Principal contraceptive biological actions	Pro	Contra & side effects	Authors
Surgical	Irreversible				
Castration Male (neutering)	Gonadectomy Orchiectomy: removal of the testis - permanent	Ceased gametogenesis Ceased steroidogenesis	100% effective;	Permanent loss of genetic information; behavioral and hormonal changes; Irreversible; Expensive; Specialized skill required; Problematic application and logistics in the field; Weight gain Weight gain associated disease	(GARDE et al., HOWE, 2006; TOBIAS; JOHNSON, 2012)
Male	Vasectomy: vas deferens are severed, tied and sealed,	Preventing sperm passage	100% effective; No behavioral and hormonal changes; considered reversible (questionable)	Expensive; Specialized skill required; Problematic application and logistics in the field	(GARDE et al., ; DEMATTEO; SILBER; PORTON, 2006; HOWE, 2006; TOBIAS; JOHNSON, 2012; EGBETADE; GADSBY, 2014)
Female (spaying)	Ovariohysterectomy, (oopho-salpingo-hysterectomy): (removal of the entire internal reproductive organs (ovaries, salpinges, uterus);	Ceased gametogenesis Ceased steroidogenesis	100% effective;	Permanent loss of genetic information; Expensive; Irreversible; Specialized skill required; Problematic application and logistics in the field Weight gain Weight gain associated disease	(GARDE et al., ; HOWE, 2006; TOBIAS; JOHNSON, 2012)
Female	Tubal Ligation: separation/tying of fallopian tubes	Prevents passage of the oocyte	100% effective; No behavioral and hormonal changes; considered reversible	Expensive; Specialized skill required; Problematic application and logistics in the field	(GARDE et al., ; HOWE, 2006; TOBIAS; JOHNSON, 2012)

Table 4.10 - Contraceptive Methods Overview, Biological actions, Pro, Contra, & side effects, Authors

Method/Type	Technique/Agents	Principal contraceptive biological actions	Pro	Contra & side effects	Authors
	Reversible				
Physical separation	Certainly the oldest method, where females and males maintained separated by barriers, without any possible contact, besides visual and olfactory	n/a	Effective; Inexpensive (if space avail.) No special skills needed; Little risks Reversible	Space requirements Psychological impacts Expensive (if no space avail.)	(ASA; PORTON, 2005a)
Castration	Examples: Zinc glutamate intratesticular treatment	Provokes severe testicular changes: fibrosis\ complete degeneration of the seminiferous epithelium shrinkage of the tubule, sperm stasis	Fast technique; low costs treatment; no special skills requested	Irreversible Permanent loss of genetic information; behavioral and hormonal changes; Wildlife? Environmental Impacts?	(JENSEN et al., 2010; BRITO et al., 2011; YODER et al., 2011; FAGUNDES; OLIVEIRA; TENORIO, 2014)
Chemical					
Male	phosphodiesterase (PDE) 3 inhibitor ORG 9935 (Feed bait)	Ovulation of a non-fertilizable, immature oocyte No affect on the development or rupture of the follicle Subsequent development of a functional corpus luteum	Reversible Possible to use in bait/feed Normal menstrual cycles		
Female	20,25-Dia-zacholesterol (Feed bait)	Believed to not affect pregnant animals inhibits the conversion of desmosterol to cholesterol, which is needed for steroidogenesis, hence, indirectly inhibits reproduction	Contrast to hormonal agents, PDE3 inhibitor prevents final meiotic maturation of the oocyte without affecting ovulation and normal function of the CL Male & female application	Behavioral and hormonal changes	

Obs.: The term "chemical" stands here for agents with toxic effects on tissues and organs, other than hormones, synthetic hormones, and immunocontraceptive agents.

Table 4.10 - Contraceptive Methods Overview, Biological actions, Pro, Contra, & side effects, Authors

Method/Type	Technique/Agents	Principal contraceptive biological actions	Pro	Contra & side effects	Authors
Mechanical	Reversible				
Obstruction Male Vas plug	Vas-occlusive contraception Injected silicone into the vas deferens forms a barrier once hardened, thus, preventing sperm passage: azoospermia	n/a	No behavioral changes No hormonal changes Long-term effect?	Questionable effectiveness Questionable long-term effect? Specialized skills required May provoke lesions/scar tissues, impedes reversibility Inflammation at injection site	(ASA; PORTON, 2005a; PATTON; JÖCHLE; PENFOLD, 2007)
Female Intrauterine Device (IUD)	Medicated or non-medicated copper IUD compromises sperm motility Hormone prepared IUD is more effective	If medicated (e.g. progestins) see associated progestin effects	Reversible Long-term effects Low costs	Requires special skills for placing IUD May cause irritation and lesions, pyometra, uterine perforation	(FORDYCE et al., 2001; KILLIAN; RHYAN; THAIN, 2006; KILLIAN et al., 2008; MALCOLM et al., 2010)
Female Sponge	Treated with spermicide More common in animal application with steroid analogs, like progestins Example: Releasing Intravaginal Device (PRID), serving for estrous synchronization	Depending on agent, (e.g. progestins) see associated progestin effects	Reversible Low cost	Requires special training for placement	(WALSH et al., 2008)

Figure 4.20- Cattle IUD CIDR

Source: ZOETIS

Figure 4.21 - Intravaginal sponge

Source: INTA Balcarce, Arg.

Figure 4.22 - A. styrene maleic anhydride polymer is injected into the vas deferens, partial occlusion of the vas, B. Intra-vas plugs.

Source: Kanakis (2015)

Table 4.10 - Contraceptive Methods Overview, Biological actions, Pro, Contra, & side effects, Authors

Method/Type	Technique/Agents	Principal contraceptive biological actions	Pro	Contra & side effects	Authors
Hormone based	Reversible	Steroid hormonal agents interfere with the reproductive endocrine system, either in a stimulating or in an inhibiting fashion, binding to receptors within the hypothalamus and/or pituitary glands, resulting in the suppression of gonadal activities.	Reversible Different forms of applications Widely available Low cost Easy application Highly effective (species depending) Adequate for Wildlife? Field application?	In general effects depending on species, dose and exposure time: Hormonal change Behavioral change, might cause severe side effects. Also depending on generation (age) of steroid agent, or if used in combination with other hormones	
Steroid hormones	<p>Progestin based examples: Medroxyprogesterone acetate (MPA) Levonorgestrel (LNG); Norethindrone acetate (NET); Medroxyprogesterone acetate (MPA); *Norgestimate (deacetyl form) (NGM); *Desogestrel (3-ketodesogestrel form) (DGL); *Gestodene (GDN); Dienogest (DNG); *Drospirenone (DRSP); Nestorone (NES); Nomegestrol acetate (NOMAc); Timegestone (TMG); 19-nor progestogen</p>	<p>In female: Promotes thickening of cervical mucus, which acts as a barrier, Inhibits sperm movement past the cervix; leaves uterus flaccid Inhibits follicular maturation and ovulation by inhibiting secretion of gonadotropins (prevention of the ovulatory LH surge) Alteration of the uterine endometrium, potentially impeding embryo implantation; Slows egg transport; Interfering with sperm transport by lowering smooth muscle contraction in the female tract In male: Inhibition of testosterone secretion</p>	<p>Reversible As implant allows for long-term effects</p> <p>*Newer generations of progestins, like:</p> <ul style="list-style-type: none"> • Gestodene • Desogestrel • Norgestimate <p>are more receptor selective, have antimineralocorticoid and antiandrogenic activity (less to no side effects) while inhibiting ovulation and endometrial hypertrophy.</p>	<p>Effect are species, dose, and time of exposure depending:</p> <p>* at the same token, long-term treatments (exposure) might lead to serious adverse effects</p> <p>Suppresses local immune system, leave organs predisposed to infections (endometritis, pyometra,)</p> <p>As a growth promoter, may cause endometrial hyperplasia, stimulate tumor/cancer growth</p> <p>Hydrometra</p> <p>Might provoke other metabolic diseases (e.g. diabetes)</p> <p>Species are known to have heightened sensitivity to progestins:</p> <p>Carnivores (especially felids)</p>	(NAVE et al., 2000, 2002a, 2002b; CHITTICK et al., 2001; LOOPER et al., 2001; PATTON et al., 2001; WOOD; BALLOU; HOULE, 2001; MUNSON et al., 2002; RAPHAEL et al., 2003; ASA; PORTON, 2005a; LORETTI et al., 2005; SITRUK-WARE, 2006; ASA; PORTON; JUNGE, 2007; CHUEI et al., 2007; HALL-WOODS et al., 2007; PATTON; JÖCHLE; PENFOLD, 2007; PENFOLD et al., 2007; WHEATON et al., 2007, 2011; COULSON et al., 2008; MORESCO; MUNSON; GARDNER, 2009; CRAWFORD; BOULET; DREA, 2010; HYNES et al., 2010, 2011; MCCAIN et al., 2010; LIU et al., 2012)

Table 4.10 - Contraceptive Methods Overview, Biological actions, Pro, Contra, & side effects, Authors

Method/Type	Technique/Agents	Principal contraceptive biological actions	Pro	Contra & side effects	Authors
Hormone based	Reversible	Steroid hormonal agents interfere with the reproductive endocrine system, either in a stimulating or an inhibiting fashion, binding to receptors within the hypothalamus and/or pituitary glands, resulting in the suppression of gonadal activities.	Reversible Different forms of applications Widely available Low cost Easy application Highly effective (species depending) Adequate for Wildlife? Field application?	In general effects depending on species, dose and exposure time: Hormonal change Behavioral change, might cause severe side effects. Also depending on generation (age) of steroid agent, or if used in combination with other hormones	
Steroid hormones	Estrogen based examples: Diethylstilbestrol (DES) Mestranol Estradiol benzoate Stradiol cypionate	Inhibits the release of FSH, which prevents follicular development Blocks implantation	Effective "Estrogen only" contraceptive preparations are basically no longer available	Due to the higher dosage needed to achieve contraception, associated with serious health risks: Provokes uterine disease Over stimulating of the uterine lining Bone marrow suppression Aplastic anemia ovarian tumors	(ASA; PORTON, 2005a; CARLSON; GESE, 2010)
Steroid Hormones	Androgens based: Testosterone Miborelone Methyltestosterone Fluoxymestherone	Inhibits LH secretion (via negative feedback due to high plasma concentrations of testosterone)	Effective (use its discouraged in wildlife)	Masculinization in females Osteoporosis Tumor/cancer growth Stimulates aggressive behavior Organ toxicity	(ASA; PORTON, 2005a; MUNSON, 2006)

Method/Type	Technique/Agents	Principal contraceptive biological actions	Pro	Contra & side effects	Authors
Combination of Hormones	Reversible				
Steroid hormones synergistic effects	Progestin & Estrogen combination based	Inhibit LH surge, which in turn, inhibits ovulation Testicular atrophy	Suppose to have fewer side effects (species depending)	Number of studies indicating severe side effects in human females, and in carnivores this synergistic effect augments the potential risk of severe side effects	(ASA; PORTON, 2005b)

Table 4.10 - Contraceptive Methods Overview, Biological actions, Pro, Contra, & side effects, Authors

Method/Type	Technique/Agents	Principal contraceptive biological actions	Pro	Contra & side effects	Authors
Non-steroid Hormone base	Reversible				
Protein, or peptide hormones	GnRH agonist Deslorelin Leuprolide	<p>Principle mechanism is the suppression of GnRH release from the Hypothalamus, with subsequent suppression of LH and FSH release, consequently, ceased gametogenesis.</p> <p>Mode of action of GnRH analogs is divided into two distinct phases: The acute phase: highly stimulated LH and FSH, may cause estrus and ovulation, depending on the exact time of administration, but can be counter-measured by the application of progestin, together with the GnRH implant, during one week. The second phase, due to chronic exposure, starts with the "down-regulating of GnRH receptors at the pituitary gland.</p>	<p>Highly effective due to high affinity to GnRH receptors</p> <p>Reversible</p> <p>Cost benefits</p> <p>Long-term effect</p> <p>Successful use in different species</p> <p><i>In situ</i> and captivity</p> <p>Believed to have little significant adverse effects</p>	In wildlife species still lacking sufficient data on its long-term toxicity;	(BERTSCHINGER et al., 2001, 2006, 2008; BAKER et al., 2002, 2004; BERTSCHINGER; TRIGG, 2002; HERBERT et al., 2004, 2005; OLIVEIRA et al., 2004; PATTON et al., 2006; CONNER et al., 2007; EYMANN et al., 2007; ALLEN et al., 2008; CARLSON; GESE, 2009; LOHR et al., 2009; KAUFFOLD et al., 2010; ASA; BOUTELLE; BAUMAN, 2012; MELVILLE et al., 2012; POWERS et al., 2012; ROSENFELD, 2012; LARSON et al., 2013; PETRITZ; GUZMAN, 2013; DOUGHTY et al., 2014; MORESCO; DADONE, 2014; GOERCKE-PESCH; GROEGER; WEHREND, 2015)

Table 4.10 - Contraceptive Methods Overview, Biological actions, Pro, Contra, & side effects, Authors

Method/Type	Technique/Agents	Principal contraceptive biological actions	Pro	Contra & side effects	Authors
Non-steroid Hormone base	Reversible				
Neuro/amide peptide hormones	Melatonin and GnIH (Gonadotropin-Inhibitory Hormone)	<p>Photoperiod is transduced into a melatonin signal whose duration is inversely proportional to day length.</p> <p>Increase on Melatonin effects the release of GnIH, which in turn suppresses GnRH release</p> <p>Melatonin administration inhibits reproductive activities</p>	Novel potential application as contraceptive agents	n/a	(TSUTSUI et al., 2012; NUÑEZ FAVRE et al., 2014; KRIEGSFELD et al., 2015)
Neuro/amide peptide hormones	Kisspeptin				(KAUFFMAN; CLIFTON; STEINER, 2007; MEAD et al., 2007; OAKLEY; CLIFTON; STEINER, 2009; TASSIGNY; COLLEDGE, 2010; CALLEY et al., 2014; CLARKE; DHILLO; JAYASENA, 2015)

Table 4.10 - Contraceptive Methods Overview, Biological actions, Pro, Contra, & side effects, Authors

Method/Type	Technique/Agents	Principal contraceptive biological actions	Pro	Contra & side effects	Authors
Immuno-contraceptives	Reversible Various	Based on the same concept of vaccines for disease prevention, whereby an animal is inoculated with either dead or attenuated pathogens or other toxin-like antigens, provoking an immune reaction, producing antibodies to a) combat the intruders, and b) increase resistance to potential future exposure			(KILLIAN; MILLER, 2000; CURTIS et al., 2002; MAGIAFOGLOU et al., 2003; NAZ et al., 2005; COOPER; LARSEN, 2006; KILLIAN; RHYAN; THAIN, 2006; HARDY; BRAID, 2007; GRAY et al., 2010; CROSS et al., 2011; KIRKPATRICK, 2011; NAZ, 2011; MILLER; FAGERSTONE; ECKERY, 2013; KAUR et al., 2014; NAZ; SAVER, 2015)
Two most used and studied immune-contraceptives: GnRH and ZP vaccine	Gonadotropin-Release Hormone Vaccine (GnRH)	GnRH vaccine, inhibiting the secretion of LH and FSH gonadotropins, and	Effective in many species Reversible Inexpensive Applicable in wildlife and the field Long-term effects Ease of application Little known side effects	Risk of species-specific side effects Lack of knowledge on toxicity impacts	(HENNESSY, ; SOMGIRD et al., ; MILLER; JOHNS; KILLIAN, 2000a; MILLER; RHYAN; KILLIAN, 2003; MILLER; RHYAN; DREW, 2004; KILLIAN et al., 2006, 2009; MILLER, 2006; CURTIS et al., 2008; KEMP; MILLER, 2008; MASSEI et al., 2008, 2012, 2015; MILLER et al., 2008a, 2008b; GIONFRIDDO et al., 2009, 2011; SAENZ et al., 2009; WU et al., 2009; CAMPBELL et al., 2010; YODER; MILLER, 2010; COWAN et al., 2011; LEVY et al., 2011; POWERS et al., 2011; SNAPE; HINDS; MILLER, 2011; BOEDEKER et al., 2012; DONOVAN et al., 2013; SCHULMAN et al., 2013; KRAUSE et al., 2014; QUY et al., 2014; RANSOM et al., 2014; PHRALUK et al., 2015)

Source: Rosenfield (2016)

Table 4.10 - Contraceptive Methods Overview, Biological actions, Pro, Contra, & side effects, Authors

Method/Type	Technique/Agents	Principal contraceptive biological actions	Pro	Contra & side effects	Authors
Immuno-contraceptives	Zona Pellucida Vaccine (ZP)	ZP vaccines (most common pZP (porcine Zona Pellucida); which blocks sperm receptors on the surface of the oocyte.	<p>Effective in many species</p> <p>Reversible</p> <p>Inexpensive</p> <p>Applicable in wildlife and the field</p> <p>Long-term effects</p> <p>Ease of application</p> <p>Little known side effects</p>		(BARBER; FAYRER-HOSKEN, 2000; MILLER; JOHNS; KILLIAN, 2000b; RUDOLPH; PORTER; UNDERWOOD, 2000; MILLER et al., 2001, 2009; FRAKER et al., 2002; KIRKPATRICK; TURNER, 2002, 2007, 2008; MILLER; KILLIAN, 2002; DEIGERT et al., 2003; LYDA; HALL; KIRKPATRICK, 2005; MUNSON et al., 2005; DELSINK; ALTENA, 2006, 2007; HERNANDEZ et al., 2006; CURTIS et al., 2007; LANE et al., 2007; LOCKE et al., 2007; PERDOCK; DE BOER; STOUT, 2007; TURNER et al., 2007; RUTBERG; NAUGLE, 2008; KIRKPATRICK et al., 2009; KITCHENER et al., 2009; BERTSCHINGER; DE; ALTENA, 2010, 2010; DRUCE; MACKEY; SLOTOW, 2011; GUPTA et al., 2011; KIRKPATRICK; LYDA; FRANK, 2011; AHLERS et al., 2012; KIRKPATRICK; RUTBERG, 2012; RUTBERG et al., 2013; MASK et al., 2015)
	EPPIN (epididymis protease inhibitor) vaccine	Several proteins present on the spermatozoa are important for the Fertilization process, e.g. sperm maturation, capacitation, and motility. Blocking these proteins with antibodies are known to impair fertilization process.	No available for wildlife field studies		(SPINASANTA, ; KARANDE, 2004; O'RAND; WIDGREN, 2004; WANG et al., 2007; LI et al., 2009)

4.2.3 "The new kids on the block" GnIH & Kisspeptin

Besides the latest, and rather successful development of immunocontraception method, more discoveries of novel contraceptive methods continue to appear and amaze.

The reasons behind seem obvious, as newer technologies become available, allowing deeper insights into the molecular world, and biological processes, anatomy and, in this discipline, reproductive physiology, all this new gained knowledge opens many new doors for discoveries. And just when you thought that this must be it, there possibility can't be any smaller molecules participating in the control of the reproductive processes, a new, never heard of, agent is being discovered.

One of the latest top two hot contestants, with great potential as contraceptive agents, must be the discovery of two peptides, quite specific, and yet, total opposite effects on the hypothalamic-pituitary-gonadal axis.

Gonadotropin-inhibitory hormone (GnIH) and Kisspeptin hormone.

4.2.4 GnIH (Gonadotropin-Inhibitory Hormone)

In the year 2000, GnIH was discovered in Japanese quails (TSUTSUI et al., 2010), then believed only to exist in avian species.

In their studies, GnIH receptors were identified in the pituitary gland, as well as in various other brain regions, such as the hypothalamus, suggesting that GnIH (depending on seasonality), directly controls, in an inhibitory fashion, the secretion of LH and FSH from the pituitary gland, and suspecting its controlling influence on GnRH secretion. Since then, it has been well documented that GnIH is present in a number of photoperiodic species (TSUTSUI et al., 2013), including humans.

The key purpose of any species is to maximizing reproductive success, and free-living wildlife specifically is under constant challenge to survive, depending on food availability and ambient conditions. In his 2013 publication, Kazuyoshi Tsutsui explained that as a result, animals must have a mechanism, transducing environmental conditions into neural signals, with the purpose to drive sexual motivation and reproductive activities. Figure 4.23 depicts the impact of external stimuli on the neuroendocrinological system.

Avian and mammalian reproduction is under the influence of environmental signals, such as photoperiodic variations (light/darkness) due to seasonality and stress situations (e.g. prey/predator), causing a signal transduction (converting external signals into internal signaling) of neuroendocrine hormones, like GnIH, melatonin, and stress hormones from the adrenal cortex, such as glucocorticoids, impacting the control mechanism of reproductive processes.

Gonadotropin Inhibitory Hormone (GnIH) when secreted, exerts an inhibitory effect on the synthesis and secretion of GnRH, but also directly on the pituitary, suppressing the secretion of LH and FSH, in avian and mammalian species. Furthermore, GnIH is believed to impact the reproductive behavior negatively, by potential actions within the brain.

Depending on the species, melatonin stimulates GnIH expressions, for example in quail and rats, while in hamsters, or sheep, melatonin suppresses GnIH expression. On the other hand, stress might induce GnIH expressions in other avian and mammalian species, acting as a reproduction disruptor of GnIH in birds and mammals. GnIH may, therefore, be seen as a stress-mediated reproduction blocker (TSUTSUI et al., 2013).

4.2.4.1 Kisspeptin

Previously, only thought of as a tumor metastasis suppressant, hence, first named "metastin", and as Oklay explained (2009), now renamed: KISS for its role as a suppressor sequence (ss); the letters "KI" were appended to the prefix "SS" to form "KISS" in homage to the location of its discovery, Hershey, Pennsylvania, home of the famous "Hershey Chocolate Kiss". Once discovered and studied, laboratory tests confirmed that in genetically manipulated mice, if this gene were to be deleted, would provoke severe hypogonadotropic hypogonadism, emerging as a key stimulating regulator of GnRH secretion.

As illustrated in figure 4.24, studies have shown that kisspeptin receptors are expressed in neurons of the arcuate nucleus and the hypothalamic anteroventral periventricular nucleus, synaptically in contact with GnRH neurons, which in turn, express receptor for steroid hormones (E_2 , P_4 , and T), suggesting an interaction of kisspeptin neurons from the arcuate nucleus in the negative feedback mechanism of gonadal hormones, controlling gonadotropin hormone secretion, while kisspeptin neurons of the anteroventral periventricular nucleus are believed to promote preovulatory gonadotropin surges (OAKLEY; CLIFTON; STEINER, 2009).

Figure 4.24 - kisspeptin receptors in neurons of the arcuate nucleus and hypothalamic anteroventral periventricular nucleus, synaptically in contact with GnRH neurons,

Source: Concept Oakley (2009); Graph: Rosenfield (2016)

The identification of the mechanisms of GnRH, as well as Kisspeptin, strongly suggests potential applications as novel contraceptive methods.

4.3 A BRIEF ASPECT ON PATHOLOGY

In order to understand the basic etiological mechanism of hormone-based contraceptive agent's, a thorough understanding of the intrinsic physiology of the natural hormones is imperative, the same holds true, considering all the newer methods, such as immunocontraception, and a basic understanding of the immune system.

In the case of progesterone, and as the name implies, a pro-gestational hormone, with the purpose of continuing the preparation for, (initiated by estrogen hormone), and maintaining a possible pregnancy (MCDONALD, 2003; CUNNINGHAM; KLEIN, 2007).

Reiterating briefly, the organ that experiences the biggest metabolic/anabolic, chemical and morphological changes is the uterus. While losing its tone, the endometrial lining is going through a hyperplasia phase, especially if a blastocyst implantation occurred, also initiate the production and secretion of nutritional fluids. At the same time, the immune system suffers a suppression, as to prevent a rejection of a foreign body, the fetus. Furthermore, progesterone is needed for the final formation of the alveoli at the terminals of the mammary gland ducts, which in turn, are under the influence of estrogen, in preparing for lactation.

Besides being responsible for the uterine and mammary gland changes, progesterone also participates in the endocrinological control, via a negative feedback, of the hypothalamus, suppressing the secretion of GnRH, as well as on the pituitary gland, inhibiting LH and FSH liberation. For the purpose of contraception, synthetic progestins are simulating the same mechanisms as the endogenous progesterone:

In female: Inhibits follicular maturation and ovulation by inhibiting secretion of gonadotropins (prevention of the ovulatory LH surge); With the uterus's relaxed myometrium and a suppressed local immune system, due to the progestin effects, the uterus is rather predisposed to develop endometritis and even might progress into pyometra. Progestins also promote a thickening of the cervical mucus, becoming more viscous, which then acts as a physical barrier, together with the lower smooth muscle contractions in the female tract interferes with sperm transport.

And in male: Inhibition of testosterone secretion, which suppresses spermatogenesis (aspermato-genesis) (ASA, 2005; ASA; PORTON, 2005)

4.3.1 The Side Effects

All hormone-based contraceptive products potentially provoke adverse effects. The severity depends on the species, the individual, gender, in females if nullipara or multipara, its overall condition, and age, type of agent used, as well as if combination of agents were used, its dosage, and the duration of the treatment. As stated by Asa (2005), most commercially contraceptive products were developed and tested for safety only for human applications, even though the test are performed on lab animals, the safety approval cannot simply be extended to free-ranging wildlife, nor animals in captivity. Although hormone-based contraceptives have changed significantly in their makeup and dosage, promising safer treatments, again, for humans, they still carry the potential risk of side-effects.

One key element to consider for an agent to cause undesirable effects is based on the use of progestins together with estrogen, or progestin only, and if the dosages might provoke a total suppression of steroidogenesis and secretion as the effects of hormones do not cancel out one another but are synergistic in action (ASA, 2005).

4.4 CONCLUSION

Repeating Kirkpatrick's, et al, 2001, semi-standard suggestion, an ideal contraceptive method would be one that could offer a high rate of effectiveness (90+ %); capable of remote delivery (eliminating stress for the animal by minimizing handling); guarantee reversibility of contraceptive effects (securing genetic information); May not be of any risk to pregnant animals (provoking abortion, or interfere with the development of the fetus); Must not present any noteworthy side effects; Provide long-term infertility effects; Contraceptive agent won't pass through the food chain; Minimizes any impact on the animal's natural behavior, and its social role in the group; Last, but not least, must be acquirable for low cost, KIRKPATRICK; RUTBERG, 2001), as large volume contraceptive programs can be a huge financial burden to local governments.

Based on the analysis of this comprehensive study, it can be concluded, that, as of this moment, the most promising contraceptive method for application in wildlife, *in situ* as well as in captivity, are the immunocontraceptive vaccines, specifically, the GnRH vaccines. This statement is rooted in the analysis of the number of studies done, the number of species investigated, the results of long-term observations conducted and then weighed in against the proposed standard-like characteristics of an ideal contraceptive method by Kirkpatrick, 2001.

Predominantly, it's the products safety reports, its desirable long-term effects, allowing to be applied via long-distance, as an "one-shot vaccine", applicable in male and female alike, reversible, no risk to pregnant animals, or their fetus, no food chain risk, no significant behavioral alterations, and, relatively inexpensive.

However, it is furthermore evident, that the true safety of contraceptive methods, their toxicity impacts, short,- and long-term, especially in wildlife species, is not known. Naturally, due to the shortage and difficulties of the of research subjects for empirical studies, as well as for economic reasons, let the truth be told, Wildlife research does not enjoy the same funding interests as, for instance, commercial animal reproduction.

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5 IMMUNOCONTRACEPTION IN WILDLIFE POPULATION CONTROL? IN FACT, THE BEST ALTERNATIVE?

Abstract

Let's be candid for a moment, the "best" population control is the one where humans are not involved and only nature is in control, meaning predators taking care of prey. But for this to occur, nature has to be in equilibrium, a state that is becoming increasingly a rare reality. Leading from human caused to human controlled. And for this to be applied, the question is, what would be the best method? When it comes to the perfect method for contraception, especially when considering wildlife population control, we do want it all! First and foremost, no risk to the individual's health, including no adverse effects on a pregnant female or its fetus. Little impact on the social behavior and group dynamics. It needs to be reversible, long acting, applicable to both genders, easy to apply in free-ranging animals, "one-shot administration (no boosters), deliverable from long distances, and it must be inexpensive. Besides, it should not pass through the food chain, or become a major environmental pollutant, after an animal's death. Having said that, it seems we are getting closer to the aspired perfect contraceptive and for the time being, namely immunocontraceptive. As of 2011, immunocontraception has been successfully tested in more than 85 wildlife species, a positive achievement that is hard to be argued against.

Keywords: Wildlife population control; Immunocontraception; GnRH Vaccine; pZP Vaccine.

5.1 INTRODUCTION

Let's be candid for a moment, the "best" population control is the one where humans are not involved and only nature is in control, meaning predators taking care of prey. But for this to occur, nature has to be in equilibrium, a state that is becoming increasingly a rare reality. Leading from human caused to human controlled. And for this to happen, the question is, what would be the best method?

Well, when it comes to the perfect method for contraception, especially when considering wildlife population control, we do want it all! First and foremost, no risk to the individual's health! Including no adverse effects on a pregnant female or its fetus. Little impact on the social behavior and group dynamics. Also, it needs to be reversible, of course, we don't know when we might need the population to increase again, or must reintroduce a rare species back into nature, or simply, the necessity to maintain genetic diversity within a given population and by exchange of gametes between zoos, to avoid inbreeding. It should not pass through the food chain, or become a major environmental pollutant, after an animal's death. Moving right on, it must allow for the application in the field, from a distance and only one time (no need for "boosters", or any form of continuous application). While offering long-term effects, meaning, years of infertility efficacy, applicable in both genders and by all means, it should be inexpensive, making it suitable to be employed in wildlife population management, on a larger scale (KIRKPATRICK; RUTBERG, 2001).

At the same token, any manipulation to "control" population will have a negative impact, ranging from an acceptable and less favorable to severe detrimental, doesn't matter how close to perfect a contraceptive method might be, if not physically, than psychologically. Nevertheless, in nowadays human-wildlife conflict-stricken times, there is always the lesser iniquity to be considered.

Having said that, it seems we are getting closer to the aspired perfect contraceptive, and for the time being, namely immunocontraceptive. As of 2011, immunocontraception has been successfully tested in more than 85 wildlife species (KIRKPATRICK; LYDA; FRANK, 2011).

What is so intriguing to this contraceptive method? For one, it works and has been validated for many species and studies on new species is steadily growing. It works so well, that we can consider it a long-term contraceptive, depending on the

species treated, 1 - 5 years. It can be administrated by darting, so in-field use and from a distance is a proven technique. In many species, it does not require a booster to provoke a sufficient immune reaction. Therefore, the "one-shot" request is satisfied as well. Its cost per application is reasonable, considering a possible future increase in demand, might even lower the price, as a consequent of streamlined fabrication. Now, last, but not least, besides some injection side inflammatory reaction, and some formation of tumors at the same location, which does not represent any further health risk to the animal, no long-term adverse effects have been reported, assuring the most important request to be satisfied (FRAKER et al., 2002; TURNER et al., 2007; KIRKPATRICK et al., 2009; MILLER et al., 2009; KIRKPATRICK; LYDA; FRANK, 2011; RUTBERG et al., 2013).

5.2 OVERVIEW OF THE IMMUNE SYSTEM FROM THE ASPECT OF IMMUNOCONTRACEPTION

One of the most important systems when it come to maintaining homeostasis in a living organism and, perhaps, one of the most complex systems to study, the immune system. It is a perfect machine to fight infections and foreign materials and at times, even too perfect for its own good, which still leaves us with many unanswered questions on the issue of auto-immune diseases, and yet, a concept that is part of the mechanism of immunocontraceptive.

5.2.1 The fundamental elements of the immune system in mammals

Organs and Vessels of the immune system, are made up of the primary and secondary lymphoid organs. Primary: such as the bone marrow, responsible for the production erythrocytes and B cells (by hematopoiesis). However, this process is not equal in all species, for instance in birds, the lymphoid organ is termed the bursa of Fabricius, the place of B cell maturation, and is associated with the bird's stomach. In cattle, sheep and rabbits, the primary lymphoid tissue for B cell maturation, differentiation, and proliferation is first active, during gestation, in the fetal spleen, and later resumes this function as the ileal Peyer's patch, as part of the intestines. T

lymphocytes, or T-cell, originate in the thymus, hence "T" cell, another primary lymphoid organ, that which age declines in function. T cells also must undergo a differentiation process, meaning these cells, once produced, still need to mature and capacitate, or turning immunocompetent, able to recognize and act on antigens (non-self proteins), or altered self-cells (pathogen infected cells). Some T cells represent receptors that can recognize antigen-MHC complexes.

Secondary immune organs, or lymphatic organs, are the locations where the defense cells perform their biological actions, the lymph nodes, distributed throughout the entire organism, some of them even palpable. Other immune organs include the spleen, tonsils and as well as other immune specialized tissues, like the mucous membranes, lining the regions of the organisms orifices, such as the oral/nasal, digestive, respiratory, and urogenital. Like the colon, being that the lumen is considered an external environment and being the major entry side, and host for a multitude of pathogens and antigens, requires continuous protection. Similar to sex organs and the reproductive tract, the skin, they all having physical and chemical barriers, fortified with residential leukocytes and dendritic (Langerhans) cells, responsible for capturing invading pathogens. And tertiary lymphoid tissues, which are on standby, becoming active during an inflammatory response, by recruiting lymphoid cell when initiated.

The lymphatic system is a network of vessels throughout defense cells are constantly circulating, but also serves as a collector of interstitial fluids, returning these to the circulatory system of the blood (DELVES; ROITT, 2000; TIZARD, 2009).

Furthermore, the immune system's response can be divided into an innate (immediate) immunity and an adaptive immunity (delayed and specialized response):

Innate immune cells are capable of recognizing pathogenic microorganisms, like viruses, bacteria, protozoa, fungi, as well as other non-infectious invasions (any antigen molecule, that can induce an immune response). Innate immune cells are neutrophils, eosinophils, basophils, mast cells, monocytes, dendritic cells, and macrophages, each with specialized functions, responding to broad, or unspecific threats as the first responder, acting quickly to a general threat, while at the same time, activating the adaptive immunity.

The adaptive immune cells include the T-cells and the B-cells, each one highly specialized and equipped with special receptors, different to the innate cells, allowing them to respond to specific signals, rather than broad ones, with the capability to "adopt" to ever changing ambient situations, and pathogen mutations. The adaptive immune system also serves as the disease agent memory, or databank, facilitating its response in speed and intensity to the same pathogens, should they invade in the future. T-cells, constantly circulating throughout the organism, are responsible for the recognition of non-self, or altered cells. Once such a cell has been recognized, and the T cell attached to this non-self intruder, T cells transform into T effector and helper cells, triggering and regulating a whole cascade of immune responses, like signaling and recruiting other immune cells into action, including their activation as T killer cells, destroying and eliminating the pathogens, or pathogen-infected cells, while B-cells produce antibodies with specificity for antigens. The activation of antibody releasing B cells depends on the antigen, once bond to one of the very many B cell's surface receptors, will trigger the B cell's differentiation into a plasma cell, capable of liberating specific antibodies.

And then there are the APCs, the antigen presenting cells, responsible for representing captured antigens to antigen-sensitive lymphocytes (DELVES; ROITT, 2000; TIZARD, 2009).

5.2.1.1 Antibodies

Antibodies also called immunoglobulin (Ig), are monomer and Y-shaped, glycoprotein molecules, circulating through the blood and transported into the interstitial space, serving in identifying and marking pathogenic microorganisms and antigen molecules for destruction. They consist of polypeptide chains with two heavy chain regions and two light chain regions, all held together by covalent disulfide bonds. The light chain regions are subdivided into constant domains and variable domains, the later responsible for antigen recognition and binding. The variable domains are further divided into two sections, a framework region, and a hypervariable region. The hypervariable region is capable of diverse reformation, allowing for antigen-specific recognition, potentially protecting against numerous antigens, see figure 5.25 and figure 5.26.

5.2.1.2 Antibody Form and Types

Following a brief overview on the basic structures, figure 5.25, types of antibodies, figure 5.26, and functional description, in table 5.11

Figure 5.25 - Antibody - antigen binding site

Source: Fvasconcellos (2007)

Figure 5.26 - Antibody Isotypes

Source: Martin Brändli (2006)

Table 5.11 - Antibody Isotypes

Name	Description (in mammals)
IgA	Resident to specialized tissues, like the mucous membranes lining the regions the oral/nasal, digestive, respiratory, and urogenital regions
IgD	Functions mainly as an antigen receptor on B cells that have not been exposed to antigens.[15] It has been shown to activate basophils and mast cells to produce antimicrobial factors
IgE	Binds to allergens and triggers histamine release from mast cells and basophils, and is involved in allergy. Also protects against parasitic worms
IgG	In its four forms, provides the majority of antibody-based immunity against invading pathogens.[5] The only antibody capable of crossing the placenta to give passive immunity to the fetus.
IgM	Expressed on the surface of B cells (monomer) and in a secreted form (pentamer) with very high avidity. Eliminates pathogens in the early stages of B cell-mediated (humoral) immunity before there is sufficient IgG
Name	Description (non-mammals)
IgY	(Birds and reptiles),
IgW	(Fish, like in sharks)

Source: Rosenfield (2016)

5.3 VACCINATION

Vaccination, or immunization, stands for one of the most important discoveries when it comes to the protection of human and animal health. Everybody is familiar with the concept of vaccination in association with disease prevention.

The principle lies within the organism's defense system, its capability to recognize and destroy foreign invaders, pathogenic or antigens (non-self proteins), whereby a vaccination deliberately provokes an immune response by inoculating the organism with a specific non-pathogenic, but highly immunogenic (strong immune response) antigens.

With the objective to prepare the organism's defense system for a potential exposure to a pathogen, which is accomplished by a "pre-exposure" to possible different vaccine types, like killed microorganisms, or attenuated (inactivated), DNA recombinant, antigen subunits, attenuated, or conjugated, all triggering an immune response, without causing a real infection, with the objective to create long-term immune memory to a specific pathogen. This will allow a faster and more efficient response, should a future contact occur, preventing a severe infection.

For an optimized immunization effect, a vaccine should be able to stimulate both, the innate, as well as the adaptive immune response (TIZARD, 2009; MOSER; LEO, 2010).

The following graph on page 120, figure 5.27, depicts a cascade immune response after an application of an vaccine with an adjuvant component.

5.3.1 Vaccine and Adjuvant Mechanism

Figure 5.27 - Vaccine/Adjuvant Immune Response Mechanism

Source: InvivoGen, Vaccine Adjuvants - Review (2011)

Legend: Upon activation by cytokines, B cells differentiate into memory B cells (long-lived antigen-specific B cells) or plasma cells (effector B cells that secrete large quantities of antibodies). Most antigens activate B cells using activated T helper (Th) cells, primarily Th1 and Th2 cells.

5.3.2 Adjuvants

Adjuvants, (lat. *adiucare*: to aid), are an important additives to vaccines, additions, improving the innate immune response (antibody and T lymphocyte), by increasing the inflammatory response, as it would happen if real infection would take place, which in turn, is important for an optimize adaptive immune response, although, adjuvants are known to have their own set of potential health risks (LYDA; HALL; KIRKPATRICK, 2005; MUNSON et al., 2005).

Adjuvants may include organic components which may be liposomes, (a spherical vesicle with a minimum of one lipid bilayer, used as a vehicle for pharmaceutical drugs), or lipopolysaccharides (LPS), part of endotoxins, other antigens, parts of a bacterial cell walls, RNAs, or DNA strands, or alum (hydrated potassium aluminium sulfate), and emulsions (oil-in-water/water-in-oil), for instance Freund's Incomplete Adjuvant (IFA).

Some adjuvants also function as a delivery system, causing the formation of depots at the injections site that traps the antigens, and allowing only a slow release of the vaccine components, maintaining the immunogenic stimulus, thereby enhancing the immune response, increasing recruitment and activation of antigen presenting cells (APCs). A concept that permits the one-shot, long-term effect of a vaccine (ASA; PORTON, 2005; SITRUK-WARE, 2006; MILLER et al., 2008; REED; ORR; FOX, 2013)

5.3.2.1 Immune Response with and without Adjuvants

The following graph. figure 5.28, shows the comparison of the immune response, depending on the vaccine given without, or if administered with an adjuvant.

Figure 5.28 - The immune response to vaccination with and without adjuvant

Source: Alberta Di Pasquale (2015)

Legend: Highly purified vaccine components frequently lack PAMPs, which means that the initial innate immune response is not activated such that an effective downstream adaptive response occurs. It is thought that the primary mechanism of action of adjuvants is on the innate immune response (Figure 4). Adjuvants can act like PAMPs, triggering the innate immune response through a variety of mechanisms, to identify the vaccine components as a "threat," with activation and maturation of APCs and initiation of downstream adaptive immune activities

Obs.: "PAMPs" (Pathogen-associated molecular patterns), are molecules associated with groups of pathogens, which are recognized by cells of the innate immune system.

5.4 MODE OF ACTION OF IMMUNOCONTRACEPTIVES

What is Immunocontraception? Breaking down the term, we have:

Immunity = from the immune system, an organism's defense mechanisms, and

Contraception = blocking pregnancy, hence,

Immunocontraception = the use of the body's own defense mechanisms to block pregnancy

The meaning of "self" and "non-self" in immunology

The immune system's first mechanism of defense is recognition and identifying the organism's own protein, carbohydrate, and lipid-based structures, thus the meaning "self", and this self-recognition is fundamental to the organisms survival. If one's immune system failed to recognize its own, the defense system would be activated and attack its self, also referred to as an auto-immune disease.

Any organic structure that is being identified as "non-self," e.g. invading pathogens (bacteria, viruses, protozoa) or other antigens, like foreign non-infectious allergens, will be marked by antibodies for destruction, isolation, and if possible, elimination.

Anti-fertility vaccines are directed against the "self" proteins, involved throughout all reproductive processes, to which the own organism's defense system, normally, would remain immunologically tolerant, are now, made "non-self," by coupling a, to the organism foreign, protein. This, now self-foreign protein is being identified and marked as "non-self," triggering the immune system to produce antibodies against its own reproductive proteins and hormones, inducing contraceptive effects, and rendering the animal infertile, for as long as a sufficiently high concentration (titer) of antibodies is present.

Currently, there are two principal target for immunocontraception that were extensively tested and are being successfully employed for wildlife population control, one aiming for the ovum's *zona pellucida*, and one for GnRH receptors in the hypothalamus (BARBER; FAYRER-HOSKEN, 2000; DELVES; ROITT, 2000; TIZARD, 2009; MOSER; LEO, 2010; CROY, 2014)

5.4.1 PZP Immunocontraceptive Vaccine Mechanism

The mammalian ovum is surrounded by a non-cellular membrane, consisting of several glycoproteins, known as the zona pellucida (ZP). The glycoprotein ZP3 had been identified as the sperm-binding receptor (which permits the attachment of sperm to the ovum, to continue the next step of the fertilization process, leading to the diffusion of the sperm into the oocyte, creating a zygote).

The pZP vaccine is produced by creating a zona pellucidae antigen from porcine oocytes. Once the female is inoculated with the pZP vaccine, her immune system will respond by producing antibodies against the pig's oocyte antigen. The very same antibodies also bind to the sperm receptors on the ovum's surface, which will cause a distortion of the egg's structure, thereby blocking sperms from attaching, rendering the egg infertile without any other side effects, or behavioral impacts, see figure 5.29, (MILLER et al., 2001; LIU et al., 2005; KIRKPATRICK; RUTBERG, 2012)

Figure 5.29 - Mode of action of porcine zona pellucida vaccine

Source: I.K.M. Liu (2014)

5.4.1.1 Histopathological Evidence of The Effects of pZP Vaccine on Mammalian Oocytes

Figure 5.30 illustrates an immunohistochemical staining of horse follicle (10 ×) and of the dog ovaries (10 ×).

Anti-pZP antibodies from pooled sera of pZP-treated horses bound to zona pellucidae (ZP) (arrow) of the oocyte (Oo) in horse (A) and oocyte of dog (B). Antibodies from pooled sera of untreated horses did not display zonae pellucidae staining of oocytes in horse (B) and dog (D).

Figure 5.30 - Immunohistochemical staining of horse follicle and dog ovare

Source: I K M Liu (2005)

5.4.2 The Mechanism Of The GnRH Immunocontraceptive Vaccine

Similar in the concept of the pZP vaccine, the GnRH peptide hormone, secreted by the hypothalamus, controlling the release of gonadotropins, is normally recognized as "self" by the immune system. In the process of fabricating the vaccine, a synthetic GnRH peptide is turned into a foreign protein by coupling it to a "non-self" protein. In this case, the source of this foreign protein are limpets (aquatic snails), see fig. 5.31, utilizing its hemocyanin proteins, purified from the hemolymph, which can carry oxygen. It is a metalloprotein as it contains two copper atoms, responsible for the blue color in the keyhole limpets

Figure 5.31 - Giant Keyhole Limpet (*Megathura crenulata*)

Source: Fagerstone (2006)

An adjuvant is further added to the vaccine, in the case of GonaCon, the adjuvant is a modified, containing killed *Mycobacterium avium*, to enhance its immunogenicity dramatically.

The immune response, similar to the pZP vaccine, will produce antibodies against the foreign limpet protein, but moreover, to animal's endogenous GnRH hormones, consequently inhibiting the GnRH from binding to its receptors at the pituitary gland. Subsequently, blocking the cascade of hormone secretions, necessary for gametogenesis, see figure 9, (GRIFFIN et al, 2004; FAGERSTONE, 2006; MASSEI et al., 2008; MILLER et al., 2008; SHARMA et al., 2014).

5.4.3 Schematic Presentation of the GnRH Vaccine Mechanism

In figure 5.32 the left side graph shows the normal action of the gonadotropin-releasing hormone, coming from the hypothalamus, diffusing through the capillary membrane, reaching the pituitary gland, where the hormones LH and FSH are stimulated for release, which in turn will act on the gonads. The graph on the right side, illustrates the effect of anti-GnRH antibodies binding to GnRH hormones before reaching the pituitary gland, creating a protein complex that becomes so large, that a diffusion through the capillary membrane is hampered. As no GnRH hormones can reach the pituitary, no gonadotropins are being released, consequently gonad activity ceases.

Figure 5.32 - Normal and inhibition of GnRH function

Source: Liu (2014)

Legend: Mechanism of action of the gonadotropin-releasing hormone. Normal and inhibition of GnRH function by anti-GnRH antibody

5.5 REMOTE DRUG DELIVERY SYSTEM USED FOR FREE-RANGING WILDLIFE VACCINATION

In the endeavor to treat free-ranging wildlife, it is key to being able to remain in the distance, hidden, undetectable, aiding in the overall success in delivering drugs "intra-animal", but allowing for multiple application in different animals, or at different times. Once an animal experienced a close-up human encounter (if he makes it that close), while being shot at, and experiencing this little, but uncomfortable impact from the dart, almost certainly, that there will not be a second time.

Drug delivery darts can be, with some practicing, employed, quite accurate, up to 80 meters of distance, provided that the projector and dart are adequate in performance, correctly adjusted, and the handling is skilled. Starting at the, as sterile as possible, filling and handling of the darts, to delivering the dart into the target. Furthermore, the right dart and needle need to be selected, accordingly to drug volume quantity, viscosity, and for the species to be used with (see figure 5.33).

Beside the principal objective to deliver the drug, it is imperative to avoid any injury to the animal, mainly dart impact injuries. Some general recommendations to assist in avoiding injuries to the animal:

- Choose the proper delivery equipment (projector, dart, needle)
- Adjust to the proper power charge, to discharge the drug content of the dart
- Select the right velocity for dart delivery (limit to animals less up to 15kg)
- Avoid using needles that are longer than necessary
- Understand the capacities of the equipment and the handler

(CHANCEY, 2006; ROELLE, 2009; EVANS et al., 2015)

Figure 5.33 - Example of a pressurized darts

▲ Spring-pressurized dart. (A) The dart is ready for firing, with (1) a tight-fitting cap occluding the dart's needle while (2) the coiled spring pressurizes (3) the agent. (B) Dart in the final stage of injection. The needle's cap slides backward as the needle penetrates the skin, allowing the spring to push (4) the plunger forward, injecting the agent.

▲ Air-pressurized blow dart in flight (A) and following skin penetration (B). Note the different components: (1) drug chamber, (2) pressurization chamber, and (3) one-way valve.

Source: Erin Chancey (2006)

Figure 5.34 and 5.25 depicts the preparation of a medicated dart, a type of long-distance projector, and its target, a North American Bison.

Figure 5.34 - Dart handling and gas powered projector, and bison application

Figure 5.35 - Bison about to be darted with a tranquilizer

Source: Julie King; Catalina Island Conservation

5.6 Conclusion

As of now, all the characteristics that the concept of immunocontraception offers are closest thing to a perfect antifertility method available.

It has been studies, over extended periods, and validated for many species, able to act as a long-term contraceptive, depending on the species treated, 5+ years. It is suitable for the application on free-ranging wildlife, as a one-shot application. And just as important as the technology, is its accessible cost. At the end, the factor that determines if it find its way into population management programs, or not.

Future development on the basis of immunocontraceptive are intriguing and promise application that cater better to species-specificity, perhaps even more effective, gender specific, safer, and perhaps, even more cost efficient.

Having said that, it is also imperative to continue safety trails in as many species as possible, to assure the animal's overall health, short and long-term, while guaranteeing its full reversibility, without any adverse effects.

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6 WILDLIFE CONFLICT VERTEBRATE PEST SPECIES, AND WILDLIFE POPULATION CONTROL IN BRAZIL

Abstract

When the rest of the world hears about Human-Wildlife Conflict (HWC) news from Brazil, images of Jaguars attacking livestock or humans come to mind, although true, it represents just a partial picture. Where, in Brazil, do humans and wildlife meet? Is it, that humans are invading ("expanding") into wildlife habitats, or is it, that Wildlife invades urbanized areas? Predators are nature's population control, performing their duties on a daily basis, making sure that overabundance in prey-species would not occur. Progressively, and not just in Brazil, the number of predators will diminish; maybe even drive these magnificent animals into their extinction. The consequence will prove traumatic to the fauna's equilibrium, contributing to increase of "pest" species and human-wildlife conflicts in all its forms. One of these growing health concern are the overabundance of capybara (*Hydrochoerus hydrochaeris*), South Americas largest rodent, thought of as amplifying, or secondary hosts of zoonotic infectious diseases. Culling, as the most traditional means of population control in Brazil, has only been liberated in a unique attempt to cope with overabundant feral pig population, but when it comes to capybara control, "officially", culling is not considered an alternative. Studies on alternative contraceptive methods to control Wildlife populations are rather limited, and so far, no adequate population management program for a large-scale and in free-ranging wildlife has been put into place. But there are viable alternatives.

Keywords: Brazil; Human-wildlife conflict; Wildlife population control; Pest species; Immunocontraceptives.

6.1 AN OPINION PIECE

On The A Common Denominator And The Bearing On The Future

The "PARADOX", an argument that apparently derives self-contradictory conclusions by valid deduction from acceptable premises (CANTINI, 2014; MERRIAN WEBSTER, 2016).

When, how and why exactly did the concept of "Human-Wildlife Conflict" (HWC) come about? Although, the term is a rather modern description of human-wildlife interactions with a negative connotation, logically, it is as old as humankind itself, considering the challenges of early human survival.

Breaking down the term into its elements, it states that humans and wild animals are antagonistic in their behavior and means of survival, they are competitive and almost always, opposing in their actions. But what it really means is, Wild animals are interfering negatively with human interests, from an individual, a local community, a state, country, or even the entire global human population,- which translates into predators attacking a landowner's livestock, or even attacks on human lives; feral pigs destroying crops right before harvest; deer, while crossing Interstate Highways, causing terrible traffic accidents; synanthropic animals turning host for zoonotic diseases; or life-threatening epidemics attacking entire continents, respectively.

Makes sense, reading all the horrifying experiences with wildlife animals from all over the globe, one can't help it, but to agree, or maybe not?

And in Brazil?

When the rest of the world hears about HWC news from Brazil, images of Jaguars attacking livestock or humans come to mind, although true, it represents just a partial picture. Where, in Brazil, do humans and wildlife meet? Is it, that humans are invading ("expanding") into wildlife habitats, or is it, that Wildlife invades urbanized areas? That, perhaps, depends on whom is being asked.

Brazil - Quick Bio Overview

- Brazil is among the most biodiverse nations in the world, distributed over six biomes (figure 6.36)
- An estimated 20 percent of the Earth's biodiversity is found in Brazil.
- Brazil is home to at least 103,870 animal species, 650+ are mammalian species

Figure 6.36 - Brazilian Biomes

Source: Biodiversity in Brazil; Fact Sheet SSC, Presidency of the Federative Republic of Brazil (2012)
http://brazil-works.com/wp-content/uploads/2012/11/Fact-sheet_india_final.pdf
 Source: SSC fact sheet (2012); <http://www.iucnredlist.org/initiatives/mammals/analysis/geographic-patterns>

With this vast biodiversity, it comes to no surprise of the potential of Human-Wildlife Encounter, and most often than not, Human-Wildlife Conflicts are inevitable.

As characterized by Silvio Marchini (2015), HWC in Brazil is diversified and it is rapidly increasing, with dynamics driven by wildlife displacement, or better, rearrangement from nature to rural, to urban areas; from once "in equilibrium", Machini refers to it as "scares" to overabundant wildlife; and from native to exotic (and added by the author: invasive) species, corresponding to capybaras, large felines, and various families of the wild pigs (*Sus scrofa*), like the wild boars, port. *javal*, *porco monteiro*, as well as their close cousins, for instance, the collared peccary, port. *porco do mato*, *cateto*, (*Pecari tajacu*), respectively.

Brazilian's Top Five - for infamous calamities

In recent years, the species depicted in figure 6.37, have found their way into Brazilian news numerous times, none of which were of upbeat nature.

Figure 6.37 - *Puma concolor*; *Panthera onca*; *Hydrochoerus hydrochaeris*; *Sus scrofa*; *Pecari tajacu*;

Source: Hollingsworth, J. & K. (P.c.); Tørrissen, B.C. (P.o.); P.Hermans (H.h.); Aleks (S.s.); Crumps (P.t.); CC BY-SA 3.0, <https://commons.wikimedia.org> (2016)

For example, records from the 1990's indicate livestock attacks by Brazilian mountain lions (*Puma concolor*), port. *Puma*, *onça-parda*, *suçuarana*, the second largest feline in South America, at cattle ranches in the South of Brazil. Even though, a rather rare occurrence, as pumas, in comparison are less stronger than Jaguars and are more known to attack smaller livestock, such as sheep and goats, as they are easier preys (MAZZOLLI; GRAIPEL; DUNSTONE, 2002). More recently, now a yearly event, Pumas start to get a different kind of attention, due to quite dramatic appearances in densely populated urban areas.

Some examples, as reported by major Brazilian news agencies on occurrences inside of cities:

- 2011: Puma caught on surveillance cameras in Campos do Jordao, a high-class mountain resort in the State of Sao Paulo;
- 2011: In figure 6.38, a Puma invades private homes, North part of the Metropolis Sao Paulo.

Figure 6.38 - Puma invades private residence

Source: Helio Torchi/Futura Press (2011)

- 2012: Puma appeared in the City Centre of Caarapo, in the State of Mato Grosso do Sul.
- 2014: Puma in figure 6.39, invading a condominium, City Campo Grande, Mato Grosso do Sul.

Figure 6.39 - Puma invading a residential condo in the city of Campo Grande, Mato Grosso do Sul

Source: Marcelo Calazans (2014)

- 2016: Puma found inside a private residence's garage, City of Jau, State of Sao Paulo.

Just a few of the 10+ registered occurrences/year, involving Pumas "invading" urban areas.

The same sad statistics exist for the largest feline species in South America, the Jaguar port. *Onça pinturada* (*Panthera onca*),

One example from 2014: Shows in figure 6.40, a Jaguar invading a private residence in Nova Mutum, State of Mato Grosso

Figure 6.40 - Jaguar in the backyard of a private residence

Source: Reporter MT, Joao Ribeiro (2014)

And, in figure 6.41, Jaguars caught feeding on killed livestock.

Figure 6.41 - Jaguars after attacking livestock (cattle)

Source: Ivandro Bonfim Vaz (2013)

Sources: (MAZZOLLI; GRAIPEL; DUNSTONE, 2002; MARCHINI, 2010; SP, 2011; G1 GLOBO, 2014, p. 1; MORENA, 2014; MARÍLIA, 2016)

In the end, many of these encounters end tragically for the animal, as shown in figure 6.42.

Figure 6.42 - Sacrificed Jaguar, after killing a human

Source: Caetano Ventura (2013)

This senseless killing, together with car accidents along Brazilian's highways, killing every second 15 wildlife animals, resulting in almost half a billion dead animals by the end of the year! See figure 6.43. Of course, most of them are smaller vertebrate species. But about 1% ($\pm$ 5 million animals) belong to large vertebrates (e.g. Jaguar; Maned Wolf; Puma; Tapir; and Capybara), an unspeakable loss of wildlife, and especially, of predators, the only natural means to control prey-type wildlife.

Figure 6.43 - Visualization of traffic accident occurrences involving Wildlife in Brazil

Source: Sistema Urubu; <http://sig.bafs.cbee.ufla.br/> (2016)

Predators are nature's "contraception", and means of population control, as illustrated in figure 6.44, they are performing their duties on a daily basis, making sure that overabundance would not occur.

Progressively, and not just in Brazil, the number of predators will diminish; maybe even drive these magnificent animals into their extinction. The consequence will prove traumatic to the fauna's equilibrium, contributing to the increase of "pest" species and human-wildlife conflicts in all its forms.

Figure 6.44 - Jaguar hunting Capybara (natural population control)

Source: Zig Koch (2016)

Brazilian "Pest and Invasive" Species

What exactly does the term "pest" mean? Traditionally, it meant an epidemic disease, which was associated with a high mortality rate. E.g.: The infamous Bubonic Plague, or Black Death, in the mid-1300s. In modern times, it stands for anything that might cause pest-like destructiveness or are grounds for extreme nuisance. Mainly used for plants or animals that are detrimental to humans, either by their presence in large numbers, or causing noise, pollution, and destruction (building, agriculture or livestock), and most importantly, are potential hosts and vectors of diseases.

Translating this into the Brazilian situation, besides the classic disease related pest species, such as insects and small rodents, Brazil is being challenged with a new set of wildlife species, turning into pests. The reasons for that are multifactorial. As above mentioned, there is the growing deficit of natural predators. In fact, the most natural, most effective, and most inexpensive means of wildlife population control.

Furthermore, many prey species, by nature, are quite proliferative, meaning; they reproduce easier and have higher birthrates. And some of them, are very resistant to environmental impacts, can adapt quickly to fast-changing conditions while being capable of evolving within human habitats, making them their own. And some actually drive very well on human-caused pollution, nicely caught on camera in figure 6.45.

Figure 6.45 - A *Nasua*, or Raccoon (port. *quati*), diving into human trash

Source: Tepes, A.K., Project Noha (2011)

Brazilian's Top "Pests", that invading urban areas, or causing crop destruction

Figures 6.46 to figures 6.51 are showing day-to-day situation of wildlife invading, or coexisting in urban areas.

Figure 6.46 - Capuchin monkeys invading homes

Source: Stephen Messenger (2013)

Figure 6.47 - C. Marmoset on electrical wire

Source: Yasuyoshi Chiba (2013)

Figure 6.46 - Capybaras at a city lake

Source: Wissam Saldah (2016)

Figure 6.47 - C. Peccary transported out of a city

Source: Hédio Fazan (2016)

Figure 6.48 - Wild boars and crop destruction

Source: ijuhy (2014)

Figure 6.49 - Capybara Road Kill/Traffic threat

Source: Hédio Fazan (2016)

Invasive Species to Brazil

Calling a wildlife species invasive does not just indicate that its origin is not native to the invaded biome, but that this species proved to be able to overcome adversity on its path to the glory land, or to establish its habitats if referring to feral animals. It must then possess quite some resistance, strength, and survival skills to avoid predators, along with it must a healthy mating behavior, guaranteeing future, better-adopted generations.

Must certainly, these invasive species will compete with the locals for territory and food, chances are, they might overburden the natives, either provoking their slow extinction or are given birth to hybrids, one of nature's ways of survival.

Naturally, these species will continue to adapt to the quickly changing environments, due to human actions. And it seems very likely, that these species will contribute even more to HWC in the near future. Some of them may even turn into future trouble maker "pests".

Based on the data from the Global Invasive Species Database, Brazil has 175 as invasive listed species, including all organism types. Out of these, only 11 belong to mammals, and some are already on the blacklist as pests:

1. *Callithrix geoffroyi*; Common Name: White-fronted Marmoset
2. *Callithrix jacchus*; Common Name: Common Marmoset, White-tufted-ear marmoset
3. *Callithrix penicillata*; Common Names: Black-tufted-ear Marmoset, sagüi
4. *Felis catus*; Common Names: domestic cat, feral cat
5. *Lepus europaeus*; Common Names: Brown hare
6. *Macaca mulatta*; Common Names: Macaco, rhesus monkey
7. *Mus musculus*; Common Names: Field mouse, house mouse
8. *Rattus norvegicus*; Common Names: Brown rat
9. *Sus scrofa*; Common Names: Pig, wild boar

Source: (ISSG, 2016)

Wildlife Population Control in Brazil

A growing health concern that deals with wildlife species are the capybara (*Hydrochoerus hydrochaeris*), South America's largest rodent. The opossum, port. *gambas* (*Didelphis aurita*), and the Black Vultures, port. *Urubu* (*Coragyps atratus*) thought of as amplifying, or secondary hosts of zoonotic infectious diseases. Most recently and acutely the spread of Rocky Mountain Spotted Fever (port. *febre maculosa*), a tick-borne disease, caused by the bacterium *Rickettsia rickettsii*. As the Cayenne tick (*Amblyomma cajennense*) has a wide range of habitats and are not host species-specific. Although they have a preference for horses, they do feed on other animals, making him a perfect vector to spread *Rickettsia*. In Brazil, horse, as well as the capybara (see figure 6.52), are considered of great importance in the epidemiological chain of this zoonotic disease (LABRUNA et al., 2002; PEREZ et al., 2008; FIOL et al., 2010).

In Brazil, Capybaras are officially part of the fauna, and as such protected by federal conservation laws from use, persecution, destruction, hunting or collection Republic Presidency House Supervisory Committee for Legal Affairs, Law No. 5,197, of January 3, 1967 (*Presidência da República Casa Civil Subchefia para Assuntos Jurídicos Lei nº 5.197, de 3 de Janeiro de 1967*). Even though the overall intent was to save wildlife from extinction, an almost paradoxical and unforeseen side-effect would turn out to become one of Brazilian's biggest challenges. How to control driving wildlife super-populations without breaking the law, while satisfying moral and ethical public demands, and in the same time, trying to cope with serious epidemiological concerns.

Figure 6.50 - Group of Capybaras at the Taquaral Lagoon, with tick warning sign

Source: César Rodrigues / AAN (2015)

What has been done so far?

Wildlife population control, other than hunting, in Brazil on a large scale, hasn't been reported as of early 2016.

Culling, as the most traditional means of population control, has been liberated in a unique attempt to cope with overabundant feral pig population responsible for devastating agriculture, ate the swamps in the State Mato Grosso, circumventing the federal law and fauna protection, by using the term "invasive species" as a back doors (MARCHINI, 2010; CARVALHO, 2013; PEDROSA et al., 2015).

As far as capybaras are concerned, "officially" culling is not considered an alternative, however, depending on the municipality, permission has been granted to sacrifice animals that are positive for *Rickettsia rickettsii*. Perhaps, this can be seen as a short-term and emergency solution for a very small and local area, for the protection of human health. However, this management procedure open strong ethical and moral concerns, as the animal itself, are not affected by this disease, and clearly, stands in opposite to the law.

The city of Campinas, Sao Paulo, in a attempt to control the local capybara population, at the Taquaral Lagoon and Ecological Park Monsignor Emilio Jose Salim, with a special permits from the Chico Mendes Institute of Conservation of Biodiversity (ICMBio), plans to employ castration and vasectomy as a measure to control population and stop the spread of Rocky Mountain fever (BACCHETTI, 2015). The same strategy is also being applied throughout the country for feral dog and cat populations.

In regards to other contraceptive methods used in Brazil on Wildlife species, some limited research was conducted on a number of different antifertility agents, but none that were conducted in a large number of free-ranging species, if it all, and only a few in captive wildlife, e.g., one study tested the effectiveness of a GnRH agonist, Deslorelin as a contraceptive method in Common Marmosets (*Callithrix jacchus*), (ROSENFELD, 2012), and one project describes the translocation of Black Vultures (LUIZ FRANCISCO SANFILIPPO, 2001).

Another study conducted was with a GnRH analog in female Lions at the Sao Paulo Zoo (GUIMARÃES, 2008).

One non-lethal method of capybara management, with the objective to protect crops, was based on attracting the animal to specifically plant vegetation, bahia grass (*Paspalum notatum*), refocusing their feed-interest. While it did show promising results, it does not present a solution for population control (GUIMARÃES; RODRIGUES; SCOTTI, 2014).

There are some private companies; which declared themselves as Environmental Consultant, with services on wildlife population control, research, rescue, and relocation. In one company, that works in together with local and State government, performing a vasectomy on capybaras.

Source: <http://www.zoovetconsultoria.com.br/site/manejo-etico-de-capivaras/>

On the other hand, a fair number of studies and case reports have been published on contraceptive methods using antifertility agents in domestic animals, but due to research emphasis on wildlife, were omitted in this review.

Concluding,

For Wildlife population control efforts in Brazil, it seems advantageous to create a national organization, that would function as a centralized body, with the possibility to observe all wildlife population efforts and activities, and research to be conducted, collecting and analyzing all information while permitting access to its databank. Which currently has no central organization, and every institute, governmental department, or company is doing its little studies, instead to combining forces.

Such central organization could provide information, training, professional specialization, and post-graduate courses on wildlife population methods, perhaps even functioning as a licensing organ for individuals to become active in Wildlife Population Control.

Additionally, further large-scale research on contraceptive methods in free-ranging wildlife is necessary, to allow alternative management techniques, appropriate for each relevant species and situation, easy applicable, that are cost efficient with long-term effects, without any risk to animals health, no risk of losing valuable genetics for good, while respecting the current laws to protect fauna.

In the author's opinion, one such promising contraceptive concept is based on immunocontraception, offering most of the desired characteristics for wildlife application.

Final thought on the future dynamics of pest animals, it is the author's notion, due to the diminishing number of predators, and the adaptability of the following prey species, over the next decades, potentially grow overabundant, throughout Brazil, and turning into the next generations of pest species:

- Deer
- Primates, mainly the Common Marmosets, and
- Nasua
- Possum

Nonetheless, applying solely contraceptive methods to attempt the control of pest populations will most likely not succeed. These efforts must go hand in hand, with the biggest recommendation that can be made yet, regarding wildlife population control and the lessening of human-wildlife conflicts, is grounded on conservational efforts to recover and protect flora and fauna, bringing back nature's equilibrium, which consequently will relinquish many current predicaments, and avoid many more future dilemmas.

One such conservation effort that must be done as immediate as possible are programs for the recuperation of natural predators.

Educating the public to preserve and protect these animals, while implementing and improving wildlife management programs, at the same time providing environmental protection, adequate education and training for all professionals of wildlife management.

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7 MATERIAL & METHODS

7.1 LITERATURE SEARCH METHODOLOGY

To discover and analyze relevant studies of contraceptive methods in wildlife population control, as well as to identify trends in the development of novel concepts, an electronic search for the period 2000 - 2015, including available articles in print for 2016, was conducted. Employing English text and keyword explorations within the most appropriate databases and search engines, specialized in Environmental Science, Life-Science, Medicine, Biomedicine, Biology, Ecology, or Multidisciplinary. English was considered to be most adequate language to find most International scientific publication, as, independent of the study's origin, in most cases, at least, the abstract would be written in English. Search engines used: ScienceDirect, PubMed, Scopus, Mendeley, containing search results from Google Scholar, Scielo, Web of Science, and BioOne.

Throughout 2015, and the first weeks of 2016, the Internet was exclusively used through the University of Sao Paulo's VPN client address, rendering full access to most available articles from the International Science Community. Additionally, many articles, that were not part of the initial search engine's results, could be identified as pertinent, during the scouting of the article's bibliographies and citations. Table 7.12, shows the English keywords or strings that were applied to search through titles and abstracts:

Table 7.12 - Keyword and Search Terms

General keywords	Concept keywords	Specific keywords
Wildlife Population Management Wildlife Population Control Vertebrate Pest Control Wildlife Contraception	Lethal Methods Contraception Methods Reversible Contraceptive Methods Reversible Contraceptives	Culling; Poisoning Hormonal (Steroids, Progestins, Estrogens, Androgens, etc) Immunocontraceptives Physical separation Sterilization, Surgical, Chemical

Source: Rosenfield (2016)

Keywords not included were "Human" and "Domestic Animals", although, under certain conditions, articles studies on humans or domestic animals, but with emphasis on wildlife, also were included when deemed relevant. The number of published articles in each search engine varies greatly, most likely due to the variations in quality standards and policies, established by each service provider. For example, comparing the search results for the same, non-quoted, keywords, such as -Wildlife Population Control, and the search period from 2000 - present; from SCOPUS (figure 7.53) vs. Google Scholar (figure 7.54), demonstrates clearly the challenge that would follow in deciding what paper to include.

SCOPUS = 2205 results

Figure 7.51 - Scopus search result

Google Scholar = 1,430,000

Figure 7.52 - Google search results

Source: Rosenfield (2016)

In the attempt to bring some order into the search engine result chaos, only papers that could be identified as having certain attributes, such as being "peer-reviewed", from established publishers, as well as confident study methodologies and results, were included in this review. Furthermore, to not miss any new and important papers, keyword alerts were created in Google Scholar and in SCOPUS that would provide up-to-date information on the most recent published articles.

Search Engine Results Overview

Figure 7.53 - Search Results - Work flow

7.2 DATA EXTRACTION

For the purpose of extracting relevant data from the 213 included articles, three database programs were being employed; see figures 7.56, 7.57, and 7.58, depicting integrated tools and functionality.

Zotero - Reference Management

An open-source reference management software to manage bibliographic data and related research materials. Particularly, the most important feature, included the web browser integration and online syncing (figure 7.56).

Figure 7.54 - Zotero, direct article saving

Source: Rosenfield (2016)

Once an article was identified as relevant for further study, by clicking the "save to Zotero" the article, its metadata, and if available, the entire pdf file would be saved into Zotero's database (figure 7.57), where all article are counted and alphabetically organized and categorized, making them available for quick search, with a overview of important metadata.

Zotero Database

Figure 7.55 - Zotero Database

Source: Rosenfield (2016)

Citation tool, with automated style and bibliography insertion

As shown in figure 7.58, Zotero, also provide a very sophisticated citation manager, with thousands of citation forms, facilitating tremendously citing and creation of bibliographies.

Figure 7.56 - Zotero citation tool

Source: Rosenfield (2016)

7.2.1 Qualitative Data Analysis and Database - NVIVO10

For article gathering via WEB page Interface (direct download into the database), organizing into source type (case studies, reviews, empirical studies, books), for general text identification and coding (highlighting) of most relevant information, organizing in nodes (categories). For final text analysis, interpretation, and research report summaries, the qualitative data analysis software QSR NVIVO10, shown on figure 7.59, was employed.

Figure 7.57 - QSR NVIVO10 Desktop image

Source: Rosenfield (2016)

In detail, this program allows to import all pre-selected articles, open, read and highlight, or code, important information, and organize them into research categories, find relationships between statements, and summarize investigated topics.

7.2.2 Excel Data Mining and Extraction Form

A research specific questionnaire was created, using MS Excel Spreadsheets, (figure 7.60), where all identified data were extracted to.

Figure 7.58 - Excel Sheet Databank / Extraction Form

	Title	1st Author	Year	Type of Study	Country of study	Order	Family	Species	Common name	Treated n	Control n	Male	Female	Method	Agent
1	Does contraceptive treatment in wildlife result in side effects? A review of quantitative and anecdotal evidence.	Grey, M.	2010	Review	USA	Various	Various	Various	Various			yes	yes	Various	Various
2	Fertility control to mitigate human-wildlife conflicts: a review	Massei, G.	2014	Review	UK	Various	Various	Various	Various			n/a	n/a	Various	Various
3	AZA Wildlife Contraception Center Programme for Wild Felids and Canids	Asa, C.S	2012	Case Studies	USA	Carnivora	Various	Various	Various	> 1K			yes	Non-steroid hormone	Deslorelin acetate - GnRH super-agonist
4	Clinical, pathological and immunohistochemical study of feline mammary fibroepithelial hyperplasia following a single injection of depot medroxyprogesterone acetate	Loretti A.P.	2005	Experimental	Brazil	Carnivora	Felidae	ssp	Large felines	8			yes	Steroid Hormone contraception	Melengestrol acetate (MSA) progestin
5	Long-term effects of deslorelin implants on reproduction in the female tammar wallaby (Macropus eugenii)	Herbert C.A.	2005	Experimental	Australia	Diprotodontia	Macropodidae	Macropus eugenii	Tammar Wallaby	7	8		yes	Non-steroid hormone	Deslorelin acetate - GnRH super-agonist
6	Achieving population goals in a long-lived wildlife species (Equus caballus) with contraception	Kirkpatrick J.P.	2008	Experimental	USA	Perissodactyla	Equidae	Equus caballus	Horses	46			yes	Immunocastration	Zona pellucida Vaccine

Source: Rosenfield (2016)

The questionnaire was constructed with the following keywords: Sequential number (document count); Article Title; 1st Author; Year of publication; Type of study conducted; Country of origin; Animal related info (Order/Family/Species/Common name); Number of treated animals (n) and number of control animals (n); Gender (Male/Female); Contraceptive Method studied; Agent studied; Means of administration; Empirical field,- Lab,- or in captivity study; Confirmed contraceptive efficiency; Confirmed minimum period of efficiency; Confirmed reversibility; Confirmed Adverse physiological effects; Form of pathological findings; Psychological effects.

Due to the qualities of information on (n) for treated and control animals, this data was omitted, as the final number would not represent a qualitative result.

More over, most studies with primary interest in contraceptive effects fell short on accompanying observations (if any) on adverse effects, psychological impacts, and partially inconsistent confirmation of reversibility. Although some available data was included in the analytic process and conclusions, an additional search on articles that specifically studied contraceptive method associated side effects and proven reversibility was conducted, to qualitatively and quantitatively improve the results of this important matter.

This Excel data sheet (figure 7.61) served for data interpretation, creation of statistics and graphics, identifying dynamics and trends while arriving at final results and conclusions.

Figure 7.59 - Excel Work sheets for Statistics and Graphs

Source: Rosenfield (2016)

8 RESULTS

8.1 NUMBER OF ARTICLES ON WILDLIFE CONTRACEPTION PUBLISHED 2000 - 2015

To demonstrate the dynamics of published studies over the last 15 years, we analyzed the data and created the following publication trend graphic, based on our analysis of the 250+ papers included in this literature review. After sifting through thousands of initial results, and filtering these down to about 1500 relevant publications, from these, a total number of 213 articles that matched all selection criteria, were filtered out, ordered by publication year (2000 to 2015), and included in the contraceptive method's database. In figure 8.62, the resulting graph show the trend line, indicating a significant increase during this 15 year period. From initial four publications to over ten by the year 2005, with a record number of 23 and 22 publications in the year 2007 and 2008 respectively, followed by a slow decrease, reaching numbers between 14 to 18 publications by the end of 2015.

Figure 8.60 - Number of Publications 2000 - 2015

Source: Rosenfield (2016)

8.2 TOP ARTICLE PRODUCING COUNTRIES

As illustrated in figure 8.63, the United States, by far, is the top producing country for scientific published articles on wildlife population control and contraceptive methods with 126 papers, followed by Australia with 25, Africa 14, and the UK with 9 publications. New Zealand and Brazil produced the same number of articles ($n=5$) while Germany published 4 studies. India and China ($n=3$), Thailand, Japan, Italy and Canada ($n=2$). Slovenia, Netherlands, Hong Kong, Finland, Chile, and Argentina participated with 1 publication each.

Figure 8.61 - Number of Publications by Country

Source: Rosenfield (2016)

8.3 TOP PUBLISHED AUTHORS

Figure 8.64, indicates that the author with the most publications was Miller, L.A. (USA), Kirkpatrick, J.F. (USA), followed by Massei, G. (USA), and Killian, G. (USA). Figure 8.65 showing a North American Bison, one of the author's research subject.

Figure 8.62 - Top published authors

Source: Rosenfield (2016) *Obs.* For better graphic representation, most single published authors have been omitted.

Figure 8.63 - Research Subject: North American Bison

Source: USDA, Public domain

8.4 PUBLICATIONS BY THE TOP FIVE AUTHORS (ALL ON IMMUNO-CONTRACEPTIVES)

Miller, L.A. (PhD.) Circle M Products, Owner; NWRC-USDA Project Leader, 2012
2000: Immunocontraception of white-tailed deer with GnRH vaccine
2000: Immunocontraception of white-tailed deer using native and recombinant zona pellucida vaccines
2001: Porcine zona pellucida immunocontraception: Long-term health effects on White-tailed deer
2002: In search of the active PZP epitope in white-tailed deer immunocontraception
2003: Evaluation of GnRH contraceptive vaccine using domestic swine as a model for feral hogs
2004: Contraception of Bison by GnRH Vaccine: A possible means of decreasing transmission of Brucellosis in Bison
2006: Contraceptive effect of a recombinant GnRH vaccine in adult female pigs
2008: GnRH immunocontraception of male and female white-tailed deer fawns
2008: Original Article: The Single-Shot GnRH Immunocontraceptive Vaccine (GonaCon™) in White-Tailed Deer: Comparison of Several GnRH Preparations
2009: Factors contributing to the success of a single-shot, multiyear PZP immunocontraceptive vaccine for white-tailed deer
2013: Twenty years of immunocontraceptive research: lessons learned

Kirkpatrick, J.F. † (Ph.D.), The Science and Conservation Center, Billings, MT
2001: Fertility Control In Animals
2002: Reversibility of action and safety during pregnancy of immunization against porcine zona pellucida in wild mares (*Equus caballus*).
2005: Contraception in free-ranging wildlife
2007: Immunocontraception and Increased Longevity in Equids
2008: Achieving population goals in a long-lived wildlife species (*Equus caballus*) with contraception
2009: The practical side of immunocontraception: zona proteins and wildlife
2010: Absence of effects from immunocontraception on seasonal birth patterns and foal survival among barrier island horses
2011: Contraceptive Vaccines for Wildlife: A Review
2011: Fertility control: A new and successful paradigm for African elephant population management
2012: Immunocontraceptive Reproductive Control Utilizing Porcine Zona Pellucida (PZP) in federal Wild Horse Populations

Bertschinger, H.J. (PhD.) Prof. Emeritus, Univ. of Pretoria, Dpt. Prod. Animals Studies

2001: Control of reproduction and sex related behavior in exotic wild carnivores with the GnRH analogue deslorelin: preliminary observations

2002: Induction of contraception in some African wild carnivores by downregulation of LH and FSH secretion using the GnRH analogue deslorelin

2006: Repeated use of the GnRH analogue deslorelin to down-regulate reproduction in male cheetahs (*Acinonyx jubatus*)

2008: Immunocontrol of reproductive rate of African porcine zona p seven private game reserves in South Africa

2008: The use of deslorelin implants for the long-term contraception of lionesses and tigers

2011: Controlling wildlife reproduction

Massei, G. (PhD.) Animal Health and Vet, Lab. Ag. (AHVLA), National Wildlife Mgmt. Ctr.

2008: Effect of the GnRH vaccine GonaCon on the fertility, physiology, and behavior of wild boar

2012: Long-term effects of immunocontraception on wild boar fertility, physiology, and behavior

2012: The use of DiazaCon™ to limit fertility by reducing serum cholesterol in female gray squirrels, *Sciurus carolinensis*

2013: Nonsurgical fertility control for managing free-roaming dog populations: A review of products and criteria for field applications

2014: Fertility control to mitigate human–wildlife conflicts: a review

2015: Immunocontraception for Managing Feral Cattle in Hong Kong

Killian, Garry (PhD.) Professor Reproductive Physiology, Pennsylvania State University

2000: Behavioral observations and physiological implications for white-tailed deer treated with two different immunocontraceptives

2006: Long-Term Efficacy of Three Contraceptive Approaches for Population Control of Wild Horses

2006: Immunocontraception of Florida Feral Swine with a Single-dose GnRH Vaccine

2008: Four-year contraception rates of mares treated with single-injection porcine zona pellucida and GnRH vaccines and intrauterine devices

2009: Observations on the Use of Gonacon™ in Captive Female elk (*Cervus Elaphus*)

8.5 NUMBERS OF PUBLICATIONS BY METHOD

Within the last 15 years, the highest number of studies was conducted in the sector of immunocontraception, see figure 8.66 Reaching almost half of all published papers, 48% (n=102); while studies on steroid and non-steroid methods are nearly the number, 16% (n=33) and 14% (n=29) respectively. Figure 8.67 shows the application of a dart on North American deer.

Figure 8.64 - Numbers of publications by method

Source: Rosenfield (2016)

Figure 8.65 - Deer Darting for Immunocontraception

Source: P.Mueller, C.A.S.H. (2016)

8.6 NUMBER OF ARTICLES BY SPECIES

The largest group of a single species investigated, as depicted in figure 8.68, during the examined period were the deer (*Artiodactyls*, *Cervidae*, *Odocoileus virginianus*), commonly known as the "White-tailed Deer," native to North-America and Canada, with n=24 papers identified. The second largest group studied are wild horses, (*Perissodactyla*, *Equidae*, *Equus caballus*), also referred to as Mustangs, as well, home to North America, with n=18 published studies. The third largest group are the African Elephants, (*Proboscidea*, *Elephantidae*, *Loxodonta Africana*), and as the name implies, natives to the African continent, n=14 investigations. In sequence, studies done on various Canis species (*Carnivora*, *Canidae*, *Canis ssp.*) n=11, and Wild Boars, (*Artiodactyls*, *Suidae*, *Sus scrofa*) n=10, Birds (*Aves*) n=9, Brushtail possums (*Diprotodontia*, *Phalangeridae*, *Trichosurus vulpecula*) n=8, the big cats (*Carnivora*, *Felidae*, *Felis ssp.*), n=8, for in vivo studies on lab animals n=8. Small cats (*Carnivora*, *Felidae*, *Felis catus*) n=6, and New World Monkeys (*Primates*, *Callitrichidae*, *ssp.*) n=5; The Australian Tammar wallabies (*Diprotodontia*, *Macropodidae*, *Macropus eugenii*), the Koalas (*Diprotodontia*, *Phascolarctidae*, *Phascolarctos cinereus*), the Kangaroo (*Diprotodontia*, *Macropodidae*, *Macropus giganteus*), the humans (*Primates*, *Hominidae*, *Homo sapiens*), the Elk (*Artiodactyls*, *Cervidae*, *Cervus elaphus*), and the bear (*Carnivora*, *Ursidae*, *Ursus americanus*) with n=4 each.

Continuing Macaques (*Primates*, *Cercopithecidae*, *Macaca radiata*), showing n=3 publications, and the squirrels (*Rodentia*, *Sciuridae* *Sciurus carolinensis*) with n=2.

Studies with a number of different species, during the same research project, included n=40 publications.

All the following species (alphabetically, and common name only) participated with n=1 publication each: Antelope; Baboon; Bison; Black Lemur; Black-flying fox; Camel; Coati; Feral cows; Fringe-eared oryx; Gerenuk; Giraffe; Gorillas; Grey seals; Lemurs; Mink; Mongolian gerbil; Nile hippos; Plateau pikas; Rabbits; Ram; Red foxes; Red Pandas; Sea Otters; and Spotted Seals.

Obs.: Birds, although no mammals, have been included here, as they are considered relevant vertebrate pests.

Figure 8.66 - Number of articles by species

Source: Rosenfield (2016)

8.6.1 Top Ranked "Pest" Species by Country and Authors

Table 8.13 shows publications, based on the order of priority pest species, country of study, and key author:

Table 8.13 - Example of Top ranking "Pest" Species, based on published ranking

Rank	Country	Considered "pest" species	Publications (list not exhaustive)
1	North America (USA)	<i>Cervidae</i> , species <i>Odocoileus virginianus</i> , commonly known as the "White-tailed Deer"	(MILLER et al., 1999; KILLIAN; RHYAN; THAIN, 2006; RUTBERG; NAUGLE, 2008; FAGERSTONE; MILLER, 2010; BOULANGER; CURTIS; BLOSSEY, 2014); *Miller, et al 2000 - 2015;
2	North America (USA)	<i>Perissodactyla</i> , <i>Equidae</i> , <i>Equus caballus</i> , Wild Horse/ Mustang	(DISTEFANO, 2005; KIRKPATRICK; TURNER, 2008; PORTER; DEPERNO; KRINGS, 2014); *Kirkpatrick 2000 - 2015;
3	Africa	Order <i>Proboscidea</i> , family <i>Elephantidae</i> , species <i>Loxodonta africana</i> , African elephant	(DISTEFANO, 2005; DELSINK; ALTENA, 2007; PERERA, 2009; BERTSCHINGER, 2012; PINTER-WOLLMAN, 2012; OMEJA; JACOB; LAWES, 2014) *Bertschinger 2000 - 2015
4	North America; South America, Europe	<i>Artiodactyla</i> , <i>Suidae</i> , <i>Sus scrofa</i> , Wild boars/feral pigs	(KILLIAN et al., 2006; MILLER, 2006; CAMPBELL; LONG, 2009; MASSEI; ROY; BUNTING, 2011)
5	Australia	<i>Diprotodontia</i> , <i>Phalangeridae</i> , <i>Trichosurus vulpecula</i> , Brushtail possums	(RAMSEY, 2007; DUCKWORTH et al., 2008; LOHR et al., 2009; EYMANN; COOPER; HERBERT, 2013)
6	Australia	<i>Diprotodontia Macropodidae</i> , <i>Macropus giganteus</i> , Kangaroo <i>Diprotodontia Macropodidae</i> , <i>Macropus eugenii</i> , Tammar Wallabies <i>Diprotodontia Phascolarctidae</i> , <i>Phascolarctos cinereus</i> , Koala <i>Artiodactyla</i> , <i>Suidae</i> , <i>Sus scrofa</i> , Wild boars/feral pigs <i>Carnivora</i> , <i>Felidae</i> , <i>Felis catus</i> , Cats	(NAVE et al., 2002; DISTEFANO, 2005; DUKA; MASTERS, 2005; TANAKA; CRISTESCU; COOPER, 2009; SNAPE; HINDS; MILLER, 2011; MCLAMB, 2013; BENGEN; GENTLE, 2014)

Source: Rosenfield (2016)

Furthermore, cats, pigeon, bear, dogs and in some North American States, the Elk (Wapiti), *Artiodactyls*, family *Cervidae*, species *Cervus elaphus nelson*, (all with n=3), are becoming a nuisance to the local human population. *Bison* (*Bison bison*) + other 85 species (n=1).

8.7 NUMBER OF STUDIES, MALE VS. FEMALE

Figure 8.69, provides an overview of conducted studies by gender, where out of 215 publications:

- 117 studies were conducted on females
- 40 studies investigated both genders
- 30 papers focused on male only, and
- 26 publications have had a general (gender unspecific), or a different research topic.

Figure 8.67 - Number of studies by gender

Source: Rosenfield (2016)

8.8 STUDIES CONDUCTED ON WILDLIFE ANIMALS *IN SITU* VS. CAPTIVITY, AND LAB

To be considered "in-field", the studies would need to be performed on free-ranging wildlife (in-field), without and physical barriers, exceptions, areas with the size of national parks, smaller islands, natural reserves, or, on smaller mammalian species, with limited roaming capacities, for instance, koalas. Although confined to areas by physical barriers, the size of the area would not directly interfere with their natural behaviors, see figure 8.70.

Empirical studies conducted:

In the field n=49

In captivity n=99

In laboratories n=11

Figure 8.68 - Number of studies in vivo, in situ, captivity, or in labs

Source: Rosenfield (2016)

8.9 NUMBER OF ARTICLES VS. CONTRACEPTIVE EFFECTS

As can be seen in figure 8.71, out of the 215 publication, included in the data extraction process on contraceptive methods:

- 72 studies did not investigate the effectiveness of contraceptive methods, or had a different research focus.
- 134 studies established positive contraception effects of the methods investigated.
- 9 articles indicated negative results of the contraceptive methods tested.

Figure 8.69 - Number of Articles vs. Investigated Effectiveness

Source: Rosenfield (2016)

8.10 NUMBER OF STUDIES THAT TESTED REVERSIBILITY

In regards to the reversibility of studied contraceptive methods, referring to a complied return of ovarian activity, giving birth to and showing estrus behavior in females, and return to spermatogenesis, courtship, territorial dispute, aggression, fighting, and mating activity in the male. Of the 215 analyzed papers, figure 8.72 shows:

- 149 publications did not investigate
- 2 publications indicated a negative result on reversibility, and
- 64 studies were able to confirm reversibility after a contraceptive treatment

Figure 8.70 - Study Number that tested reversibility

Source: Rosenfield (2016)

8.11 NUMBER OF STUDIES THAT INVESTIGATED FOR ADVERSE EFFECTS

Adverse effects are any pathologic finding, directly associated and provoked by the contraceptive method tested. Severe lesions on the injection side, caused by the injection (hand, or dart), were included as a side effect. Light injuries, typical of vaccination, but without a severe inflammation response on the injection side, were not considered as an adverse effect. Illustrated in figure 8.73:

- 138, the large majority of studies conducted on contraceptive methods, did make any mentioning of adverse effect
- 49 publications indicated to have not observed any associated adverse effects during their study period
- 27 studies did observe and associated pathologic occurrences to the contraceptive method applied.

Figure 8.71 - # Articles vs. Investigated Adverse Effects

Source: Rosenfield (2016)

8.12 NUMBER OF STUDIES THAT INVESTIGATED BEHAVIORAL IMPACTS

Behavioral impacts are included in the analysis, not only as a negative psychological effect but was included as a result if any behavioral alteration was observed and investigated. Such as, change in mating behavior, increased estrus behavior, increased special impact, decreased courtship, aggression, and, for instance, loss of Musth in elephants, which would be a desired physiological and behavioral effect, see figure 8.74:

- 137 studies did not investigate contraceptive methods associated behavioral impacts or have had other investigation goals.
- 43 studies did not observe any related behavioral alterations, and
- 34 studies did investigate and could confirm behavioral changes, associated with the contraceptive method studied.

Figure 8.72 - # Articles vs. Investigated Behavioral Impacts

Source: Rosenfield (2016)

9 DISCUSSION

After this comprehensive literature review, one results stands out from all possible other conclusions, as of today, there is not 'perfect' population control method, which would allow controlling any given wildlife species without any negative impact, for the individual's health and behavior, or on the entire group's social structure. Having said that, there has been great progress in minimizing adverse physiological effects, while current indications for future technologies hold promise to deliver management tools to control populations, with much less health and psychological impacts as any current method could.

Following the analyzed and interpreted results, based on each before established research question.

9.1 ON THE NUMBER OF PUBLICATIONS

Year 2000, $n=6$, year 2007, topping at $n=23$, and 2015, $n=15$. The numbers of articles published between the years 2000 - 2015 show a clear increase. It is the author's interpretation that the reasons are multifactorial. Possible mechanisms might depend on the increase of human population and consequently in human-wildlife conflicts. Partially due to a massive population surge in vertebrate "pests", leading to a necessary pest control (population) on one side, but in the same moment, governmental conservation laws, as well as public awareness with their aversion to culling and surgical sterilization procedures on the other side. As well as ethical and professional progress on animal well-being, all increasing the demand to search for alternative methods that would satisfy both challenges. Furthermore, availability of new technologies and advances in molecular research, combined with the better understanding of reproductive physiology and for a larger spectrum of species, provided the basis for new discoveries and development of novel contraceptive agents. Many of which are a natural extension from the fast advancing biotechnologies and assisted reproductive technologies in animal science, hence, more research has been done to solve the before mentioned problems (DISTEFANO, 2005; MILLER; FAGERSTONE; ECKERY, 2013; COHN; KIRKPATRICK, 2015).

9.2 ON THE NUMBERS OF PUBLICATIONS BY COUNTRY

Out of the 213 scientific articles on contraception, with emphasis on wildlife population control, the USA, by far, is the country with the most published articles ($n=126$), reaching 59%.

Contrary to the USA numbers, Australia with 26% ($n=26$), appears to have the much bigger problem with exploding population numbers of true pest species, based on the number of and varieties of pests investigated in Australian studies. Therefore it seems plausible that Australian scientist would frantically seek for more, or better solutions (BOMFORD, 1993, 1993; DUKA; MASTERS, 2005, 2005; LAPIDGE, 2008; BENGTSEN; GENTLE, 2014; BOMMEL; JOHNSON, 2014; DICKMAN, 2014).

Africa participates with 7% ($n=14$) studies, where Elephants were the prime study subject and immunocontraceptive the method of choice to investigate, followed by non-steroid hormone concepts (OLIVEIRA et al., 2004; LOUWERENS, 2014).

Europe, with a total of 15 publications, with a research focus on species such as fox, pigeons, and wild boars (MASSEI; COWAN; MILLER, 2011; DUARTE et al., 2015).

New Zealand ($n=5$) like Australia, suffers from the same species problem, and Brazil, with the same number of publication ($n=5$), deals with feral dogs, cats, pigs, and a newcomer, the capybara (JOLLY, 1993; FERRAZ; MANLY; VERDADE, 2009).

The rest of the International scientific community with publication on studies conducted on wildlife population control by contraceptive methods is scattered out over Canada, Asia, Eastern, Northern Europe, and South America. With species ranging from Elk, Deer, feral cows, feral dogs, cats, to birds and various rodents. Figure 8.75, shows indeed feral cows in the streets of Hong Kong.

Figure 8.73 - Feral cow in Hong Kong

Source: CNN, Brad Olsen (2012)

9.3 ON THE NUMBER OF PUBLICATIONS BY AUTHORS

When comparing the numbers of most published authors, it becomes apparent, that they have a certain research relationship, all coinciding with the top published research topic, immunocontraception. Naturally, being the top authorities in this field, in many studies, out of common interest, these researchers would form cooperation, or in some cases, they are from the same institutes conducting these researches. For instance, (MILLER; JOHNS; KILLIAN, 2000; KILLIAN et al., 2009), or (MASSEI; MILLER, 2013), and (FAYRER-HOSKEN et al., 1999), indicating Dr. Bertschinger and Dr. Kirkpatrick as co-authors, and what they all do have in common was the research on pZP and GnRH vaccines for wildlife population control.

9.4 ON THE NUMBER OF PUBLICATIONS BY METHODS

Over the past decade and a half, a clear movement, away from hormone contraceptives towards immunocontraceptives methods can be observed, a clear trend indication, reaching almost half of the published studies during this 15 year period.

The rationale behind these trend dynamics is multidimensional, as can be identified in numerous articles (KIRKPATRICK; LYDA; FRANK, 2011). Doubtless, the most important reason, severe pathological side effects provoked by synthetic steroid hormone based contraceptive agents, their impacts on individual and on social behavior, and, more importantly, health risks for the pregnant animal and its fetus. Furthermore, the high cost of procurement and difficulties in the application over long-distances and the necessity for repetitive administration. For that reason, slowly but surely, demands on finding a safer alternative to hormone-based contraceptives, with long-term antifertility effects, simpler application. Especially for wildlife *in situ*, and lower dosage requirements, with better cost-benefit ratio, all these characteristics made immunocontraceptives a more interesting solution, as before mentioned (see *chapter Antifertility and Contraceptive Methods*).

Nevertheless, hormone base contraceptive methods are still the most frequently used for wildlife in captivity, and to a lesser degree, in free-living animals (ASA; PORTON, 2005). Although, the driving force behind contraceptive product availability is still the human market. But with modern pharmacological technologies, a better understanding of molecular structures of receptors, and pharmacokinetics constantly improving, offering a much better specificity of binding these target receptors, for example, steroid hormone based contraceptive agents and their corresponding progesterone, estrogen, androgens, or glucocorticoids receptors, resulting in much less potential side effects.

Figure 9.76 shows the administration of a contraceptive in White-tailed deer, and in figure 9.77, the darting of a brown bear with a tranquilizer dart.

Figure 9.74 - Captured deer / contraceptive treatment Figure 9.75 - Darted Bear

Source: NMR - Brandon Hubbard (2013)

Source: APIS/USDA (2015)

9.5 ON THE NUMBER OF PUBLICATION BY SPECIES

Analyzing the numbers of published papers by species, one can correlate investigated species with current situations on pest populations. While, at the same time, due to public influence, the demand to abandon traditional culling and to identify alternative population control methods are desired (DISTEFANO, 2005; FAGERSTONE; MILLER, 2010; MASSEI; COWAN, 2014).

Since the initiation of successfully using immunocontraceptive methods in wildlife, in 1986, four wildlife species became the principal target as a research subject. These are identified in this review, as the most frequent cited species, when it comes to immunocontraception: *Cervidae*, species *Odocoileus virginianus*, commonly known as the "White-tailed Deer," North America number one pest species, followed *Perissodactyla*, *Equidae*, *Equus caballus*, Wild Horse, Mustang or Brumbies. In Africa, not only because of increasing numbers, but more due to the animals size, strength, and long life span, the African Elephant, *Proboscidea*, *Elephantidae*, *Loxodonta Africana*. In the Americas, Europe, Australia and South America, the top pest mammal is the wild boar, or feral pig, *Artiodactyla*, *Suidae*, *Sus scrofa*.

And then, there is Australia, with the highest diversity of pest species, to name a few, like the Brushtail possum, *Diprotodontia*, *Phalangeridae*, *Trichosurus vulpecula*, the famous Kangaroo, *Diprotodontia*, *Macropodidae* *acropus giganteus*, and as cute as they are, the Koala are turning into a plague, *Diprotodontia* *Phascolarctidae*, *Phascolarctos cinereus* and as unbelievable as it may seem (fig. 9.78), the Camel, *Artiodactyls*, *Camelidae*, *Camelus dromedaries*, are indeed a pest species.

Figure 9.76 - Feral camels in Australia

Source: Department of Land Resource Management, Australia (2016)

9.6 NUMBER OF STUDIES, MALE VS. FEMALE

Interestingly enough, the majority of studies, almost traditionally, remains with females (n=117), studies on males only (n=30), studies that included both genders (n=40), and studies without gender relevance (n=26). Curiously, leading to the question, why is there such as gender biased? Is it because out of the traditional development of contraceptive methods for the human female market? Maybe due to major advances in production animal science, where many hormonal products for application in females are being developed? Or, conceivably, out of concern that treated males would lose their secondary sexual characteristics, aggressiveness, change of social group dynamics, losing dominance position? Evidently, which method to use on which gender is situation depending, nevertheless, applying contraception to males, supposing, there will be no impact on behavior and secondary sexual characteristics, seem a better solution for species, where one dominate male would mate with two, or more females.

On a very practical point of view, thinking about the application in real life situations, executed by specially trained personnel, in species without clear sexual dimorphism (figure 9.79), at a longer distance, being able to deliver the contraceptive agent into an adult animal, independently what gender has been treaded, still have an effect on population control, would be advantageous to wildlife management efforts. For example, in Latin America, where costs are a priority in the decision making process, only lesser trained wildlife employees are available, which would be performing most of the applications in a species like the capybara.

Figure 9.77 - Capybara, sexual dimorphism?

Source: Palani (2016)

9.7 STUDIES CONDUCTED ON WILDLIFE ANIMALS IN SITU VS. CAPTIVITY, VS. LABORATORY

In the field n=49; in captivity n=99; in laboratories n=11

There is no surprise in seeing the majority of studies, *in vivo*, conducted on wildlife animals in captivity, merely, due to logistic motives. As there are known and healthy individuals, in a controlled environment, with easy 7/24 access, in a secure area, and not far from needed equipment and labs, among other reasons.

While interpreting the data on studies conducted in laboratory animals, one might reach a careful notion, that research done on real novel methods were somewhat low during the last 15 years, considering that prior to product availability for studies on wildlife, new agents would pass through many laboratory process steps before being liberated for further studies in non-laboratory animals.

9.8 NUMBER OF ARTICLES VS. CONTRACEPTIVE EFFECTS

Of the 215 studies included in the contraceptive methods meta-analysis, 72 studies did not investigate the effectiveness of contraceptive methods, or had different research focus, for example literature reviews. 134 studies established positive contraception effects of the methods investigated. 9 articles indicated negative results of the contraceptive methods tested.

Post-treatment, positive results were determined by hormone analysis (gender specific reproductive hormones below physiologic effects, or baselines), diminished reproductive behavioral, and observations of any newborn addition.

Post-treatment, negative results meant, presence of newborn, now suppression of reproductive hormones evident, and maintaining mating behavior.

The results of the analysis showed 62% of contraceptive methods being effective in controlling fertility in various wildlife species.

The most successful species tested were: Horses; deer; feral pigs, followed by various ungulates, carnivores, wallabies, kangaroos, possums, primates, giraffe, elks, bison, bear, hippos and otters.

9.9 NUMBER OF STUDIES THAT TESTED REVERSIBILITY

Although, one of the most important arguments when testing a new contraceptive method, not always is it possible to provide results of reversibility in the same study, or perhaps, not even of the entire project/species. As we can see by the analysis on the number of studies that tested reversibility:

- 149 publications did not investigate, or the study period did not allow analyzing reversibility, of the contraceptive methods tested.
- 2 publications indicated a negative result on reversibility, and
- 64 studies were able to confirmed reversibility after a contraceptive treatment

One of the key reasons behind the lack of results on reversibility is due to the study duration. More often than not, are the contraceptive methods investigated so effective, that by the end of the study the animal still is infertile and long-term studies are needed to proof reversibility. Additional reasons are loss of the animal during the study or any other combination of research faux pas than can occur, especially when working with free-ranging wildlife.

9.10 NUMBER OF STUDIES THAT INVESTIGATED ADVERSE EFFECTS

138 publications, the large majority of studies conducted on contraceptive methods, did not investigate adverse effect, either because the study time was too short to observe possible side effects caused by the contraceptive used, or not part of the research goal.

49 publications indicated to have not observed any associated adverse effects during their study period, which does not necessarily preclude potential long-term health risks.

27 studies did observe and associated pathologic occurrences to the contraceptive method applied.

Most reported side effects were associated with the potent synthetic hormone based contraceptive methods. The majority occurred in females treated with progestins, showing predominantly pathologies of the reproductive tract, especially the uterus, and the mammary glands. Most pathologies found were adenomatous and cystic hyperplasia, endometrial polyps, hydrometra, pyometra, fibrosis, endometrial mineralization, as well as mammary carcinomas. However, the lesion with the highest occurrence is endometrial hyperplasia (EH), while investigative studies have documented that carnivores, and more specifically, felids seem to be especially sensitive to progestin provoked side effects, (MUNSON et al., 2002; MCALOOSE; MUNSON; NAYDAN, 2007).

Over the last decade, hormone contraceptive have changed in dosage and treatment protocols, minimizing the risk of potential side effect, nevertheless, still not 100% without risk, particularly, when long-term treatments are involved.

When investigating potential side effects caused by treatments by immunocontraceptive methods, the most mentioned lesion encountered, is on the injection side, as shown in figure 9.80, where local inflammatory response, mainly due to the adjuvant component of the vaccine, causing, from light to more severe granulomas, (GIONFRIDDO et al., 2009; KRAUSE et al., 2014)

Figure 9.78 - pZP vaccine cause abscess on a Pryor mare

Source: Pryor Wild (2010)

A not commonly investigated potential side effect is the serious risk of heart diseases. As a number of studies on hormone based contraceptives in lab animals have shown, the risk of various heart disease, such as arterioscleroses; thromboses, are quite frequent, depending on dosage and duration of the contraceptive treatments (SHUFELT; NOEL BAIREY MERZ, 2009, 2009). It seems reasonable to consider the possibilities that these evident contraceptive associated pathologies are a potential risk in wildlife application as well.

On the other hand, not a pathological issue, but nevertheless, a serious concern on the long-term effect of immunocontraceptive methods, is there a possibility to develop resistance to the vaccines (MAGIAFOGLOU et al., 2003)? As Magiafoglou continues, "There are many examples of rapid evolutionary changes in populations faced with an environmental shift, such as the evolution of pesticide resistance in insects, resistance to anticoagulants in rodents, and resistance to heavy metals in plants and invertebrates". Even more evident as a serious current problem is the evolving Super-bug, bacteria that developed resistant to many, once, very powerful antibiotics. An answer, that still needs time for investigation.

9.11 NUMBER OF STUDIES THAT INVESTIGATED BEHAVIORAL IMPACTS

As the last, but not least, analytic result, how many studies investigated contraceptive associated behavioral impacts:

- 137 studies did not investigate contraceptive methods associated behavioral impacts, or have had other investigation goals.
- 43 studies did not observe any related behavioral alterations, and
- 34 studies did investigate and could confirm behavioral changes, associated to the contraceptive method studied.

To not have observed any change in behavior while an animal is being treated with a contraceptive method, can only mean one of two things. Either, the contraceptive method does take effect, or, simply put, in the opinion of the author, no serious attention has been paid to the animal's behavior during the study phase.

Being able to successfully manipulate such a complex life force as reproductive physiology cannot occur without a change in behavior. Therefore, the studies that stated no alterations observed, seem not just anecdotal, but lack an important scientific base.

10 WHAT IS THE "BEST" CONTRACEPTIVE METHOD FOR WILDLIFE? POPULATION CONTROL

A question, to which a straight answer doesn't exist, as it does depend on many factors. Starting with the species, even the individual animal, male or female, if *in situ*, or in captivity, location, time of year (referring to the reproductive characteristics of that species), and the present situation. However, considering this day and age, there is only one "right" attitude to take, when it comes to even animal well-being, even more so, considering how heavily the newly developed public stance, morals and ethics are weighing in virtue of strong disagreement on lethal population control measures. Which, indeed, should only be considered, if nothing else "more adequate" provides the solution (CURTIS et al., 1993). It seems therefore, quite reasonable to suggest that the methods to avoid, in succession are:

- 1) **"Culling"** the gathering and killing of animals. To avoid because of moral and ethical issues and also to circumvent the loss of genetic materials for good. However, there might be moments where culling simply cannot be avoided.
- 2) The use of **chemicals** to kill free-ranging wildlife. Highly toxic agents used as pesticides, will, inevitably lead to environmental pollution, and putting eventually other wildlife species and even humans at risk of exposure, directly, by contact or consumption, or perhaps via ground water contamination. This method, for many reasons, must be avoided as the long-term consequence will be more severe than any transitory solution. Again, there might be emergency situations that call for extreme measures, such as mass-poisoning for animal populations that are just too great in number.

3) Evidently, any contraceptive agent that has the capacity to effect the endocrinological system will, inadvertently, cause a homeostatic imbalance, and consequently, cause disease, some more so than others. Still, as of today, all methods with intrinsic actions are potentially pathologic and on top of this category are still **hormone base contraceptives**. Now, the severity of the agent's impact depends on many factors, such as the duration of treatment, the dosage and also, on the species to be treated, even an individual's unique response and should, therefore, be evaluated on a case by case basis.

Each method will have its advantages and disadvantages, or acceptable vs. non-acceptable effects, decision subject to species and its application in free-ranging animals, or for animals in captivity.

Trying to get closer to the answer of what is the right method, materialized from this review's interpretation and its analysis, which, in the opinion of the author, is the **concept of immunocontraception technologies**, see figure 10.81.

Although the youngest concept of all methods investigated, with more than 20 years and numerous successful studies conducted, the results can't be pushed aside as still being too early, or there are still more long-term studies necessary to be conceded as a proven concept. In direct comparison with other methods of bioactive or intrinsic actions, immunocontraception satisfies most of the characteristics of the "perfect-contraceptive" wish-list, in fact, why not consider as a new standard, when it comes to wildlife population control.

Figure 10.79 - pZP Vaccine prepared darts

Source: PM Cynthia Smalley (2016)

10.1 CONTRACEPTIVE METHODS VS. ENVIRONMENTAL CONSIDERATION

Another serious and growing environmental concern is called bioaccumulation and is due to in nature ceased animals treated with agents that maintain biologically active and potent, even more so, in treated free-ranging wildlife.

It is not just an empty threat anymore, but a fact that these biological agents enter the food chain, either as a prey for its natural predators, or when hunted for human consumption, or once a treated animal's corpse decomposes and its substances enters the ground water, lakes, reaches fishes and plants, eaten by scavengers, where it potentially leads to bioaccumulation/concentration in fishes, plants, or other mammals (VAN DER OOST; BEYER; VERMEULEN, 2003; RUNNALLS, 2010; RAO, 2011; MEFFE; DE BUSTAMANTE, 2014; ORLANDO; ELLESTAD, 2014).

Presenting the question, how would immune-based contraceptives behave once liberated into the environment, or if consumed?

No specific literature has been found on this topic as of February 2016.

11 CONCLUSION

Research conducted on traditional contraceptive methods, steroid hormone and non-steroid hormone agents showed important improvements. Offering newer generations of steroid hormones, or combination of steroid hormones, that would require less concentrations for similar infertility effectiveness, yet with fewer side effects. However, hormone treatments are complex when it comes to the application in wildlife, as the success, and adverse effects depend on many factors. From species to individual's history, when working with females, to the right timing of application, dosage, and duration. Synthetic steroid hormone agents are still the most frequently used when considering population management for captive wildlife. And yet, they remain the method of choice and the potential risks of severe side effects.

An ongoing focus in a number of studies, investigating associated pathological effects from contraceptive methods in wildlife.

Non-steroid hormone seem to be the next best alternative, as they offer long-term infertility effects, with fewer health risks, and provide better functionality when it comes application in free-ranging, as well as captive wildlife. In some circumstances, treatment with steroid,- and non-steroid hormones applied at the same time has shown to counter some initial "flair" effects, known to occur from GnRH analog, for instance, and progestins would counter, until down-regulation takes over.

Immunocontraception, by far the most studied subject and scrutinized infertility method for wildlife and feral species, over the last ten years, and there is a big reason for it. The majority of empirical research done, proved repeatedly to be effective in rendering various species infertile, and that for extended periods of time, as stated by Miller (2009), in some cases more up to 7 years. In many studies, due to time constraints, reversibility was not always an option for investigation, nevertheless, the majority of studies, could conform reversibility. Observed adverse effects were a few, and if apparent, none were severe. Most frequent side effects observed were vaccine injection site injuries, and the development of some persistent granulomas, this due to extensive inflammatory reactions to the adjuvants. A necessary "evil" to initially stimulate the immune response effectively enough, and together with slow-release technologies, keeping the antibody titer adequately high to maintain the antifertility effect, without the need for "booster" applications.

Apparently, current and indicated future studies, keep their focus on immunocontraceptives, with some promising new concepts in the near future.

12 DISCUSSION

Specifically for Brazil, the author recommends strongly the formation of a permanent national organization and research institute that would centralize and collect data on crisis areas, epidemiological impacts due to developing pest species. History (database) on population control methods and contraceptive agents applied success rate and pathological effects while offering services for crises evaluation on target species and areas, and recommendations for solutions and methods, even execution of such services. Developing new technologies, and strategies, while managing, organizing and financing population control methods specific research.

Offering, furthermore, education, seminars, and training for professionals and technicians; together with the possibility of selling of special equipment. Upholding with the overall goal, which is to reduce the impacts of invasive and pest animals on Brazilian's people and animal health, economics, and environments, by combating and prevention, and early detection of existing and new threats from invasive species or elevate to a pest-like status of high proliferative feral and wildlife species. Promoting Wildlife Conservation, protection, and well-being, perhaps develop into a South-America Headquarters for Wildlife (Pest) Species Population Control.

Currently, there are two major Institutions, offering similar services and databanks that are accessible for information on which contraceptive method would be the most adequate for a given species. In addition to having a health surveillance program, collecting information on contraceptive agents related complications and associated pathologies. They are also serving as a "know-how exchange", open for anyone willing to share their professional experiences with colleagues worldwide.

These are:

- St. Louis based, AZA Wildlife Contraceptive Center, with close to 35,000+ entries.
<http://www.stlzoo.org/animals/scienceresearch/reproductivemanagementcenter/contraceptionrecommendatio/contraceptionmethods/>
- European Association of Zoos and Aquariums (EAZA), with emphasis on European needs.
<http://www.egzac.org/aboutus.aspx>

However, as these two are members of each other's organization, they do share basically the same information.

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APPENDIX

Partial content extraction from of the Excel data base:

Contraceptive Methods Data Analysis Extraction Form

Contraceptive Methods Meta-Analysis Extraction Form

#	Title	1st Author	Yr.	Type of Study	Countr/ study	Order	Family	Species	Common name	Treat. n	Contr. n	♂	♀	Method	Agent	Admin.	Field Exp.	Lab	Captiv.	Contracep. Effic.	Min. Eff./m	Revers.	Adver. effec.	Form Pathol	Behav. Impact
1	Does contraceptive treatment in wildlife result in side effects? A review of quantitative and anecdotal evidence.	Gray, M.	2010	Review	USA	Various	Various	Various	Various			yes	yes	Various	Various	n/a	n/a	n/a	n/a	yes	n/a	yes	yes	n/a	yes
2	Fertility control to mitigate human-wildlife conflicts: a review	Massei, G.	2014	Review	UK	Various	Various	Various	Various			n/a	n/a	Various	Various	n/a	n/a	n/a	n/a	yes	n/a	yes	yes	n/a	yes
3	AZA Wildlife Contraception Center Programme for Wild Felids and Canids	Asa, C.S.	2012	Case Studies	USA	Carnivora	Various	Various	Various	> 1K			yes	Non-steroid hormone	Deslorelin acetate - GnRH super-agonist	Implant	yes		yes	yes	24	yes	yes	n/a	n/a
4	Clinical, pathological and immunohistochemical study of feline mammary fibroepithelial hyperplasia following a single injection of depot medroxyprogesterone	Loretti A.P.	2005	Experimental	Brazil	Carnivora	Felidae	sap	small cat	8			yes	Steroid Hormone contraception	Melengestrol acetate (MGA) progestin	Injection			yes	yes	n/a	n/a	yes	Feline mammary fibroepithelial hyperplasia (FMFH)	n/a
5	Long-term effects of deslorelin implants on reproduction in the female tammar wallaby (<i>Macropus eugenii</i>)	Herbert C.A.	2005	Experimental	Australia	Diprotodontia	Macropodidae	<i>Macropus eugenii</i>	Tammar wallaby	7	8		yes	Non-steroid hormone	Deslorelin acetate - GnRH super-agonist	Implant			yes	yes	24	yes	No	n/a	No
6	Achieving population goals in a long-lived wildlife species (<i>Equus caballus</i>) with contraception	Kirkpatrick J.F.	2008	Experimental	USA	Perissodactyla	Equidae	<i>Equus caballus</i>	Horses	46			yes	Immunocontraception	PzP Vaccine	Injection	yes			yes	12	n/a	no	n/a	no
7	Immunocontraception of mammalian wildlife: ecological and immunogenetic issues	Cooper D.W.	2006	Review	Australia	Various	Various	Various	Various			n/a	n/a	Immunocontraception	Various	Injection	n/a	n/a	n/a	yes	n/a	n/a	yes	juvav recti	no
8	Reproductive Cycles and Contraception of Black Lemurs (<i>Eulemur macaco macaco</i>) With Depot Medroxyprogesterone Acetate During the Breeding Season	Asa C.S.	2007	Experimental	USA	Primates	Lemuridae	<i>Eulemur macaco</i>	Black Lemur	5	7		yes	Steroid Hormone contraception	Melengestrol acetate (MGA) progestin	Injection			yes	yes	n/a	n/a	no	Darkening of pelage	no
9	Fertility Control in the Eastern Grey Kangaroo Using Levonorgestrel Implants	Nave C.D.	2002	Experimental	Australia	Diprotodontia	Macropodidae	<i>Macropus giganteus</i>	Kangaroo	21	14		yes	Steroid Hormone contraception	Levonorgestrel (LNG) Progestin	Implant	yes		yes	yes	27	n/a	no	n/a	no
10	Contraceptive effects of levonorgestrel implants in a marsupial	Nave C.D.	2000	Experimental	Australia	Diprotodontia	Macropodidae	<i>Macropus eugenii</i>	Tammar wallaby	24	17		yes	Steroid Hormone contraception	Levonorgestrel (LNG) Progestin	Implant			yes	yes	36	yes	no	n/a	no
11	Reproductive seasonality and the effect of the GnRH agonist deslorelin as a contraceptive in captive male Black Flying-foxes (<i>Pteropus allecto</i>)	Melville D.F.	2012	Experimental	Australia	Chiroptera	Pteropodidae	<i>Pteropus allecto</i>	Black-flying foxes	5	6	yes		Non-steroid hormone	Deslorelin acetate - GnRH super-agonist	Implant			yes	yes	5	yes	no	n/a	no
12	Long-term suppression of fertility in female giraffe using the GnRH agonist deslorelin as a long-acting implant	Patton, M.L.	2006	Experimental	USA	Artiodactyla	Giraffidae	<i>Giraffa camelopardalis reticulata</i>	Giraffe	2			yes	Non-steroid hormone	Deslorelin acetate - GnRH super-agonist	Implant			yes	yes	15	no	no	n/a	no
13	Efficacy and Health Effects of a Wildlife Immunocontraceptive Vaccine on Fox Squirrels	Krause S.K.	2014	Experimental	USA	Rodentia	Sciuridae	<i>Sciurus niger</i>	Squirrel	59	33m/24f	yes	yes	Immunocontraception	GnRH Vaccine	Injection	yes			yes	n/a	n/a	yes	abscesses at injection site, limping and weight loss	no
14	GonaCon Immunocontraceptive for Deer	NWRC USDA	2008	Review	USA	Artiodactyla	Cervidae	<i>Odocoileus virginianus</i>	Deer				yes	Immunocontraception	GnRH Vaccine	Injection	n/a	n/a	n/a	yes	n/a	n/a	n/a	n/a	n/a
15	Regulation of a small, discrete African elephant population through immunocontraception in the Makalali Conservancy, Limpopo, South Africa	Deisink A.K.	2006	Experimental	Africa	Proboscidea	Elephantidae	<i>Loxodonta africana</i>	Elephant	23			yes	Immunocontraception	PzP Vaccine	Injection	yes			yes	12	yes	no	n/a	no