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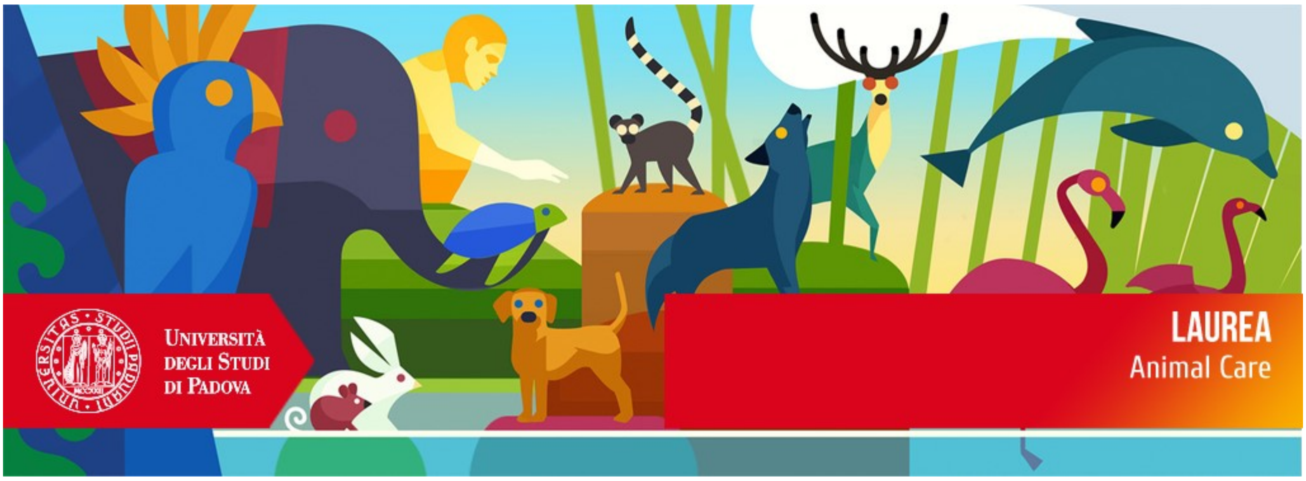

Contraception methods in wild animal species: a literature review

Supervisor

Prof. Milani Chiara

Laureanda/o/Submitted by

Segalla Emma

Matricola n./Student n.

1193310

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1. SUMMARY

Human interactions with the environment have altered the delicate equilibrium that defines relationships between animals and the ecosystem. As the population growth rates have been modified, humans have to support an equilibrated coexistence between species. The implementation of contraception can be used to control the number of animals without the need to cull them. A wide variety of contraceptive methods have been developed, ranging from synthetic hormones to Immunocontraceptive vaccines. Each formulation stops reproduction with a different approach, giving the opportunity to select the most appropriate ones for animal species that are supposed to be treated. Contraceptives have also been used to treat conditions, usually related to reproductive diseases or hormonal imbalances. Elephants can be regarded as a case study for the implementation of contraception in wild animals. With their numbers rapidly increasing in reserves as a result of human management, contraceptives have been employed to reduce the population growth rate. Immunocontraceptive vaccines have been tested, proving their efficacy in reducing the fertility of female elephants and consequently the population growth rate.

2. INTRODUCTION

As urbanization and agriculture take away more and more natural habitats from animals every year, it is becoming increasingly obvious that some form of animal control is needed to maintain the welfare of humans, animals, and the environment. An overabundance of animals can lead to ecosystem degradation, ruined food crops and danger and illnesses to humans, as high animal densities increase contact rates between animals and thus allow the spreading of diseases. Another threat is posed by invasive species, able to upset the balance of an ecosystem and cause harm to humans. In the past years the methods of choice were hunting, trapping, and poisoning, but with the popularization of animal welfare advocacy these methods are seen negatively by the public and may be illegal in places like urban areas or national parks. In the same way, institutions that host animals (zoos and aquariums) might need a way to control the growth rate of animal groups in their care. If lethal control cannot be the solution, to avoid causing unneeded suffering to the animals, the only other option to slow population growth is to stop reproduction (Turner er al., 2013).

After reaching this conclusion, it's important to define that a suitable contraceptive method should have the following characteristics (as reported by Massei et al., 2014):

1. **No or negligible side effects to the animal's behaviour, welfare and physiology.** This also includes the case in which an animal is pregnant or lactating. This factor is not as essential in species that do not have long gestation times, as the energy they invest in gestation is relatively low. In highly social species it's important that the social structure and sexual behaviors are intact, while in solitary species this would not overly influence their welfare.
2. **Highly effective, with a duration of at least one reproductive season after being delivered in a single dose.** The need for the method of the contraception to be reversible depends on the target species: a permanent contraceptive could be used on overabundant species, or species that cause human-animal conflict. On the other hand, a reversible contraceptive would be more useful in controlling the population growth, while allowing occasional births.
3. **Suppress reproduction in both sexes.** While it's sufficient to stop females from getting pregnant, the efficacy of the contraceptive in both sexes ensures that, even if it is not possible to administer it to the whole population, the effect is increased.
4. **No effect on the food chain.** This is meant to prevent affecting involuntarily other species, like predators, that could indirectly enter in contact with the contraceptive.

5. **Species specificity.** This characteristic is meant particularly for drugs that can be delivered in an oral formulation. The consumption of contraceptive by different species could lead to an imbalance of relative species abundance and consequently changes in species interactions and the functioning of the ecosystem. In the same way, it should not be toxic to the animals receiving it as well as to the person performing the delivery.
6. **Remotely deliverable.** Capturing an animal to administer an injection or place an implant creates stress for the animals and requires trained personnel to sedate and handle the animals.
7. **Inexpensive to produce and deliver.** Contraception is highly cost-ineffective compared to lethal control, especially when the target animal is sought after by hunters, but it can be more cost-effective than other methods when culling of the animals is more expensive.
8. **Stability under field conditions.** The drug should be easily transportable, and, in case of oral delivery, it shouldn't deteriorate too quickly when left for the animals.

In the following chapters the principal methods of contraception will be examined, highlighting the most important characteristics about their use on wild and captive animals.

3. PROGESTINS

Progestins were originally used as contraceptives for humans and later utilized on animals. They are available in many different formulations with various routes of administration.

Active ingredient:	**Commercial name:**	**Formulation (route of administration):**
Altrenogest	Regu-mate® (Merck Animal Health, USA)	Oral solution
Levonorgestrel	Norgestone® (Bayer AG, Germany)	Pill
Medroxyprogesterone acetate	Depo-Provera® (Pfizer, USA)	Intramuscular injection
Etonogestrel	Nexplanon®/Implanon® (Organon, USA)	Subcutaneous implant
Melengestrol acetate	MGA (ZooPharm division of Wildlife Pharmaceuticals, Colorado, USA.)	Intramuscular implant and oral solution

Table 3.1 Most used progestin-based drugs.

Progestins have an anti-estrogenic activity (https://eazarmg.org/documents). They interfere with many steps of the reproductive process, providing contraception by:

- Promoting the thickening of the cervical mucus, making it difficult for the sperm to penetrate.
- Altering the motility of the oviducts and the uterus, reducing ova and sperm transport.
- Altering the endometrium, so that less glycogen is available for blastocyst survival.
- At higher doses, by suppressing the surge of lutenizing hormone (LH) necessary for ovulation (Beck, 1991)

Progestins that are formulated in oral solutions or pills should be administered daily, as the half-life of the hormones is very short. Injectable formulations and implants are designed to release their contents over an extended period of time. The recommended dose for intramuscular injections is 2.5-5 mg/Kg BW every 2-3 months, with smaller species needing a higher dose compared to larger ones and New World monkeys requiring as much as 20 mg/Kg BW monthly. Implants are positioned subcutaneously and are effective for 3 years, although individual variability can increase the length of efficacy. It’s important to routinely check the position of the implants on the animals as they can move and get lost due to overgrooming of the animals or a wrong implantation. The effect of these contraceptives can begin as soon as 1-3 days, but in the case in which the stage of the animals' estrus cycle is unknown it is advised to keep males and females separated or use an alternative method of contraception for at least one week. The treatment for seasonal breeders should start at least one month before the expected beginning of breeding season. However, some species such as canids, mustelids and ursids should begin treatment more than 2 months before breeding season as estradiol increase during proestrus can intensify the deleterious effects of progestins on the uterus and mammary glands.

Progestins should not be administered to pregnant animals as they can cause a prolonged gestation, stillbirths, and abortions. Most of these contraceptives do not pose any risk in lactating animals, with the only exception being Nexplanon®, because etonogestrel is excreted in the milk.

Progestin based contraceptives are reversible after the cessation of the treatment. Signs of estrus can be observed as soon as 5 days after the end of the treatment but, depending on the individual and the taxa, it can take up to several months before normal ovulations return (https://eazarmg.org/documents, https://www.stlzoo.org/animals/scienceresearch/reproductivemanagementcenter/contraceptionrecommendatio/contraceptionmethods).

Since progestins alter the hypothalamic-pituitary-gonads hormonal cascade, they can interact with behaviors and mechanisms regulated by sexual hormones. Their use has been linked to mood changes, lethargy, and depression in some species. They can cause an alteration of elephant's social structure and have been reported altering lemur scent and potentially affecting their social dynamics. Progestins can induce a longer breeding season, resulting in weight loss and increased injuries to males. As some progestins bind readily to androgen receptors and are antiestrogenic, females could experience male like qualities such as increased aggression and development of male secondary sexual characteristics (Asa et al., 2005).

Carnivores are highly sensitive to progestin-induced diseases, with a significant risk of developing mammary and uterine diseases like cystic endometrial hyperplasia, endometrial mineralization, hydrometra, and pyometra that may cause permanent infertility. For this reason, they are contraindicated in animals with a previous or current history of uterine inflammation. Progestins can also cause weight gain and diabetes in carnivores (Asa et al., 2005).

Some progestin formulations are able to be absorbed through the skin, so it is recommended to wear gloves when handling those products (https://www.merck-animal-health-usa.com/species/equine/products/regu-mate). It is uncertain what consequences the low-level chronic exposure to these contraceptives through the food chain are, but they could include effects on human male fertility and fetus development, so they are deemed inappropriate for animals destined for consumption (Powers, 2011).

4. GNRH AGONISTS

Deslorelin, commercially called Suprelorin® (Virbac, France), is a synthetic gonadotrophin-releasing hormone (GNRH) analogue. It differs from native GNRH because of an aminoacid substitution at position 6 in the GNRH decapeptide, site where the molecule is most susceptible to cleavage, increasing the half-life of the synthetic hormone and the time of receptor occupancy (Magon, 2011). Suprelorin® is formulated in implants consisting of low-melting point lipids and biological surfactant that slowly but constantly release deslorelin over a long period of time (Herbert et al., 2005).

GNRH stimulates the production of luteinizing hormone (LH) and follicle stimulating hormone (FSH) and is secreted in a pulsatile rhythm that allows receptors binding during pulses. GNRH agonists mimic the function of GNRH but bind to the receptors for a much longer time. This causes an initial flare up response because the gonadotropins stored in the pituitary are released and can result in oestrus and ovulation in females, while males may experience a temporary enhancement of testosterone and semen production (Massei et al., 2014). After 3 to 4 weeks, it induces a hypogonadotropic and hypogonadal state in response to a desensitization sustained by the loss of GNRH receptors. GNRH agonists temporarily suppress the reproductive endocrine system by preventing the production of pituitary and gonadal hormones (Magon, 2011). This results in effects similar to those induced by ovariectomy or castration but can be reversed after the implant is depleted or removed (https://www.stlzoo.org/animals/scienceresearch/reproductivemanagementcenter/contraceptionrecommendatio/contraceptionmethods/suprelorin-deslorelin).

Suprelorin® is delivered as a subcutaneous implant, usually positioned between the scapulae, but alternative placements do not interfere with the efficacy of the contraceptive and can facilitate a later removal (Cowl et al., 2018). Implants can have different dosages, 4.7 mg or 9.4 mg, to be used depending on the species. If the animal requires a bigger dose, it is possible do administer more than one implant, but this cannot be used to increase the duration of the effect. Duration can decrease if the implant is broken, so particular attention should be observed during administration. The 4.7 mg implant has a duration of at least 6 months, while the 9.4 mg provides a minimum of 12 months of contraception, but the duration is heavily influenced by the species, the individual and the weight. There is a latency of effectiveness of up to 3 weeks, during which there is a flare up reaction triggered by the administration of deslorelin. This initial stimulation phase can be managed by separating the sexes for 3 weeks, administering oral megestrol acetate pills 7 days before and 7 days after the administration or leaving preexisting progestogen implants in place for up to a week;

leaving them for a longer time can compromise the efficacy of deslorelin. Seasonal breeders should be treated more than 2 months before the expected beginning of breeding season. Deslorelin should not be used on pregnant animals, as it can cause abortion and impede proper mammary development, but there are no contraindications once lactation has been established. The use in prepubertal and juvenile animals can cause a delayed epiphyseal closure of the long bones, but reproductive cycles begin normally after the treatment is ceased. As GNRH is a well conserved hormone among mammals, GNRH agonists are effective across a wide variety of taxa, but there are some exceptions like male bovids or marsupials (https://www.stlzoo.org/animals/scienceresearch/reproductivemanagementcenter/contraceptionrecommendatio/contraceptionmethods/suprelorin-deslorelin).

Once the administration of a GNRH agonist is discontinued either because the implant is removed or is completely expended, the animal becomes fertile again, however there is a wide range of variation of the timeframe this happens in (Lucas, 2014). This variability could be the result of different sensibility of individual animals to downregulation induced from GNRH (Herbert et al., 2005). In males the return to fertility following the use of deslorelin has to account for the recovery of steroidogenesis, the length of the spermatogenic cycle and the return to mating behavior, which can vary depending on the individual animal and the species. After the return to fertility of females, natural oestrus occurs and no differences on fertility or litter sizes has been reported (Lucas, 2014).

Deslorelin can cause the suppression of physical secondary sexual characteristics, especially in males who may lose muscles and become the size of females (https://eazarmg.org/documents). Weight gain can be observed as a result of increased appetite especially in felids (Munson, 2006). In females GNRH agonists change the number, size, and weight of follicles (Gray et al., 2010), while in males there is a decrease in ejaculate volume, sperm concentration and motility (Lucas, 2014). These changes are completely reversed after the implant's contents are depleted or it is removed. During the initial flare-up reaction species with induced ovulation may ovulate and experience a pseudo-pregnancy, and in males the stimulation of testosterone can cause increased aggression and sexual interest towards females (https://eazarmg.org/documents).

Deslorelin has been used in birds for the mitigation of unwanted associated reproductive behavior, such as aggression, feather destructive behavior and masturbation. It has also been employed for the treatment of reproductive diseases, as it can be used to decrease egg production, but the effect tends to be of shorter duration compared to mammals, most likely due to a difference in metabolism in avian species. Deslorelin implants have succeeded in controlling the increased aggression in male bearded dragons (Mans et al., 2014). The efficacy of GNRH agonists on reptiles varies drastically among species: Deslorelin implants were able to suppress female green iguanas' reproductive activity but had no effect on female veiled chameleons (Cermakova et al., 2019).

5. IMMUNOCONTRACEPTION

5.1. GNRH VACCINE

The GNRH vaccine is a synthetic incomplete analogue of GNRH conjugated to a carrier protein in an aqueous non-oil-based adjuvant (https://www.improvac.com/nz/product-overview.aspx).

The GNRH vaccine stimulates production of antibodies against the GNRH molecule. This leads to a diminished FSH and LH production by the anterior pituitary and consequently a reduction of ovarian follicular development for the females, and inhibition of spermatogenesis and testosterone secretion from the testes in the males (Massei et al., 2020).

There are currently two GNRH vaccines being used:

- Improvac® (Zoetis, Belgium), whose duration of effect is relatively short, requiring boosters every six months or yearly, depending on the species.
- Gonacon® (US Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS), Wildlife Services' National Wildlife Research Center (NWRC)), which has a formulation that induces production of antibodies for a longer period of time, allowing for multiple years of contraception with a single injection.

The GNRH vaccine is delivered as a subcutaneous or intramuscular injection. For seasonal breeders it is recommended to deliver the vaccination at least six weeks before the onset of the breeding season. After a group of animals is immunized, keeping males and females separated for a period of six weeks allows time for the effect of the vaccine to take place, otherwise an alternative contraception method is suggested to be put in place (https://eazarmg.org/documents).

The antibodies produced in response to the GNRH vaccine are relatively short lived; the brief duration of these antibodies allows an easy reversal of the vaccine effect and return to fertility.

The GNRH vaccine interferes with the pituitary-gonadal axis, so the production of FSH and LH from the pituitary gland, and estradiol, progesterone and testosterone from the gonads is interrupted. (Miller et al., 2013). This causes the effects of the immunization to resemble the ones of castration, but with limited duration. Animals do not perform reproductive behaviors while immunized; this could be beneficial for contraceptive programs aimed at animals that interact closely with humans (Imboden et al., 2006). While this kind of vaccine can be used in both males and females, studies demonstrate that females tend to be immunized for a longer period of time (Miller et al., 2013).

Miller et al. (2000) states that the biological efficacy of the GNRH vaccine can be measured by several factors:

- The immune response to the vaccine, measuring the antibody titers.
- The reduction of serum progesterone in females and testosterone concentration in males.
- Ultrasound and palpation of the females to confirm pregnancy or the absence of it.
- Behavioral responses during mating period.
- Natality response.

GNRH vaccines show, apart from the effects on reproduction, no significant consequences on the health of treated animals. Body condition can be improved in the short term, but this effect does not last in the long term (Massei et al., 2012). In vaccinated females the number, weight and size of the follicles change (Gray et al., 2010), while in males plasma testosterone and size of the testes are reduced.

A GNRH vaccine controls more effectively the production of LH than FSH, which results in a return of the vaccinated animals to breeding behavior before their return to fertility. GNRH vaccines do not induce estrus, ovulation, or production of semen (Herbert et al., 2005). Literature has demonstrated that these types of vaccine are safe in most pregnant animals, but they can potentially cause abortion in animals that rely on pituitary LH to maintain the corpus luteum supporting a pregnancy (Kirkpatrick et al., 2011).

The efficacy of any vaccine relies on the capability of an animal to have an immune reaction to the substance administered; given the differences among individuals, it is possible that the GNRH vaccine will not have an effect on animals with compromised immune systems or non-responders. If animals that do not respond to vaccination are the only ones that breed, it could lead to a natural selection for nonresponse or immunocompromised animals, rendering ineffective the vaccine (Herbert et al., 2005). Immunocontraceptive vaccines do not pose a risk of entering the food chain, as they are readily broken down when ingested (Massei et al., 2014).

The severity and frequency of injection site reactions differs among species and might be related to poor health and high parasite load of the animal. This adverse effect can be prevented by decreasing the dosage of the vaccine or the amount of adjuvant injected. The reactions can present themselves as swelling at the vaccination site and development of sterile abscesses and granulomas (Massei et al., 2014), lasting for at least three years (Powers et al., 2014).

GNRH receptors are present in many body tissues, like the cerebellum, cerebrospinal fluid, bladder, and cardiac tissue (Kirkpatrick et al., 2011). It is still uncertain if this kind of vaccine has any negative interactions with non-reproductive tissues, but it’s worth mentioning that, because the GNRH vaccine influences the reproductive process further upstream than other vaccines, the range of physiological mechanisms interrupted is wider, and can possibly be more problematic.

Vaccination of immature males can cause them to not grow to the same size as non-treated ones, have a higher amount of fat deposition, and they tend to appear more feminized, not developing secondary sexual characteristics; sex-specific processes, like antler growth and shedding can be altered (Hampton, 2017). In some species, like deer, boar and horses, the GNRH vaccine prevents reproductive behaviors (Miller et al., 2013); females do not show signs of sexual libido and males do not attempt to mount them (Killian et al., 2000). On the opposite side of the spectrum, female elk can perform precopulatory behaviors for a longer time period (Powers, 2011).

Seen as the GNRH vaccine interacts with a wide range of physiological mechanisms, it can be utilized for purposes other than contraception. Improvac® was originally developed for the use in domesticated livestock: it can help improve carcass characteristics by increasing lean meat, feeding efficiency and live weight. This vaccine is especially used on pigs to avoid the development of boar taint in males; boar taint, an unpleasant odor and taste of the meat, is caused by the accumulation in the fat tissue of androstenone and skatole, a pheromone and a byproduct of intestinal bacteria, whose breakdown by the liver is inhibited by testicular steroids (https://www.improvac.com/nz/technical-information.aspx).
As GNRH vaccines downregulate the synthesis of testosterone, they can be used to reduce male aggression during reproductive season (Bertschinger et al., 2005). This can be especially helpful in dangerous animals, such as elephants, who enter a frenzied state called musth during rutting season. Gonacon® has been proven to be an effective vaccine for preventing adrenocortical disease (ACD) in ferrets. Adrenocortical disease, caused by a continuous production of LH resulting from a lack of negative gonadal hormone feedback, is a common problem in neutered ferrets and leads to enlarged adrenal glands, presenting itself with symptoms such as alopecia, muscle atrophy, aggression, and hyperplasia of the adrenal glands. Although the GNRH vaccine is not as effective as a GNRH agonist for the treatment of ACD, it is successful in preventing the onset (Miller et al., 2013).

5.2. PZP (PORCINE ZONA PELLUCIDA) VACCINE

The PZP vaccine consist of two main components: the antigen and the adjuvant.

The antigen is derived from pigs' oocytes. The process of extraction starts with frozen-thawed ovaries that are sliced and the oocytes separated by filtering through increasingly finer screens with phosphate buffered saline. The resulting oocytes are then ground up, and the zonae separated with a screen, rinsed and heat-solubilized (Liu et al., 1989).

There are two adjuvants used for vaccination with the PZP vaccine. For the first inoculation, Freund's Modified Adjuvant (FMA) is recommended; FMA is comprised of 85% Drakeol 5NF(white mineral oil), 15% Arlacel A (mannide monooleate emulsifier), and 0.1% *Mycobacterium butyricum* dry cells. (https://www.sigmaaldrich.com/IT/it/product/mm/344289), while for the boosters Freund's Incomplete (FIA) adjuvant is used. FIA's components are the same as FMA, but lack the microbial component.

The zona pellucida is an extracellular matrix surrounding the plasma membrane of the oocyte. Among the different glycoproteins that constitute the zona pellucida the fraction ZP3, in particular, is the one responsible for sperm recognition, binding and acrosome reaction. The PZP vaccine stimulates antibodies production against the zona pellucida glycoproteins. As a result, the sperm can no longer penetrate the oocyte and fertilization is thus prevented (Sacco et al., 1987).

The PZP vaccine is used exclusively on females. The intramuscular injection can be delivered remotely, advantageous for the treatment of wild animals, due to the small volume needed. A booster is required 2-4 weeks after the initial inoculation. The initial treatment is delivered with Freund's Modified adjuvant, while the boosters use Freund's incomplete adjuvant.

Depending on the species, more boosters are required at intervals varying from every five months to once a year; evidence shows that Ovidae and Capridae can retain antibodies for up to 3-4 years from the initial dose and the first booster (Kirkpatrick et al., 2009). The vaccine is recommended for the use on ungulates, elephants, bears and pinnipeds. On the other hand, the PZP vaccine is ineffective on suids.

In captive animals it is recommended to keep males and females separated until the effect of the vaccinations has taken place, for a minimum of two weeks after the last injection.

The vaccination for seasonal breeders should start at least two months before the onset of breeding season, to allow the delivery and the effect of the vaccine, first injection plus the booster, to take place (https://www.stlzoo.org/animals/scienceresearch/reproductivemanagementcenter/contraceptionrecommendatio/contraceptionmethods/pzp-vaccine).

It was demonstrated that this vaccine is not dangerous to pregnant animals. A study conducted on wild mares that had been treated for multiple years, observed that not only the pregnant horses did not suffer complications from being inoculated with the vaccine, but the resulting foals later reached maturity and had foals themselves (Kirkpatrick et al., 1995).

Animals that will be used for breeding programs should not be treated for longer than 3-4 years; this is due to the fact that treatment lasting longer than 5 years has been associated with ovarian failure, damages to the ovarian follicles and cessation of ovarian function.

Reactions at the injection site are rare, but don't exceed the 1%. The incidence of reactions, as demonstrated by Kirkpatrick et al. (2011), is higher in animals treated remotely than in those hand-injected, most likely due to the bacteria and debris dragged in the injection site by the dart.

The PZP vaccine stops fertilization in a direct way and the zona proteins, the immunogens, have an absolute lack of cross-reactivity with unrelated hormones and tissues. Compared to other methods, it regulates fertility more downstream, so the processes that are disrupted are minimized and do not have consequences on other mechanisms (Kirkpatrick et al., 2011).
Seen as this vaccine only interferes with fertilization and not hormones, it will not change behaviors, but in seasonal breeders it can lengthen the duration of the reproductive season.

It has been recorded that animals immunized with PZP tend to have a better body condition score than unvaccinated ones, most likely due to the lack of energy expended during pregnancy and caring for the newborn.

6. EXAMPLES OF CONTRACEPTION USED ON ELEPHANTS

Although both African elephants (Loxodonta africana) and Asian elephants (Elephas maximus) are threatened in their range countries, contraception plans are required to manage their numbers. Around 70% of African elephants occur in southern Africa, and their rate of population increase is about 4-5% each year. Many smaller populations are held in fenced reserves. These space constrictions can be detrimental not only to the elephants, but also to other animal species and to the ecosystem as a whole. To fully grasp the dangers posed by an overabundance of elephants, it is necessary to understand how these animals can influence the environment they are part of. They are able to uproot trees in search of food, create paths through dense forests and also act as seed dispersers while traveling long distances looking for water sources. Elephants are a keystone specie for the environment they occupy, helping in maintaining a healthy balance for the benefit of the flora and the fauna, but their overpopulation can cause habitat degradation, consequently having a negative impact on the species that share the same space (Benavides Valades et al., 2012).

In large, open conservation systems overpopulation rarely occurs, as stochastic events such as fires, droughts and predation can maintain elephant populations at a sustainable level by eliminating the old, weak, sickly, and young individuals as well as slowing down the natality rate by stressing the females. However, especially in smaller reserves, these natural stochastic events are altered by the human management: the spread of large fires is prevented, droughts are mitigated by the continuous supply of water and food and predator-prey ratios are artificially modified. Thanks to this the mortality rate of elephants held in the reserves is lower and there is a stable birth rate, leading to an eruption of the population. The space constraints imposed by the fenced reserves, together with the high population numbers, can limit the expression of natural behaviors, can prevent natural movement patterns, can lead to negative interactions with human communities and can ultimately lead to population crashes. Therefore, it is imperative to adopt a population management plan to maintain the well-being of the elephants, the other animal species, and the ecosystem (Druce et al., 2011).

Culling elephants, although the most straightforward way to reduce their numbers, is not considered an acceptable solution, in part because of the public opinion but also for more technical reasons. If the management of a reserve decided to cull a number of individuals, it could cause severe disruption of the herds and, as elephants are very intelligent animals with strong social bonds, a general decrease of their welfare. A similar scenario would present itself if the animals were translocated instead of being culled, with the addition of the need to host the removed animals in a different location. To avoid herd disruption, it would be necessary to translocate the whole herd, but this can result in the overpopulation of the receiving reserve, while incurring in an exorbitant expense (Fayrer-Hosken et al., 2000).

If culling or moving animals are not viable methods of population control, reducing the birth rate is the only method to maintain a stable population of animals. Surgical sterilization of wild elephants is quite difficult and can pose many risks to the wellbeing of the animals. There are many concerns that come with such an invasive procedure:

- Health of the animals: a lot of safety issues can arise from a surgery, such as the need to sedate the animal and the successive healing period, which could have negative effects on the welfare of the elephant.
- Difficulty of execution: elephants are massive animals and due to the intraabdominal location of the testes in males, both sexes require a particularly invasive procedure to be surgically sterilized. Laparoscopic vasectomies have also been performed, but they still necessitate of specialized operators and expensive equipment (Lueders et al., 2017).
- Efficiency: the surgical sterilization of a high enough number of elephants would require such a large amount of time, money, and people that it is not considered a suitable method for the maintenance of elephant population.

Thus, the only solution remaining is implementing a contraception plan.

Hormonal contraceptives have been tested on one occasion, when 10 elephant cows were treated with subcutaneous estradiol implants. The unsuitability of this method was soon declared as the animals, although perfectly contracepted, entered a state of continuous estrus lasting at least 12 months, which resulted in constant harassment of the treated cows by bulls, distressing family groups and endangering young calves (Delsink et al., 2012).

Immunocontraceptive vaccines have proven to be an adequate method of contraception for these animals, with the first easily discernible benefits being their inexpensiveness, compared to other methods, and the possibility to be delivered from a distance. Numerous studies have been conducted testing the efficacy of Immunocontraceptive vaccines.

In a report by Benavides Valades et al. (2012), two injections of GNRH vaccine were not able to induce anestrus in the eight female elephants treated. The inefficacy was likely due to either the incomplete delivery of the vaccine while darting the elephants, the excessive length of time elapsed between the first inoculation and the booster, or the wrong timing of delivery, as the stage of the estrous cycle of the animals was unknown at the time of vaccination. GNRH vaccines can be employed not only as contraceptives, as for example:

Boedeker et al. (2012) report the use of this vaccine on an Asian elephant suffering from a reproductive tract pathology. In the wild, adult females experience comparatively few reproductive cycles as they are usually either pregnant or lactating, while captive ones develop reproductive pathologies such as endometrial hyperplasia and myometrial tumors, resulting in periodic mucohemorragic vaginal discharge. After four boosters of GNRH vaccine it was possible to observe that both ovaries appeared small and inactive, the uterus had decreased in size and no clot or active hemorrhage associated with the previously mentioned reproductive tract pathology were present.

A more widespread use of GNRH vaccines in elephants is the control of musth in bulls: during musth, a periodic condition caused by a spike in testosterone, males become aggressive and have been known to injure and kill several handlers in captive situations. In a case reported by Lueders et al. (2014) a young male Asian elephant was treated for more than 4 years with GNRH vaccine injections (see Table8.1). The concentrations of serum testosterone of the animal remained baseline from the second injection for the duration of the observations and he showed no sign of musth. Along with the arrest of sperm production, a significant decrease in the size of the penis, the testicles, and the accessory sex glands were observed. Although these examples show the successful contraceptive activity of GNRH vaccines, it is important to consider the fact that these trials have been carried out on a small number of animals, and more research is needed in order to understand the potential to employ this kind of vaccine on a larger scale.

As we can observe from Table 8.1 numerous trials of vaccination with PZP vaccine have been performed. Fayer-Hosken et al. firstly demonstrated the similarity of PZP and elZP (elephant zona pellucida), confirming that the vaccine could induce an immune reaction towards elZP. They then performed vaccination trials on 3 elephants, observing the significant increase in anti-PZP antibodies. These trials set the basis to begin following field trials. The first trial, described by Fayer et al., was somewhat disappointing, as the contraceptive efficacy was only 56%, but by decreasing the interval of administration of the boosters in the second trial the efficacy rose to 80%. Several more field trials have been performed, resulting in a contraception efficacy of 100% (Bertschinger, H., 2011). While the PZP vaccine has been proven to be a reliable and practical method of contraception in elephants, there are some important facts that should be noted. First of all, the price: although the vaccine in itself is quite expensive, the greatest cost incurred during the vaccination of wild elephants are professional fees, in particular hiring a helicopter; this implies that the application of this method of contraception in large reserves requires a considerable amount of funds, not always available (Bertschinger, H., 2011). Another important aspect to consider when implementing a contraceptive plan in an elephant population is the effect it will have on the psychological wellbeing of the animals: the prolonged use of immunocontraception, preventing the birth of calves in a population, could give rise to several social problems such as a lack of allomothering, due to the lack of newborn calves, and the depression of adult females because of their continual oestrus cycles. As adult female elephants spend most of their life either pregnant or lactating, it could be stressful not being able to calve for a long period of time. To preserve the wellbeing of the animals it is possible to employ a rotational Immunocontraceptive plan: in order to increase the inter-calving interval for the individual female, but at the same time allow for the social needs of the herd to be met, it is possible to allow one female to give birth every 2-3 years, rotating the births between the females (Druce et al., 2011).

Reference	**Contraceptive method**	**Sex**	**N° animals**	**Interval of booster administration**	**Results**
Fayer et al., 1999	PZP vaccine	Females (2 African, 1 Asian)	3	First booster after 4 weeks Second booster after 10 months	Blood levels of anti-PZP antibodies sufficient for effective contraception
Fayer et al., 2000	PZP vaccine	Females	21	First booster after 6 weeks Second booster after 6 months	9 pregnant, 10 not pregnant
	PZP vaccine	Females	10	First booster after 2 weeks Second booster after 2 weeks	2 pregnant, 8 not pregnant
Bertschinger et al., 2018	PZP vaccine	Females	18	Two boosters at 2-3 week intervals Annual boosters	Contraceptive efficacy of 100%
Bertschinger et al., 2012	PZP vaccine	Females	108	Two boosters 3-6 weeks apart Annual boosters	Contraceptive efficacy of 100%
Benavides Valades et al., 2012	GNRH vaccine	Females	8	Single booster after 5-7 weeks	Failed to induce anestrus
Lueders et al., 2014	GNRH vaccine	Male (Asian)	1	Boosters on month 0, 1, 2, 3, 4, 5, 11, 17, 23, 29, and 53	Basal levels of testosterone, no spermatozoa in ejaculate, no sign of musth
Boedeker et al., 2012	GNRH vaccine	Female (Asian)	1	Boosters at 14 weeks, 8, 12 and 20 months	Cessation of ovarian ciclicity

Table 8.1: results of trials with different Immunocontraceptive vaccines and booster intervals; the trials have been performed on African elephants unless specified.

7. CONCLUSION

The subject of animal contraception is complex and constantly evolving. It is not possible to rule one method as the universally better option as it is important to consider a wide range of aspects before choosing what is more suitable to each situation. It is imperative, when designing a contraceptive plan for an animal or a population, to carefully examine the advantages and disadvantages that different contraceptive methods have to offer. The ability of a contraceptive method to affect hormone production and hormone-driven reproductive behavior, as an example, can be beneficial if the animals pose a risk to humans they are in close contact with. On the other hand, the repression of natural behaviors could be unsuitable for animals living in the wild. If the social needs of a population are not met the resulting social disruption has the potential to generate an overall decrease in animal welfare. For this reason, each situation in which a contraceptive is supposed to be applied should be carefully weighed to choose the most suitable method. To establish this, professionals involved should be able to identify the characteristics of an animal, such as its social needs, and the overall objective of the contraceptive plan. As some methods can reduce the future fertility of the animal or lead to physical changes, such as feminization or masculinization of certain individuals, they could decrease the chance of the treated animal to reproduce in the future. This would be highly inappropriate for animals that will be allowed or required to breed after a long period of contraception. On the other hand, a contraceptive method that is not reversible would be considered the optimal solution to avoid the need to deliver the medication multiple times. It is also worth considering the expenses created by a contraceptive plan, as time and money invested in the delivery of a contraceptive could influence the decision-making process.

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