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*RESEARCH ARTICLE*

# REVIEW ON CHEMICAL STERILIZATION OF MALE DOGS.

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***Abstract***

The aims of this review were to illustrate the spectrum of fertility inhibitors or chemical sterilizations available for male dogs. The uncontrolled growth of a dog population can have a negative impact on public health and can create socioeconomic, political, and animal welfare problems. Consequently, several cities of the world, tried spay and neuter operations to prevent the persistent increase in the canine populations. Although surgery is the most effective and safe procedure, it is also expensive so use of non-surgical, sterilization methods that would make male sterilization inexpensive, easy and fast for sterilization of large number of male dogs within short period of time to effectively contribute to curb the growth of the stray dog population were introduced. Chemical sterilization methods so far employed included hormonal methods, immunocontraceptives and Inorganic Chemo-sterilants (chemo-sterilants such as calcium chloride - $CaCl_2$, zinc gluconate neutralized by arginine (Neutersol) and hypertonic sodium chloride -NaCl solution). At present the most promising methods are the immunocontraception/sterilization vaccines and Inorganic Chemo-sterilants. Intratesticular injection of calcium chloride ($CaCl_2$), Zinc gluconate (Neutersol), and 20% NaCl hypertonic solution showed a promising result as chemical sterilants. The review concluded that the main challenges for the future are evaluating the feasibility, effectiveness, sustainability, and effects of mass nonsurgical sterilization campaigns on dog population size and impact as well as integrating nonsurgical fertility control with disease vaccination and public education programs. Accordingly, it is recommended that immunocontraceptives and male sterilants need to be given further attention they deserve for application of dog population management. Therefore chemical sterilization methods have to be experimentally explored for their efficacy and potential applicability under local condition for the development of additional more cost effective sterilization tool than surgical sterilization.



## Introduction:-

The global dog population is estimated to be around 700 million (Hughes and Macdonald, 2013). It has been estimated that approximately 75% of the global dog population are free roaming or stray dogs, living mostly in Latin

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America, Africa and Asia (Matter & Daniels, 2000; WHO/WSPA, 1990). National and international organizations working on dog population management and welfare often classify dogs in the following categories, according to ownership and degree of confinement: 1) owned and permanently confined within household premises; 2) owned by a single household but free to roam; 3) "community owned," with several households or people providing food and shelter but free to roam; or 4) ownerless and free-roaming. As a consequence of uncontrolled reproduction, canine overpopulation remains a problem facing many countries throughout the world, where it creates locally overabundant populations of animals that are often in poor health and have a high turnover because of low survival rates (ICAM, 2007).

The uncontrolled growth of a dog population can have a negative impact on public health and can create socioeconomic, political, and animal welfare problems (Downes *et al*., 2009; OIE, 2016; Salamanca *et al*., 2011). Problems caused by free-roaming dogs include diseases transmitted to livestock and humans, predation on livestock, bites, road traffic accidents, and nuisance such as barking and soiling of parks and recreational areas (Jackman and Rowan, 2007; Macpherson *et al.*, 2013).

Among the zoonoses, rabies is of particular concern for humans and livestock. Dogs are the main vector (75% - 99%) of rabies transmission to humans (Garde *et al*., 2013). It is estimated that canine mediated rabies kills between 30,000 and 60,000 people annually worldwide (Garde *et al*., 2013). The majority of deaths and post bite vaccinations occur in Asian and African countries, which can barely afford this economic burden (Knobel *et al*, 2005). Dogs also pose serious threats to wildlife and biodiversity as predators, competitors, transmitters of disease, and by interbreeding with native species (Hughes and Macdonald, 2013; Young *et al*., 2011).

There are two techniques for sterilization known as surgical and chemical. Surgical castration removes the testes from the scrotum via an incision in male animals. This method is effective, but infection or bleeding can become a problem and it is also time consuming, not cost-effective and needs skilled surgeons. Moreover, this method is not effective for large-scale application, especially for controlling large population size of undesirable mammals in the community like stray dogs. Besides this, postoperative care and management of the animal are also required to prevent infection (Jana *et al*., 2005). Although vasectomies and vasal occlusion are less invasive surgical procedures than castration, these procedures also carry similar anesthetic risks and postsurgical complications (Jana and Samanta, 2007).

In order to minimize post-operative complications and costs associated with conventional surgical castration, other in situ noninvasive approaches have been used which include immune-castration and chemical castration and more recently natural oil castration (Dube *et al*., 1987; Jana and Samanta, 2007 and Abshenas *et al*., 2013). Chemical orchidectomy with chemical agent has been suggested as a fast and low cost alternative which could be used in a wide range of canine populations, especially in poor regions where problem is more intense (Soto *et al*., 2007).
Advantages of nonsurgical chemical sterilization are apparent reduction of pain and stress, and elimination of hemorrhage, hernia, infection, myiasis and other surgical sequelae (Cohen *et al*., 1990). Consequently, several attempts have been made in search of promising effective chemical agent, which included intratesticular injections (ITIs) of a various chemicals to promote castration in various species such as primates (Kar *et al*., 1965), rodents (Emir *et al*., 2011), canine (Pineda *et al*., 1977; Jana and Samanta, 2007; Oliveira *et al*., 2012), feline (Pineda *et al*., 1984), bovine (Neto *et al*., 2014), donkey (Ibrahim *et al*., 2016) and caprine (Jana *et al*., 2005).

The chemical agents tried to produce castration include hormones such as androgen (Matsumoto, 1988), progestagens (Swerdloff *et al*., 1992), androgens plus progestagens (Wu and Aitken, 1989; Dube *et al*., 1987) and agonists for gonadotrophin releasing hormone (Trembley and Belanger, 1984) as well as chemical like cadmium chloride (Parizek, 1960), Danazol (Dube *et al*., 1987), glycerol (Wiebe *et al*., 1989), lactic acid (Fordyce *et al*., 1989), ferric chloride and ferrous sulphate (Kar *et al*., 1965), calcium chloride (CaCl) (Jana and Samanta, 2007; Ibrahim *et al*., 2016), Zinc gluconate (Neutersol), and 20% NaCl hypertonic solution (Kwak and Lee, 2013) have been used for induction of chemosterilization.

The use of non-surgical, inexpensive and easy sterilisation methods of a large number of male dogs would effectively contribute to curb the growth of the pet population. Alternative methods to surgical sterilization that are effective, easy to administer, safe, and affordable would offer immense benefits, allowing animal welfare organizations, public health programs, and governments to reach further with limited resources (Briggs, 2012). Several new methods of inhibiting fertility in male dogs have been under extensive research. However, a comprehensive review on their efficacy, possibility for large scale application and limitations of each chemical technique is lacking. Therefore, the objective of this paper is to review on the available experimented chemical sterilization methods used on male dogs and to assess the possibility of their applicability in our local situation for adoption and exploitation of the best techniques.

## Literature Review:-

### Stray Dogs:-

Definitions of stray dogs are inherently problematic and judgments regarding when a dog is considered to be a stray varies from country to country and may be subject to local and national regulations. Indeed any dog, found unaccompanied by a responsible person in a public place may, in some countries, may be considered as stray and collected accordingly. Conversely, at the other end of the scale, unwanted dogs, from which owners have revoked all care giving responsibilities, may be considered to be genuinely ownerless and in some instances they could be feral (Gueseva, 2013). Feral dogs, those that are truly independent of human care givers are rarely considered to be salient contributors to the problem of strays as their survival rates are invariably low and their reproductive success is likely to be poor (ICAM, 2007).

Somewhere between the two examples, dogs may be cared for by one or more members of a community, allowed to roam and permitted to reproduce. Nevertheless, they are genuinely dependent upon human caregivers, as they provide access to the resources essential for their survival. The reproduction rates of these dogs and their rearing success has the potential to be high because care given by humans offers the necessary protection for puppy survival (ICAM, 2007).

Some of the explanations given for why some countries have larger stray dog populations than others are related to economic development, quality of political institutions, and comprehensive pet ownership laws of a country. First, as the wealth of a country increases the size of the stray dog population within its borders decreases. Second, democracies tend to have a small number of stray dogs. Third, countries with well-regulated dog ownership legislation have fewer stray dogs (Gueseva, 2013).

### Dog classifications:-

The dog population can be divided into different subpopulations (Beck, 2000, ICAM, 2007; OIE, 2009; WHO and WSPA, 1990): 1) Dogs that never roam (owned dogs that are fully dependant and restricted/supervised); 2) Free roaming dogs/stray dogs (owned dogs that are fully or semi-dependant, sporadically or continuously roaming or dogs that are lost and therefore free roaming); 3) Neighborhoods/community dogs that are semi- or fully dependant and semi- or unrestricted; 4) Feral dogs that are independent and unrestricted (Elina, 2010).

### Problems associated with stray dogs:-

Stray animals, often experience poor health and welfare, related to a lack of resources or provision of care necessary to safeguard each of their five freedoms. Furthermore, they can pose a significant threat to human health through their role in disease transmission. A summary of the problems arising from stray dogs is given in Table 1.

**Table 1:-** Problems associated with stray dogs (Gueseva, 2013).

| **Factor** | **Dogs** |
|---|---|
| Public Health<br>1. Zoonosis – Disease transmission<br><br>2. Bite incidence | >100 zoonotic diseases identified; pathogens transmitted from dog to human<br>– varying degrees of severity<br>– varies with location<br>- Dogs may be responsible for bite occurrences – varies from region to region, severity of bite – rabies transmission |
| Environmental contamination | - Deposition of excreta near or in areas inhabited by people<br>- Potential genetic contaminators of wild Canidae populations |
| Nuisance factors | - Noise: Barking, howling, aggressive interactions<br>- Odour/aesthetics: Territorial urine marking, faecal contamination and deposition of urine during elimination in the environment. |
| Wildlife | - Predating smaller wild mammals |
| Damage to property & livestock | - Result from accidents<br>- Predation of livestock or game |
| Animal welfare | - Injury resulting from car accidents |

| | |
|---|---|
| | - Injury from aggressive confrontation during competition for limited resources<br>- Malnutrition due to limited availability of suitable food sources<br>- Disease susceptibility<br>- Inhumane culling methods, stray control measures<br>- Persecution/deliberate abuse by members of the community |

The most important canine zoonoses include rabies, leptospirosis, Chagas disease, echinococcosis, and leishmaniasis (Garde *et al*., 2013). Many of these canine pathogens are shed in the feces (e.g. *Echinococcus granulosus*) or urine (e.g. *Leptospira*) of infected animals, making fecal and urine contamination of public spaces an important route of pathogen transmission to humans (Himsworth *et al*., 2010).

Rabies is caused by fatal encephalitis in most mammals including humans. Animals like dogs, bats, raccoons, skunks and foxes act as reservoirs and the virus is transmitted through bites and licking. The incubation period can vary between 2 weeks and several years, with an average of 2-3 months (WHO, 2005). The epidemiology of dog and human rabies depends on the human-dog relationship and the structure and density of the dog population (WHO, 1987; Rinzin, 2015).

In addition to disease risks, dog bites and attacks are also public health and safety concerns. The consequences of dog bites include physical injury, the psychological effects inflicted upon the victim, permanent or temporary disability and disfigurement, as well as the economic cost associated with medical treatment (Palacio *et al*., 2005; Tenzin *et al*., 2011). In severe cases, dog bites and aggression can result in human death (Matter and Arbeitsgemeinschaft, 1998; Quiles *et al*., 2000; Palacio *et al*, 2005).

It is important to develop long-term, sustainable strategies to deal effectively with stray animal population control. This is essential not only to protect humans from coming into contact with those animals but to protect the health and welfare of the animals themselves. Experience shows that effective control involves the adoption of more than one approach (WHO/WSPA, 1990; ICAM, 2007). In male dogs, castration has numerous advantages in addition to sterilization, mainly related to removal of the main source of testosterone. Castration controls urine marking in most males and reduces the tendency to roam in search of in-season bitches (Duffy and Serpell, 2006).

**Methods of Chemical Contraception and Sterilization:-**

Reproduction control utilizing chemical or immunological methods offers a humane and less expensive alternative to surgical sterilization (FAO, 2014). Contraception in dogs can be achieved through chemical reproductive control, which prevents pregnancy by temporarily or permanently sterilizing these animals (Kutzler and Wood, 2006; Munson, 2006). Chemical fertility control can be achieved through contraception, which prevents the birth of offspring but maintains fertility or by sterilization, which renders animals infertile (Kutzler and Wood, 2006; Munson, 2006). Chemical sterilization methods so far employed include hormonal methods, immunocontraceptives and Inorganic chemo-sterilants such as calcium chloride - $CaCl_2$, zinc gluconate neutralized by arginine (Neutersol), sodium chloride -NaCl solution.

**Hormonal methods:-**

Steroidal hormones (e.g. progestin, estrogens, and androgens) administered via oral tablets or by parenteral injections, have been used as reproductive inhibitors in owned dogs (Rojas *et al*., 2011; ACC&D, 2013; Massei and Miller, 2013) and have the ability to temporarily control the reproduction of dogs by delaying their estrous cycles. GnRH is one target for fertility inhibitors. GnRH controls the release of the pituitary gonadotropins, Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH), which in turn control the production of sex hormones and ultimately ovulation, spermatogenesis, and sexual behavior (Massei and Miller, 2013).

Other hormonal methods are based on Gonadotropin-releasing hormone (GnRH) agnostics (proteins that act like GnRH and stimulate the production and release of FSH and LH). These methods can postpone puberty in both sexes and can also control the reproductive cycle (Romagnoli *et al*., 2009; Rojas *et al*., 2011; Massei and Miller, 2013; Kisiel, 2017). Among GnRH agonists, deslorelin (Suprelorin, Virbac), can suppress reproduction in male dogs for at least a year (Trigg *et al*., 2001). Deslorelin is currently registered only for use in male dogs in Australia and Europe (Massei and Miller, 2013).

Another GnRH agonist is azagly-nafarelin (Gonazon, Intervet International B.V.). Gonazon has shown to decrease the concentration of testosterone for at least 6 months (Goericke-Pesch *et al.*, 2010). Gonazon is currently approved in the European Union, but has not been yet brought into the European Union market (Massei and Miller, 2013; Kisiel, 2017).

**Immunocontraceptives:-**

Inmunocontraceptive vaccines are another option and work by inducing antibody production against proteins or hormones essential for reproduction (e.g. gonadotrophins and GnRH) and therefore, can prevent pregnancy (Rosado, 2009; Miller *et al*., 2009; McLaughlin and Aitken, 2011; Massei and Miller, 2013). GnRH based vaccines (different than GnRH agnostics), target GnRH and prevent ovulation and spermatogenesis. Zona pellucida (ZP)-based vaccines inhibit egg–sperm binding and fertilization. These vaccines are commonly used for population control in wildlife species but may have additional application for the control of domestic dog populations (Miller *et al*., 2009).

GonaCon is a GnRH-based vaccine, recently registered as a contraceptive for white-tailed deer, horses, and feral donkeys in the United States (Killian *et al*., 2008; Miller *et al*., 2008; Massei and Miller, 2013). This product was shown to induce infertility for at least 1 to 6 years after a single injection. GonaCon has not yet been proven effective in dogs; however, early trials of ZP-based vaccines in dogs have shown promising results. Early formulation of GonaCon tested in dogs "showed abscesses and draining at the injection site after the injection" (Massei and Miller, 2013).

A new formulation has been produced since then and tested in a pilot study carried out on captive dogs in Mexico (Massei and Miller, 2013; Vargas-Pino *et al*., 2013). This new formulation did not result in abscesses at the injection sites. Currently GonaCon is not commercially available for dogs; however, the US Department of Agriculture, Animal and Plant Health Inspection Service are actively investigating the licensing, manufacturing and distribution of this product (Massei and Miller, 2013; Kisiel, 2017). The research results indicate that immunization techniques vary in effectiveness and in duration of azospermia. Adverse vaccination reactions are also observed as another disadvantage of this method (Rosado, 2009).

**Inorganic Chemo-sterilants:-**

Inorganic Chemo-sterilants are non-surgical approach to male contraception which has been developed as an alternative to surgical castration in male dogs. An ideal Inorganic Chemo-sterilizing agent would be one that effectively arrests spermatogenesis and androgenesis as well as libido with absence of toxic or other side effects (Ibrahim *et al*., 2016). Advantages of Inorganic Chemo-castration are apparent reduction in pain and stress as well as elimination of hemorrhage, hernia, infection, myiasis and other surgical sequelae. It is simple or technically not demanding and inexpensive suited for mass-scale sterilization (Koger, 1978; Ibrahim *et al*., 2016). The low cost, ease of use, and cultural acceptance of a sterilization method that does not require removal of the testes make Inorganic Chemo-sterilants a valuable tool for large-scale sterilization campaigns, particularly in areas lacking clinical facilities or skilled staff (Kutzler and Wood, 2006; Levy *et al*., 2008; Soto, 2009; Massei and Miller, 2013).
Researchers have also tried various Inorganic Chemo-sterilants agents such as cadmium chloride (Parizek, 1960), ferric chloride and ferrous sulphate (Kar *et al*., 1965), danazol (Dixit *et al*., 1973), Bacillus Calmette–Guerin (BCG) (Naz and Talwar, 1981), glycerol (Wiebe and Barr, 1984) and lactic acid (Fordyce 1989) for chemical castration by intra testicular injection in laboratory and domestic animals. All these agents, after intratesticular injection, exhibited pain, pyrexia and even severe inflammation (orchitis). Some agents, e.g., cadmium chloride, glycerol, lactic acid, caused selective destruction of testicular tissue (Parizek, 1960; Immegart and Threlfall, 2000) with reversible testicular tissue damage (Heath and Arowolo, 1987).

In some cases, the interstitial portion regenerated after an initial phase of testicular atrophy, and this led to secondary male behavior, which caused management problems of the animals (Fordyce, 1989). Due to the above complications caused by the use of aforementioned chemicals, an effective chemosterilizing agent is yet to be established. Very recently, an attempt has been made to induce sterilization by intratesticular injection of calcium chloride ($CaCl_2$) (Jana and Samanta, 2007), Zinc gluconate (Neutersol) (FDA, 2003), and 20% NaCl hypertonic solution (Kwak and Lee, 2013) in male animals. These are injected in the testes, epididymis, or vas deferens and cause lack of sperm in semen and thus infertility.

**Calcium chloride ($CaCl_2$):-**

Nonsurgical male sterilization techniques have been evaluated as a means to avoid the potential health complications, expense, expertise and facilities required for surgical sterilization procedures. One of the most promising is calcium chloride ($CaCl_2$), which has been utilized to chemically castrate a variety of species since 1977 (Koger, 1977).

Calcium chloride ($CaCl_2$), delivered as intratesticular injection, is being researched as Inorganic Chemo-sterilants for dogs. Following intratesticular injection of $CaCl_2$, necrosis, fibrosis and degeneration of seminiferous tubules and Leydig cells occurs, reducing or eliminating the production of spermatozoa, testosterone and sperm counts in a dose-dependent manner in male dogs (Jana and Samanta, 2007). Although $CaCl_2$ did not affect dogs' food intake, chronic stress, or blood parameters, swelling of the testicles persisted for 3 weeks following injection and the behavior of the animals returned to normal (although no definition of normal was provided) a month after treatment (Jana and Samanta, 2007). More studies are ongoing to standardize and validate formulation, dosage, and administration protocol for $CaCl_2$ (ACC&D, 2013; Massei and Miller, 2013).

Intratesticular injection of calcium chloride has been applied to a variety of animal species including rats ( Jana and Samanta, 2002; Jana and Samanta, 2006), dogs (Jana and Samanta, 2007; Leoci *et al*., 2014; Vanderstichel, 2015), cats (Jana and Samanta, 2011), goats (Jana *et al*., 2005; Mohammed and James, 2013), bulls (Koger, 1978; Mitra and Samanta, 2000; Canapolat, 2006) and donkey (Ibrahim *et al*., 2016), in a variety of formulations and concentrations (Jana and Samanta, 2011).

Dr Jana and colleague Dr PK Samanta have been credited with pursuing the method after it was forgotten in the 70s and bringing it to the 21 century. They concluded that an intratesticular injection of $CaCl_2$ was effective and economical for the sterilization of male dogs. It is free from pain and chronic stress and will contribute to a simple alternative (Jana and Samanta, 2007). Also Jana and Samanta, (2011) reported that: A single bilateral intra-testicular injection of calcium chloride solution is effective, economical, easy to perform and does not require to removal of testis in cats. It causes permanent sterilization and is a simple alternative method to surgical castration (Jana and Samanta, 2011).

More recently, two peer-reviewed papers were published in Scandinavian journal Acta Veterinaria Scandinavica in October 2014. Researchers conducted a year-long study on 80 dogs and found that one injection of 20% calcium chloride in ethyl alcohol solution was optimum for rendering them azoospermic and lower testosterone levels by 70%. No side effects were recorded (Leoci *et al*., 2014).

Single intratesticular injection of calcium chloride in male dogs is effective on male reproductive system and may be used as substitution of surgical castration. In this year (2017), Soumendra and Shyamal study were evaluated the effect of intratesticular calcium chloride ($CaCl_2$) injection on reproductive system of male albino rats and they concluded that, intratesticular injection of $CaCl_2$ has its major role on male reproductive system impairing various key reproductive parameters including testicular histology keeping other general parameters intact. So it can be declared that, intratesticular $CaCl_2$ injection has potent sterilizing activity (Soumendra and Shyamal, 2017).

Procedure of Intratesticular injection of $CaCl_2$ solutions: Each intratesticular injection can be performed using a sterile 21-gauge needle directed from the caudo-ventral aspect of each testis approximately 1 cm from the epididymal tail and towards the dorso-cranial aspect of that testis, so that the solution can be deposited over the entire route by linear infiltration while withdrawing the needle from the proximal end to the distal end. Necessary care should be taken to avoid the seepage of the solution from the injection site. All the dogs should be restrained through a gentle handling and proper care. The intratesticular injections should be given very carefully like any other intramascular injections. The animals can also restrained through proper handling procedure in prior experiment in the case of vaccination and blood collection, etc. The animals should keep under routine clinical observations (Jana and Samanta, 2007; Leoci *et al*., 2014)

**Zinc gluconate neutralized by arginine (Neutersol):-**

The ideal method of chemical sterilization needs to meet three key criteria to be regarded as a good alternative to surgical sterilization. First, it has to be effective in a high percentage of treated animals. Second, it should have a high margin of safety, without adverse effects for the environment. Third, it has to be permanent and irreversible following a single treatment. The first product obviously fulfilling these criteria was zinc gluconate. It was first described by Fahim (1993), who injected Neutersol (Pet Healthcare International, Inc., Columbia, MO, USA), a Zinc Gluconate-based chemical sterilant, into the epididymides of dogs. Subsequently, Neutersol was injected into the testes of puppies and in the testes of adult dogs (Oliveira *et al*., 2012).

Neutersol®/ Esterisol®, is a zinc-gluconate solution neutralized to a pH of 7 by arginine (Kutzler and Wood, 2006). The procedure involves injecting a predetermined amount of zinc solution based on scrotal width into each testis of puppies 3-10 months of age (Kutzler and Wood, 2006). Histological findings within 2.5 months of injection induced almost complete fibrosis of the seminiferous tubules and Leydig cells (Kutzler and Wood, 2006; Tepsumethanon, 2005).

Zinc gluconate neutralized by arginine (Neutersol, Addison Biological Laboratory Inc.) was approved in 2003 by the US Food and Drug Administration for chemical Sterilization of male puppies. Injected in to the testicles, this chemical causes sclerosis of the testes and sterility. Neutersol induced sterilization in 99.6% of the 223 male puppies aged 3 to 10 months (Wang, 2002). Although the treatment with this chemical does not require general anesthesia, sedation is recommended to prevent movements of the dog during injection. Correct injection technique was found critical for the safe use of Neutersol in order to avoid ulceration of the scrotum and painful swelling of the testes (Kutzler and Wood, 2006).

Though Neutersol® is not currently available in the U.S., a similar compound, Esterisol®, is on the market in Mexico. However, neither compound produces a decline in testosterone long-lasting enough to significantly reduce nuisance behavior. Studies using these models of male contraception report no or minimal signs of discomfort following injection, but a transient increase in testicular diameter may follow the injection, resulting scrotum swelling. Additional local and systemic reactions reported after intra-testicular injections include scrotal ulceration and dermatitis, scrotal self-mutilation, preputial swelling, vomiting, diarrhea, anorexia, lethargy and leukocytosis (Kutzler and Wood, 2006; Jana and Samanta, 2011). Also, unlike surgical castration, this kind of chemical sterilization does not eliminate gonadal sources of testosterone (Kutzler and Wood, 2006; Jana and Samanta, 2011).

Ark Sciences introduced Esterilsol in Mexico in 2008 and began selling the product to private practice veterinarians, government programs, and nongovernmental organizations (NGOs). In 2010 Esterilsol received regulatory approval in Bolivia, Panama, and Colombia. In Mexico, Colombia, Bolivia, and Panama, Esterilsol is approved for use in dogs three months and older. In Colombia it is also approved for use in cats. International distribution is temporarily on hold while regulatory agencies review paperwork from the new manufacturer. Ark Sciences has announced plans to extend distribution to additional countries. In some countries in which Esterilsol is not approved by regulatory agencies, it has been used on a limited basis in field research projects with government permission.

In a study carried out in the Galapagos, severe injection-site reactions occurred in 3.9% of the 103 dogs treated with zinc gluconate; basal testosterone concentration in treated dogs decreased initially but was similar to untreated dogs 2 years after treatment (Barnett, 1986; Levy *et al*., 2008). Thus, secondary male characteristics such as roaming, marking, aggression, or mounting may be displayed (Massei and Miller, 2013).

Zinc gluconate is currently available in Mexico, Colombia, Bolivia, and Panama as Esterilsol and in the United States as Zeuterin (both through Ark Sciences, New York). The cost of Esterisol is about US$15 per dog (medium size) (ACC&D, 2013; Massei and Miller, 2013). A similar formulation has regulatory approval in Brazil as Infertile (Rhobifarma Indústria Farmacêutica) (ACC&D, 2013; Massei and Miller, 2013).

A study carried out with Esterilsol in Mexico found that this compound induced azoospermia (absence of sperm) or aspermia (absence of semen) in 52 of the 53 dogs administered a single dose per testis (Esquivel, 2006). Ulcers related to poor injection technique occurred at the injection site in 2.6% of the dogs; however, their incidence decreased when proper injection technique, such as using new needles for each injection, were employed. A similar study conducted in Brazil (Soto *et al*., 2009) for dogs concluded that zinc gluconate could be regarded as a permanent sterilant with no observed sign of behavioral alteration or severe discomfort following intratesticular injection (Soto *et al*., 2009).

Another study in Isabela Island concluded that, The low cost, ease of use, and cultural acceptance of a castration technique that does not require removal of the testes make zinc gluconate a valuable option for large-scale use, particularly in remote locations lacking sophisticated clinical facilities or skilled surgeons and staff (Levy *et al*., 2008).

Use and effectiveness: Esterilsol is administered via an injection to each testicle with either a 28 gauge, ¾ inch or a 30 gauge, ½ inch needle, depending on the dose determined for the individual dog. Ark Sciences plans to distribute the needles with the product. (These needle sizes are not readily available through veterinary suppliers). Dosage is determined by measuring each testicle with a caliper provided with the product. The correct dose is indicated on the caliper and is based on the maximum width of each testicle. Esterilsol is labeled for use in dogs with an individual testicular width of 10-27mm; some dogs' testicles will not fall into this range. Ark Sciences recommends light sedation to ensure that the dog holds still during the injection (Anesthesia is not necessary. Reversible sedation is commonly used so that dogs are awake and alert in as little as 15-20 minutes after the Esterilsol injection/zinc neutering) (ACC&D, 2012).

Experienced practitioners report that the process of measuring the testicular width (to determine dose), preparing the injections, and administering the injection into each testicle takes two to five minutes. Following proper administration protocol is critical to reduce the risk of injection site reactions. In the clinical trial results presented to the U.S. Food and Drug Administration (FDA), this formulation was found to cause permanent sterility in 99.6% of treated dogs (ACC&D, 2012). Further investigation is needed to identify risk factors for adverse reactions to zinc gluconate and to develop strategies for avoidance.

**Sodium Chloride Solution:-**

Sodium Chloride Solution - Hypertonic saline is a solution that is inexpensive and easy to administrate. In a study, 20% hypertonic saline solution was injected bilaterally into the rat testes at different areas with a total amount of about 0.5 to 1 cc in each testis and Reported that the coagulation necrosis was observed in all testes (Emir *et al*., 2008; Ibrahim *et al*., 2016).

Kwak and Lee, (2013) revealed that severe degenerative changes in testicular seminiferous tubules and massive infiltration of immune cells in hypertonic saline group. Additionally researchers indicated that "intratesticular hypertonic saline injection seems to be an alternative method in the future to its rivals such as orchiectomy and medical castration" but that further laboratory work would be required to ascertain the potential utility of this approach in dogs (Emir *et al*., 2008).

In other study, it was observed that 20% sodium chloride could be used for chemical castration in young dogs. It was suggested that intra testicular injection of hypertonic saline could be an effective method for nonsurgical sterilization of the non adult male dogs but not adult dogs (Ibrahim *et al*., 2016).

Also other study, Intratesticular injection of 20% NaCl hypertonic solution induces coagulative necrosis of Leydig cells and seminiferous tubules and extensive testicular fibrosis. These observed lesions compromise testicular development and testosterone synthesis leading to sterility when performed during the first 20 days of life. The NaCl ITI represents a viable alternative to surgical orchiectomy in calves (Neto *et al*., 2014).

The use of Inorganic Chemo-sterilants in male dogs is an attractive option because it removes the disadvantages and costs of surgical sterilization and post-operative care (Rojas *et al*., 2011). Furthermore, in regions where surgical castration of male dogs is not culturally accepted (e.g. Romania, the Bahamas) chemical castration offers a reasonable alternative (Fielding *et al*, 2002; Cocia and Rusu, 2010; Garde *et al*., 2016). Hence, chemical castration could be an attractive option for developing countries with limited resources for surgical dog management programs (Garde *et al*., 2016).

## Conclusions and Recommendations:-

With the worldwide overpopulation of dogs, a variety of options are needed for population control. The use of fertility inhibitors is gaining acceptance to control populations of companion animals and wildlife. For dog population management, nonsurgical or chemical sterilization is increasingly advocated as deserving priority for development because of its potential to be more cost effective than surgical sterilization. The benefits of using sterilization (both surgical and nonsurgical) alongside vaccination to manage dog populations include the reduction in population turnover, which also results in the population maintaining herd immunity, improved health, potential reduction of bite rate, and increased owner compliance as owners bring their dogs to sterilization centers.

Chemical sterilization methods so far employed include hormonal methods, immunocontraceptives and Inorganic Chemo-sterilants such as calcium chloride - $CaCl_2$, zinc gluconate neutralized by arginine (Neutersol), sodium chloride -NaCl solution). Immunocontraceptives and Inorganic Chemo-sterilants deserve further attention for dog population management. Although females are the primary target to manage populations through fertility control, male sterilants could also be employed, particularly if less expensive or with longer effectiveness than those used on females.

This review indicated that the past decade saw a significant increase in studies concerning fertility inhibitors for dogs. Despite the fact that several studies mentioned specially by intratesticular injection of calcium chloride (CaCl), Zinc gluconate (Neutersol), and 20% NaCl hypertonic solution, that these drugs could be used to decrease dog population size and impact. If nonsurgical fertility control is chosen to manage dog populations or their impact, social acceptance, humaneness, effectiveness, feasibility, costs, and sustainability of this method should be evaluated at an early planning stage. For instance, owners of roaming dogs as well as other stakeholders should be consulted and informed on the possible benefits of fertility control. This framework is based on the assumption that a set reduction of dog population size or the elimination of a disease such as rabies, within a predefined timeframe can be achieved by using nonsurgical fertility control as an additional tool to education and vaccination.

The following list outlines desirable characteristics for chemical sterilization methods specifically recommended to male dog population managements:- causes permanent loss of fertility; causes permanent loss of sexual behaviour (therefore reduces ‘nuisance’ behaviour of animals for people in the community and might decrease displays of some forms of aggressive behaviour); requires single practical delivery (oral delivery by injection or via bait would be most practical); is safe and has no deleterious side effects for the target and non-target species (including humans) in case of accidental exposure or self injection; has good efficacy (high success rate in treated animals); is technically feasible; Is stable in formulation, to allow for storage and handling under field conditions; allows large-scale manufacturing; Is affordable and cost effective. Based on the above conclusion the following recommendations are forwarded:

1. The above mentioned drugs or fertility inhibitors could be used to decrease dog population size and impact in general especially for developing country.
2. The review also showed the relative lack of research or knowledge related to fertility inhibitors in developing country as Ethiopia and suggested that more work is required in these country.
3. Stray dogs control legislation should be implemented in the country and in the world as whole.

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