

Considerations on contraceptive techniques for vulnerable mares

Considerações acerca de técnicas contraceptivas para fêmeas de equídeos sob vulnerabilidade

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ABSTRACT

Brazil currently has several public policies that aim to prohibit animal traction. Despite this scenario, this is still a subsistence activity widely used by many of the population, so such public policies are inefficient. For these policies to have the desired effect, actions are needed to control the population of these vulnerable draft horses through low-cost, effective, and definitive contraceptive surgical techniques or the use of specific medications. The present review aims to evaluate the most viable contraceptive techniques in population control of these animals, considering the scarcity of resources, difficulties in regular veterinary monitoring, effectiveness, and animal recovery time.

Keywords: Ovariectomy. Colpotomy. Contraception. Vulnerability. Animal traction.

RESUMO

O Brasil, na atualidade, possui diversas políticas públicas com o objetivo da proibição da tração animal. Apesar deste cenário, esta ainda é uma atividade de subsistência muito utilizada por uma grande parcela da população, de modo que tais políticas públicas se mostram ineficientes. Para que estas políticas tenham o efeito desejado são necessárias ações de controle populacional destes equídeos de tração sob vulnerabilidade através de técnicas contraceptivas cirúrgicas ou não, de baixo custo, eficazes e definitivas. A presente revisão tem como objetivos avaliar as técnicas contraceptivas de maior viabilidade no controle populacional destes animais, considerando o cenário de escassez de recursos, dificuldades de acompanhamento veterinário regular, tempo de eficácia e tempo de recuperação do animal.

Palavras-chave: Ovariectomia. Colpotomia. Contracepção. Vulnerabilidade. Tração animal.

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Introduction

Horses are seasonal polyestrous mammals, with females showing estrus and ovulatory activity during the brightest peak of the year (Maia et al., 2019; Oliveira & Souza, 2003). Considering this specific peculiarity and the need for contraceptive techniques in different situations, innovative research and extension work have been conducted since the 1970s, focusing on developing efficient and safe techniques.

Among the situations where contraception is indicated, we can list the minimization of the undesirable consequences of estrus in equine athletes (Kane, 2018), the use of castrated females in embryo transfer (Hinrichs et al., 1985; Hinrichs et al., 1986, 1987; Prado & Schumacher, 2019), and population control of wild horses in reserves. Furthermore, population control of working horses in urban areas, which is very common in cities in developing countries, has been a concern in public policy, One Health, and animal welfare.

In Brazil, laws such as Law No. 10531 of 09/10/2008 in Porto Alegre, Rio Grande do Sul (RS), Law No. 17918 of 09/06/2013 in Recife, Pernambuco (PE), and Law No. 11285 of 01/22/2021 in Belo Horizonte, Minas Gerais (MG), aim to abolish urban traction work in vulnerable communities. However, these laws have not been effective in practice, highlighting the need for the determination of techniques for reproduction control and gradual reduction in the population of these animals (Belo Horizonte, 2021; Curitiba, 2006; Escodro et al., 2012; Kane, 2018; Porto Alegre, 2016, 2008; Recife, 2013, 2021).

Studies indicate that up to one-third of foals are sired by stallions not associated with the original herd, so sterilizing males in one area does not prevent females from becoming fertilized (Bowling & Touchberry, 1990). Therefore, it is concluded that the most effective method for the population control of wild equines is the sterilization of females. This

can be achieved through highly effective contraceptive vaccines, eliminating the need for surgical procedures (Hobbs & Hinds, 2018).

Therefore, the initial efforts for equine population control through sterilizing mares and jennies were conducted in the United States. Since then, various contraceptive methods have been described for females, including the use of hormones, intrauterine devices, and various surgical techniques (Kane, 2018; Smith & Mair, 2008).

The objective of this review is to provide relevant information about the main contraceptive techniques in female equines, aiming to establish guidelines for population control of equines under conditions of vulnerability and urban labor in developing countries, with a focus on the development of public policies for the Brazilian context.

Equines traction in developing countries

It is common in urban centers of developing countries for low-income families to rely on the income generated by equine traction activities. Despite the high level of technology in transportation, horse-drawn carts are still commonly used in the labor activities of waste pickers, recyclable collectors, debris removal, material delivery for construction, and other similar tasks in the outskirts of major cities. As a result, these equines do not have favorable conditions for regular veterinary care or sanitary control. They are marginalized by society and neglected by governments when creating policies for regulation and concern for animal welfare. There is a high rate of reported animal cruelty cases in this sector (Ferraro et al., 2008; Maranhão et al., 2006; Rezende, 2004).

Sousa Júnior & Santos (2019) reported that at least 300 million animals are used for cart-pulling worldwide. Lima et al., (2020) published an estimate of approximately 5 million horses in Brazil, with 3.9 million being working animals primarily engaged in rural activities, without specific mention of urban traction horses. Escodro et al., (2012) estimate that approximately 10-20% of Brazil's equine population is involved in urban traction and labor activities. With such a significant number of equines playing a role in the daily life of cities, there is a need to study the socioeconomic conditions of this cart-pulling population and implement public policies that address the welfare of the people involved and their animals. This includes considerations for public health and animal welfare aspects.

In Brazil, laws like Law No. 10531 of 09/10/2008 in Porto Alegre, RS, Law No. 17918 of 09/06/2013 in Recife, PE, and Law No. 11285 of 01/22/2021 in Belo Horizonte, MG, aim to abolish urban animal traction work, establishing

deadlines for this to happen. However, these deadlines have already been extended in Porto Alegre and Recife through Laws No. 12117 of 09/06/2016 and Decree No. 34432 of 03/19/2021, respectively. Still, no activities promote animal welfare or population control, which would be the first step toward achieving a future without equine cart-pullers in cities (Belo Horizonte, 2021; Curitiba, 2006; Porto Alegre, 2016, 2008; Recife, 2013, 2021).

Knowing all the problems this population faces, creating public policies that address unique health and financial sustainability is paramount. These policies aim to reduce social inequality and animal mistreatment, reducing public health risks caused by the spread of zoonoses, uncontrolled reproduction, and accidents on public roads (Escodro et al., 2012; Freitas, 2007; Rezende, 2004).

In light of the above, population control of equines in cities through the sterilization of both males and females, similar to what is recommended for dogs and cats, should be a primary measure to be adopted. Females with foals are expected to be seen in urban areas (Figure 1). To achieve this, the validation of equine sterilization laws, along with concurrent actions to promote health and animal welfare, such as rabies vaccination, deworming, measures to control other potentially zoonotic diseases, and load control based on zoometric profiling, should be implemented under veterinary and zootechnical supervision (Albuquerque Mariz et al., 2018).

Contraceptive techniques

Pharmacological techniques

Initial contraceptive studies in mares and stallions used steroid hormones. However, despite good functioning in mares, there were difficulties administering medications and a growing concern about environmental and food chain contamination (Kane, 2018).

Pharmacological suppression of estrous behavior can also be achieved by administering progesterone or progestogens. However, this is a costly and challenging treatment due to the need for constant administration and the possibility of interrupted therapy, particularly in the case of capturing wild equines in reserves and the lack of protocol control in urban working mares in developing countries (Kane, 2018).

Other pharmacological methods to suppress reproduction are based on extending the lifetime of the corpus luteum and include repeated administration of oxytocin during the diestrus phase of the cycle, manual follicle rupture between days 16 and 22 after ovulation, administration of equine chorionic gonadotropin (eCG) to induce ovulation during the luteal phase, and intrauterine administration of a small amount of vegetable oil during diestrus. While some of these methods may be effective, they are not feasible within the context of urban equines, making it difficult to maintain these actions (Hedberg et al., 2006; Rebordão et al., 2017; Stout et al., 1999; Wilsher & Allen, 2011).



Figure 1 – A female equine with her foal walking freely on the streets of Maceió, state of Alagoas.
Source: Author archive.

Vaccines for equine reproduction control

Some methods using immunobiologicals have been developed, such as vaccines based on the zona pellucida of pigs (PZP). The PZP vaccine contains a glycoprotein antigen extracted from pigs' ovaries (*Sus scrofa domesticus*). It is then mixed with a Freund's adjuvant, which induces an immune response in the mare, producing antibodies against the zona pellucida proteins. The antibodies formed by the vaccine have a blocking effect on the fertilization process of the ovum, rendering it non-functional. When a booster shot is administered 30 days after the initial dose, before the breeding season, it has an efficacy of 80 to 90% in prevention (Kane, 2018).

However, issues with this method have been observed, such as the need for an annual application, which often becomes less reliable after the third application due to heavy handling, extra care, neglect, and forgetfulness. Another significant concern is that by creating antibodies against the zona pellucida, problems began to arise in the mares' ovaries, including degeneration and rejection of ovarian tissues, leading to immunotoxicity in the treated mare (Kane, 2018). Another medicine is GonaCon™, developed as a contraceptive method, GonaCon is currently the only vaccine available on the market for use in horses that works against gonadotropin-releasing hormone (GnRH). The effectiveness of this method appears to be the best among contraceptive vaccines, but they are still being studied for different dosages. However, it has restricted availability and high costs and is impractical for vulnerable urban horses or those in preservation reserves (Kane, 2018).

Based on the assessment, it becomes evident that the viability of using pharmacological techniques for reproductive control of female equines, including preservation reserves and vulnerable communities, is low. Implementing programs focused on collective veterinary medicine is challenging. However, there is an indication of hormonal and vaccine use for sport and performance animals.

Intrauterine devices

In addition to the injectable treatments, new intrauterine devices (IUDs) are being tested. Initial studies of IUDs were promising, with reports of retention and efficacy exceeding 80% with marble IUDs without adverse health effects on mares (Daels & Hughes, 1995). Besides their high effectiveness, a recent study by (Gradil et al., 2021) evaluated the use of IUDs with independent capsules, where there was no contraceptive failure, and their effects could be reversed up to 30 days after device removal. However, the mechanism responsible for the effectiveness of the

IUD occurs due to a low-intensity local inflammation, necessitating regular evaluations. After prolonged use, issues such as endometritis and pyometra can occur (Gradil et al., 2021; Morris et al., 2017).

Various devices, such as the Copper T Intrauterine Device, a silicone ring, glass beads, and magnetic capsules, have been tested in equines. However, most have shown high costs and complications associated with prolonged use without maintenance (Gradil et al., 2021; Hobbs & Hinds, 2018).

These devices are designed to prevent embryo implantation and simulate pregnancy, thus extending the lifetime of the corpus luteum and preventing undesired reproductive behavior in mares (Morris et al., 2017).

As previously mentioned, this method has been widely used for mechanical interference with pregnancy, extending the lifetime of the corpus luteum. However, several complications have been reported, including device fragmentation in the uterus (in the case of glass devices), device migration through the endometrium and myometrium to encapsulate in the uterine serosa, chronic endometritis, pyometra, and intermittent abdominal discomfort (Amorim et al., 2016; Graham & Freeman, 2014; Klabnik-Bradford et al., 2013; Morris et al., 2017; Turner et al., 2015)

Surgical techniques

Ovariectomies are the recommended and performed surgeries for reproductive control in female equines for three reasons: one or both ovaries have physiological changes preventing reproduction and affecting their health; for management reasons, such as improving unstable behavior during estrus or addressing colic episodes related to the estrous cycle; and in population control of the wild population (Pettrizzi et al., 2020). In the study by Kamm & Hendrickson (2007), removing both ovaries was performed to improve abnormal behavior, such as aggression, excitability, kicking, biting, training issues, problems with other horses, and frequent urination during estrus. This resulted in improved behavior in 83% of the mares. Few reports or legislative references propose surgical techniques for population control of equines in urban areas.

Several techniques have been described for removing one or both ovaries. Some of these techniques can be carried out with the mare in a standing position using neuroleptoanalgesia protocols involving alpha-2 agonist drugs, local anesthetics, and opioids. This should be a feasible approach for surgeries in vulnerable female equines working in urban areas, considering its cost-effectiveness, ease of execution, and quicker recovery compared to general anesthesia (Moraes, 2017; Smith & Mair, 2008).

Patient preparation and neuroleptoanalgesia

Neuroleptoanalgesia is tranquilization with high-intensity analgesia without the animal losing consciousness, resulting in reduced excitability. Since there is no single drug capable of providing all the necessary advantages for restraint and analgesia, the use of neuroleptoanalgesic combinations becomes necessary to achieve safety, quality, and a reduction in drug doses (Ginelli, 2013).

Equine anesthesia presents particularities and complications associated with recumbency and general anesthesia compared to other domestic animals. Therefore, the proposed scenario should prioritize a neuroleptoanalgesia protocol for satisfactory animal handling in a standing position (Viveiros, 2011).

A preoperative clinical examination is performed to ensure the mare's health for patient preparation. Then, the mare undergoes fasting for approximately 12 h, and preventive antibiotic therapy is administered, typically with 20,000-40,000 IU/kg of sodium benzylpenicillin and 6.6 mg/kg of gentamicin. Non-steroidal anti-inflammatory drugs (NSAIDs) are also used, with flunixin meglumine at a dose of 1.1 mg/kg or phenylbutazone at 4.4 mg/kg administered intravenously. Antimicrobials are continued for three to seven days postoperatively and anti-inflammatories for up to 5 days.

In addition to antibiotic therapy, it is recommended that vulnerable female equines without a vaccination regimen administer tetanus antitoxin (5000 IU/IM/one application) 24 h before the procedure. The preparation also involves cleansing the anus and vulva and shaving the left and right flanks (Seabaugh & Schumacher, 2014).

Alpha-2 adrenergic agonists such as xylazine (1 mg/kg IV) and detomidine (0.04 mg/kg) have gained wide acceptance for tranquilization and muscle relaxation in equines. However, to achieve the necessary analgesia during ovariectomy, it is necessary to combine them with opioids such as epidural morphine (10 mg/kg) in the intercoccygeal space (Cc1-Cc2). Additionally, a local block with 2% lidocaine or 0.5% bupivacaine without vasoconstrictor is administered subcutaneously in the musculature and peritoneum at the incision site, with a volume of 20 to 60 mL, depending on the size of the animal (Nascimento et al., 2020).

Local anesthetics can also be infiltrated into the ovarian pedicle to reduce pain during surgical traction (Nascimento et al., 2020).

Ovariectomy by laparoscopy

Laparoscopy has been widely used nowadays as it is a minimally invasive technique performed using a camera

that provides adequate visualization of the area to be operated. Ovariectomy is one of the procedures for which laparoscopic techniques have been used effectively in a standing position. Various techniques are described, often differentiated by hemostatic maneuvers on the ovarian pedicle and a unilateral or bilateral paralumbar incision (Colbath et al., 2017; Moraes, 2017).

Establishing hemostasis of the ovarian pedicle during laparoscopic ovariectomy involves placing a surrounding ligature, ultrasonic shears, a vessel sealing device, a staple, electrocoagulation, laser energy, and a harmonic scalpel (Moraes, 2017).

The laparoscopic approach has a description similar to that of various authors (Bouré et al., 1997; Colbath et al., 2017; Lee & Hendrickson, 2008; Smith & Mair, 2008; Velloso Alvarez et al., 2022). It involves introducing the laparoscope into the abdominal cavity using a trocar. Some authors recommend pneumoperitoneum, which involves applying carbon dioxide gas before trocar insertion. This helps separate the abdominal wall from the viscera, provides the operator with a wide view, and facilitates trocar passage due to positive intracavitary pressure (Colbath et al., 2017).

Regardless of the chosen side, precautions should be taken when inserting the trocar. If it is done on the left flank, the spleen may be damaged, and if it is done on the right flank, care should be taken to avoid damage to the cecum. (Adams & Hendrickson, 2014). There are also recommendations for using a spiral-shaped trocar that gradually opens each layer of tissue, preventing damage from overly deep punctures (Shettko & Hendrickson, 2021). Furthermore, fasting (only from solid food; water can be administered up to a few hours before surgery) is recommended for 12 to 24 h. The longer the fasting time, the less gas production in the intestinal loops, which can improve surgical visualization (Hendrickson, 2006).

According to the surgical technique description by (Adams & Hendrickson, 2014), three vertical incisions of 1 to 2 cm in length are made in each paralumbar fossa for use as entry ports. The first port is located in the middle of the height of the 18th rib. With a trocar and a laparoscopic cannula, the abdomen is inflated with carbon dioxide (CO₂) gas to a 10-15 mmHg pressure. The second entry port is made 6 cm dorsally and 3 cm cranially to the first entry port, and the third entry port is made 6 cm ventrally and 3 cm caudally to the first entry port. A 30°, 10 mm diameter, and 57 cm long laparoscope is inserted into the abdomen through the most dorsal entry port.

A clamp for grasping and manipulating the ovary is inserted in the most ventral part, and the 5 mm diameter,

30 cm long ultrasonic scissors with a 1 cm grasping tip are inserted into the central port. The grasping tip of the ultrasonic scissors cuts part of the ovarian pedicle and is repositioned repeatedly until the ovary is completely severed. Hemostasis is successfully achieved using proper ligation.

After the left ovary is severed, the pedicle is inspected to ensure hemostasis. As previously described, the left ovary is grasped with forceps until the right ovary is severed through the right paralumbar fossa. The right ovary is positioned caudal to the left within the abdomen between the descending colon and the bladder. The laparoscope is reinserted on the left side at the most dorsal entry port of the left paralumbar fossa, identifying the right ovary. Another forceps is inserted through the middle entry port and grasps the right ovary (Petrizzi et al., 2020).

A new incision is made between the two most ventral entry ports to remove the ovaries from the abdominal cavity. The incisions in the paralumbar fossa are sutured with standard suture material using 2-0 nylon, and the incision for ovary removal is sutured in two layers, using 0 polyglyconate for the muscle layer and 2-0 nylon for the skin (Lee & Hendrickson, 2008).

A similar technique was described by Colbath et al. (2017), but it involved unilateral access through the left paralumbar fossa, requiring the displacement of the descending colon to reach the contralateral ovary using additional dorsal access. At the end of the procedure, all equipment is removed sequentially and slowly to avoid the abrupt release of abdominal gas (Silva et al., 2000).

Compared to laparotomy under general anesthesia, laparoscopy in the standing position has benefits, including lower costs, direct visualization of the abdominal viscera, safe hemostasis, tension-free manipulation of the ovaries, and smaller flank incisions. These advantages reduce complications and hospitalization time associated with ovariectomy (Lee & Hendrickson, 2008).

Paralumbar laparotomy

This technique involves paralumbar access to the ovaries and their removal. The paralumbar fossa is a triangular depression in the dorsolateral area of the abdomen. The paralumbar fossae are smaller than the animal's size, allowing for excellent visualization of adjacent abdominal organs. It is a surgical technique that can be quickly performed by an experienced surgeon without the need for general anesthesia or specific instruments and can be done in the field. However, this technique has the disadvantage of exposing the abdominal cavity and a higher possibility of environmental contamination due to the size of the

incision, longer healing time, and more incredible difficulty in exposing the ovaries to ligation (Freeman & Lyle, 2015; Graham & Freeman, 2014; Shettko & Hendrickson, 2021; Ximenes, 2018).

A study by Barros et al. (2018) compared laparotomy and laparoscopy techniques for ovariectomy in donkeys, noting differences in the degree of inflammation through the analysis of acute-phase proteins in peritoneal fluid. The results showed that the inflammatory reaction was more significant in the laparoscopy group, and the authors attributed greater discomfort to patients undergoing the technique during the insufflation of CO₂ gas. On the other hand, Seabaugh & Schumacher, (2014) suggested that ovariectomy by laparoscopy technique is safer than ovariectomy performed with standard methods and allows the mare to return quickly to normal function.

To perform the technique, the flank is prepared by shaving and surgical antisepsis, and the proposed incision site is desensitized with local anesthetic solution while the mare is sedated. This is achieved using a fan block and subcutaneously infusing local anesthetic solution along the proposed incision line (Collar et al., 2021; Creighton & Lamont, 2022).

The surgical technique involves a unilateral or bilateral incision of the paralumbar fossae. After gaining access to the abdominal cavity and identifying the ovaries, the surgeon makes an approximately 15 cm incision in the left paralumbar fossa. Subsequently, the muscles, including the external and internal obliques and transverse abdominal muscles, are dissected. With access to the abdominal cavity after breaking through the peritoneum, the left ovary is identified and enveloped with a gauze soaked in 2% lidocaine for desensitization. It is then exteriorized. With the ovary exteriorized, the surgeon achieves hemostasis and proceeds to section the ovarian pedicle. The contralateral ovary can be desensitized, exteriorized, and sectioned on the same side, or the same surgical technique can be performed on the right (Kadhim & Mohamad, 2023; Petrizzi et al., 2020).

After removing the ovaries, the flank incision can be closed in 3 to 4 layers of sutures. The mare needs to be kept at rest in a stall until the skin sutures are removed in 12 to 14 days. Broad-spectrum antibiotic therapy should be administered prior to the surgery and for at least 5 to 7 days, and therapy using non-steroidal anti-inflammatory drugs (NSAIDs) is recommended for 3 to 5 days (Collar et al., 2021).

Ovariectomy by colpotomy

Another technique for bilateral ovariectomy that can be performed with the mare under neuroleptoanalgesia

in a standing position is access through colpotomy. This technique is cheaper than other approaches for ovariectomy because it is carried out quickly with the mare in a standing position, with just one specialized instrument, the ecraseur, and just a few complications that can be avoided with good surgical technique (Prado & Schumacher, 2019).

A colpotomy ovariectomy is recommended for the removal of both ovaries through the vaginal canal, preferably in the absence of tumors. This technique is more economical and more commonly described in the bovine species (Carrasco et al., 2016; Lauder, 2018). It does not require much equipment or sutures and is a relatively quick method, depending on the surgeon's skill.

Due to its minimally invasive nature, colpotomy allows the mare to return to her daily routine quickly (Draheim & Hopper, 2023). One characteristic and disadvantage is that it is performed almost blindly, so the surgeon's skill must be high to avoid ligating areas of the intestine with feces when mistaken for the ovaries, and there is little control over intra-abdominal bleeding (Prado & Schumacher, 2019).

The surgical technique described by Prado & Schumacher (2019) involves the introduction of gauze soaked in an anesthetic solution, such as mepivacaine or lidocaine, to the bottom of the vaginal cavity, pressing for a few minutes to desensitize the area. An incision is made with a scalpel using a n.10 or n.15 blade on the dorsolateral portion of the vaginal cavity at the 10:30 or 13:30 position, approximately 2 cm in length. This incision is enlarged with fingers until a hand can easily pass through. The fascia and peritoneum are then dissected to gain access to the abdominal cavity. With the hand and forearm already inside the abdominal cavity, the surgeon will identify the ovaries, and their desensitization is performed using gauze soaked in anesthetic, being careful not to let it fall into the animal's abdominal cavity. The ecraseur chain is inserted into the cavity and positioned. With the ecraseur chain attached to the surgeon's proximal phalanges, the ovary is grasped, and the chain is slipped over the hand to wrap around the ovarian pedicle. The chain is tightened around the pedicle using the ratchet at the end of the device, taking care to ensure that the chain does not encircle any structures other than the ovary and ensuring the ovarian pedicle is not stretched during this procedure until the pedicle is severed. The contralateral ovary is removed using the same technique. After the ovaries are removed, hemostasis of the pedicles is ensured. Suturing is usually not performed, allowing the incision to heal by secondary intention (Prado & Schumacher, 2019).

The study by Velloso Alvarez et al. (2022) shows that the combination of colpotomy and flank video laparoscopy

can help avoid complications such as entrapment and sectioning of intestinal loops.

Conclusion

For the article, a review was carried out on considerations regarding contraceptive techniques for vulnerable female equids, showing the need to standardize safer techniques for the construction of public policies associated with the population control of equids in urban centers, with more effectiveness than current laws, which do not consider the sterilization of animals.

Brazilian regulations regarding traction horses do not consider sterilization techniques, demanding the reduction or extinction of those activities without providing safe techniques and a line of action.

It is clear from this review that surgical techniques are more recommended for controlling the reproductive processes in female equines, with laparotomy and laparoscopy being theoretically the safer options due to their higher number of scientific citations. However, ovariectomy through colpotomy should also be considered for future research, especially with the potential for variations that incorporate colposcopy or video laparoscopy. This approach has a lower risk of complications and a shorter surgical recovery time. An experienced surgeon can efficiently perform the technique, but it must be made with caution due to the high risk of damaging another structure close to the ovaries.

Furthermore, using intrauterine devices (IUDs) and their long-term study could represent a less costly and feasible technique within communities. However, it is essential to consider the need for regular evaluations by a veterinarian, which may make this approach less practical due to the high rate of selling and buying of these animals, lack of attendance and commitment on the part of owners to take them to veterinary appointments and the lack of public policies committing to perform such services at low cost or free of charge.

In conclusion, sterilizing female equines in urban areas is feasible. It would require innovative actions involving the quadruple helix, which combines new research from universities with support from public authorities, businesses, and civil society, represented here by animal welfare NGOs.

Conflict of Interest

The authors declare no conflict of interests, financial or otherwise.

Ethics Statement

The present study was approved by the Ethics committee on animal use under the protocol number 018/2022.

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