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Xylazine action and its physiological effects on animal reproduction: a review

Acción de la xilacina y sus efectos fisiológicos en la reproducción animal: una revisión

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ABSTRACT

Xylazine has been used to immobilize domestic and wild animals for the purpose of applying technologies aimed at reproduction, acquiring this drug special relevance in the veterinary field, for this reason, a literature review was carried out based on scientific publications related to the physiological effects of xylazine on reproductive aspects of animals, with the aim of describing its action and impact on the reproductive physiology of female and male domestic and wild animals, specifically, in assisted reproduction techniques. Xylazine, when interacting with receptors in different tissues and organs, has both adverse and favorable effects on reproductive processes. In females, xylazine can cause hypoxia and uterine contractions, while in males, when used in semen collection processes by electro-ejaculation, it can improve the amount of ejaculate as a factor that allows reducing stress levels. For the use of xylazine, it is important to consider that, in pregnant females, it can have adverse effects, and in males it is feasible to have beneficial effects in semen collection programs. Additionally, it is very important to know the desired effect to determine the dose to be used.

Keywords: anesthetic, sedative, electro-ejaculation.

RESUMEN

Se ha utilizado la xilacina para inmovilizar animales domésticos y silvestres con la finalidad de aplicar tecnologías encaminadas a la reproducción, adquiriendo este fármaco especial relevancia en el ámbito veterinario, por esta razón, se realizó una revisión de literatura basada en publicaciones científicas relacionada con los efectos fisiológicos de la xilacina en aspectos reproductivos de los animales, con el objetivo de describir su acción y repercusión en la fisiología reproductiva de la hembra y del macho de animales domésticos y silvestres, específicamente, en técnicas de reproducción asistida. La xilacina, al interactuar con receptores en diferentes tejidos y órganos, tiene efectos tanto adversos como favorables en los procesos reproductivos. En las hembras, la xilacina puede causar hipoxia y

contracciones uterinas, mientras que, en los machos, cuando se utiliza en los procesos de recolección de semen por electroeyaculación, puede mejorar la cantidad del eyaculado como un factor que permite reducir los niveles de estrés. Para el uso de la xilacina es importante considerar que, en hembras gestantes puede tener efectos adversos, y en machos es factible tener efectos benéficos en los programas de recolección de semen, adicionalmente, es de gran relevancia conocer el efecto deseado para determinar la dosis a emplear.

Palabras clave: anestésico, sedante, electroeyaculación.

INTRODUCTION

The achievement of immobilization and anesthesia in domestic animals is essential for the application of certain reproductive technologies (Trujillo-Rodríguez, 2018), such as the laparoscopic intrauterine artificial insemination technique, in which sheep and goats require sedation to prevent the procedure from affecting animal welfare (Márquez-Hernández *et al.,* 2024). In cattle, the Ovum Pick Up (OPU) procedure requires epidural administration as a muscle relaxant to perform ultrasonographic examination of the female reproductive tract, ovarian follicle counting, and follicular aspiration. Likewise, in the embryo transfer procedure in alpacas, the process requires intramuscular administration to induce sedation and avoid animal suffering (Pérez *et al.,* 2019). Similarly, in techniques aimed at the production and preservation of wild animals (Ugalde, 2014), where chemical restraint uses anesthetic drugs, α2-agonists have gained particular relevance due to their sedative, analgesic, and anticonvulsant effects.

Xylazine was the first α_2-adrenergic agonist used. This molecule was synthesized in 1962 in Germany for use as an antihypertensive in humans (Kitano *et al.,* 2018). This colorless crystal, capable of depressing the thermoregulatory center, can produce marked hypothermia (Anban *et al*., 2020). Some of the effects of this drug in animals include increased uterine pressure, premature parturition, reduced follicle-stimulating hormone (FSH) release, and stimulation of peripheral presynaptic α_2 receptors, which induces norepinephrine release, leading to atrioventricular blocks (González *et al.,* 2020). Therefore, it is plausible to assume that the administration of this drug in reproductive biotechnology programs may have adverse effects on expected fertility. Thus, the objective of this literature review is to describe the physiological action of xylazine and its repercussions on assisted reproductive techniques in domestic and wild animals.

What is xylazine and what is its action?

Xylazine is chemically described as 5.6-dihydro-2-(2.6-xylidino) (dimethyl-phenylamine)-4H-1.3-thiazine hydrochloride, or 2-(6-dimethylphenylamine)-4-H-5.6-dihydro-1.3-thiazine hydrochloride salt. It is freely soluble in water and stable in solution. It is a member of the G protein-coupled receptor superfamily, which acts as an intermediary in cellular signal transduction. G proteins are composed of three subunits: alpha, beta, and gamma, which function as a "molecular switch" that can be activated or deactivated in response to specific signals. When a chemical signal or molecule binds to a specific receptor on the cell membrane, the G protein is activated

and releases the alpha subunit, which interacts with different proteins to execute a response and initiate a new biochemical response within the cell (Alcántara-Hernández *et al.,* 2022). The G protein family is located in various tissues: the cardiovascular, respiratory, renal, gastrointestinal, and central nervous systems; adrenergic, cholinergic, and serotonergic terminal tissues; platelets, adipose tissue, pancreas, endocrine system, smooth muscle, vascular system, kidney, brain, uterus, and digestive system (Grogan *et al*., 2023); it inhibits adenyl cyclase, causing changes in transmembrane voltage and neuronal excitability (Anban *et al*., 2020). Consequently, this leads to significant changes in the ability of neurons to generate and propagate electrical signals, as well as affecting neurotransmitter release and synaptic activity, thereby influencing communication between nerve cells (Sheikhbahaei *et al*., 2018).

Upon xylazine stimulation, activation of adenyl cyclase is inhibited, thereby reducing cyclic adenosine monophosphate (cAMP) levels (Bylund, 1992), accompanied by an initial hypertension and stimulation of peripheral α_1 and α_2 receptors, followed by hypotension, which decreases cardiac output from 50 to 30 %, as a consequence of marked bradycardia and reduced adrenergic activity; this causes first- and second-degree atrioventricular blocks, with the potential to induce various arrhythmias (Cherdchutham *et al*., 2021).

The sedation and hypnosis caused by xylazine administration originate from its effect on locus coeruleus receptors by increasing GABAergic release, and analgesia through α_2 receptors located both in the *locus coeruleus* and the spinal cord (Silva *et al*., 2018). The main pharmacological activities in dogs develop within 10 to 15 minutes after intramuscular injection and three to five minutes after intravenous administration; they produce a sleep-like state, the depth of which is dose-dependent, generally lasting 60 to 120 minutes, while analgesia lasts 15 to 30 minutes (Munif *et al.,* 2021).

Forms of administration of xylazine

In veterinary medicine, xylazine is usually administered intramuscularly or intravenously in domestic animals to induce sedation and analgesia. For handling and sedation of wild animals, the application of xylazine has been diversified through the use of projectile syringes delivered with rifles, compressed air guns, or blowpipes, as they provide greater safety for the technician by allowing them to remain at a safe distance from the animal. Thus, the importance of a proper chemical restraint method for wildlife handling lies in the need to ensure the safety of both the animals and the humans involved in the process (Hernández-Silva *et al.,* 2018). Proper handling reduces stress in animals, minimizes the risk of injury, and facilitates veterinary or research procedures without causing harm. Furthermore, it ensures that wild species are treated ethically, contributing to biodiversity conservation and ecosystem balance. In this regard, xylazine has been used for sedation and other veterinary or zootechnical procedures in domestic species; it is mainly used for the safe transport of strong-tempered animals or for interventions, whether surgical or not, that require total or partial sedation of the animals. Its use has gained particular relevance in wildlife (aquatic and terrestrial) management, and due to its potent effects, its use has been

reported in the chemical restraint of several wild species, such as handling of *Leopardus guigna* (Acosta, 2007), *Choloepus didactylus* (Lescano, 2014), in the wild, reproduction of *Vicugna vicugna* (Enciso, 2009), immobilization of *Priodontes maximus* (Falzone *et al.,* 2013), *Canis latrans* (Servín, 1989), *Panthera onca* (Hagnauer, 2018), *Otaria flavescens* and *Arctocephalus philippii* (Cárdenas, 1986), and *Cyprinus carpio* (Fuentes-Rousselin *et al.,* 2016).

General aspects of xylazine pharmacokinetics

Before using xylazine, its effects must be anticipated in each individual, since this lipophilic molecule has a high capacity for binding to plasma proteins, rapid distribution throughout the body, and is metabolized in the liver by cytochrome P450 enzymes. In animal studies, more than 20 metabolites have been detected, and elimination occurs mainly through urine (>60–70 %) as metabolites; excretion of the intact molecule by the kidney is normally very low (<10 %). This hepatic microsomal cytochrome P450 system, which comprises families and subfamilies of isoenzymes, acts in the metabolism of a large number of endogenous and exogenous substrates that play a central role in drug biotransformation (Silva, 1999). Effective concentrations (0.2 to 2.0 ng mL^{-1}) are reached in the brain, and 70 % is eliminated by the kidney after xylazine has been N-dealkylated and S-dealkylated, oxidized and/or hydroxylated to 12 phase I metabolites. Phenolic metabolites are partially excreted as glucuronides or sulfates (Meyer & Maurer, 2013), and the remaining 30 % is eliminated via the enteral route (milk, saliva, and others). In cattle, elimination occurs between 10 and 15 hours; this is attributed to extensive metabolism rather than rapid renal excretion, with 1 % being excreted unchanged (Ruíz-Colón *et al.,* 2014).

There are factors that influence the pharmacokinetics of this sedative, where increasing the dose does not increase the depth of sedation but does prolong the duration of effects (Kitano *et al.,* 2018). In adult animals, metabolic pathways may be degraded, and in young animals they may be immature, which increases plasma concentrations. Each individual is genetically related to receptor proteins; in females, adipose tissue is greater and therefore plasma volume, transport mechanisms, and clearance are lower (Aparicio, 2003). The pharmacokinetics of xylazine depends on the state of metabolic and elimination pathways. Experimental studies have shown that under conditions of altered metabolism, such as in aged animals, clearance decreases, prolonging half-life and increasing systemic exposure, thereby raising the risk of respiratory depression. Therefore, in the presence of hepatic or renal dysfunction, it is reasonable to assume greater vulnerability to adverse effects, although specific studies confirming this association in domestic species are lacking (Giroux *et al.,* 2016).

Physiological effects of xylazine on female reproduction

Lemke (2007) reported that one hour after xylazine injection in domestic females (bovine, equine, and ovine), there are transient hormonal changes in prolactin and FSH secretion, as well as physiological changes, alterations in myometrial tone, intrauterine pressure, and decreased diaphragmatic activity in the fetus. Although the occurrence of reproductive problems is not well correlated with xylazine administration during different phases of gestation (bovine and equine), it should be taken into account that this drug causes a reduction in cardiac output and oxygen supply to the fetus (Sousa, 2015).

In veterinary practice, there are records of xylazine use in reproductive programs in females; however, adverse effects have been observed with the use of xylazine as an anesthetic for chemical restraint in females subjected to assisted reproduction protocols, which may impair implantation and gestation (Table 1).

One particular precedent is reported by the Zoological Society of London, where through artificial insemination, one of three female giant pandas (*Ailuropoda melanoleuca*) became pregnant; in this procedure, sedation of the animals is essential for handling (Moore, 1984). Similarly, at Ueno Zoo in Tokyo, Japan, in 1985 and 1986, two female giant pandas were inseminated, resulting in one birth per year (Masui, 1989), and in 1990, the first artificial insemination of a giant panda was performed in Mexico (Gual & Pulido, 1997), where xylazine was used to sedate the animals in order to place the female in a supine position with the head 30 degrees lower than the tail; subsequently, to open the vagina with a speculum and expose the cervix, avoiding the urethra, and to inseminate via the cervical route. These protocols used a mixture of two anesthetics: in London, the dose was calculated per kilogram of live weight (LW) as 4.5 to 6.0 mg of ketamine HCl plus 0.45 to 0.6 mg xylazine; in Tokyo, 0.1 mg kg^{-1} of diazepam and atropine plus 0.02 mg kg^{-1} of ketamine HCl were administered; and in Mexico City, 5 mg kg^{-1} of ketamine plus 0.5 mg kg^{-1} of xylazine were given. Until that time, there were no reports of xylazine causing any adverse effects on giant panda reproduction; however, there were publications in other wild and domestic species where combinations with xylazine were used and uterine contractions occurred. Therefore, after attempting to achieve pregnancy up to seven times over three seasons using artificial insemination, the Chapultepec Zoo proposed changing to 5 mg kg^{-1} of ketamine plus diazepam, 0.1 mg kg^{-1} and atropine, 0.02 mg kg^{-1}, eliminating ketamine HCl, a protocol similar to that used in Tokyo. With this modification, the giant panda became pregnant, resulting in a total of eight cubs from six births, of which four survived (Gual & Pulido, 1997).

Table 1. Effect of xylazine on reproductive programs in females

Author	Specie	Dose/Anesthetic	Observed effects
Moore, 1984	Giant panda	5 mg kg^{-1} of ketamine HCl/0.45 to 0.6 mg of Xylazine	Artificial insemination successful and gestation achieved
Masui, 1989	Giant panda	0.1 mg kg^{-1} of diazepam and atropine, 0.02 mg kg^{-1} of ketamine HCl	Artificial insemination successful and gestation achieved
Gual & Pulido, 1997	Giant panda	5 mg kg^{-1} of ketamine, 0.5 mg kg^{-1} of xylazine Changed to: 5 mg kg^{-1} of ketamine, 0.1 mg kg^{-1} of diazepam, 0.02 mg kg^{-1} of atropine	Artificial insemination successful and gestation achieved after the anesthetic protocol was changed
Dobrinski, 1994	Heifers	Xylazine	During the first trimester of gestation. Decreased fetal and maternal heart rate at three minutes after administration. Increased uterine contractility. Reduced uterine blood flow. Fetal hypoxia and bradycardia. Fetal recovery at 60 minutes. Maternal recovery at 120 minutes.
Sakamoto *et al.,*1996	Goats	Xylazine	Decreased maternal heart rate and blood pressure from five to 120 minutes. Maternal hypoxemia and respiratory acidosis. Increased intrauterine pressure. Significant decrease in uterine arterial blood flow. Decreased heart rate. Increased fetal blood pressure.
Hodgson *et al.,* 2002	Bovine (cows)	Xylazine	Decrease in heart rate and uterine artery flow, reducing oxygen supply: 59 % at five minutes 32 % at 45 minutes Reduced flow and availability of oxygenated blood to the uterus. Uterine vascular resistance lasting 30 minutes. Decreased fetal partial pressure of oxygen and carbon dioxide. Fetal stress and physiological distress, potentially increasing morbidity and mortality.

In domestic species of interest, it was demonstrated that in heifers of beef breeds, intravenous administration of xylazine (20 mg kg^{-1}) during the first trimester of gestation decreases fetal and maternal heart rate three minutes after administration; the fetus recovers at 60 minutes, while the mother remains depressed 120 minutes later. Uterine contractility increases markedly at three minutes after xylazine administration but returns to normal 120 minutes later; however, uterine blood flow is reduced, resulting in fetal hypoxia and bradycardia (Dobrinski, 1994). Likewise, administration of xylazine (0.04 mg kg^{-1}) in cows between days 219 and 241 of gestation causes a decrease in heart rate and uterine artery flow, reducing oxygen supply to 59 % at five minutes and 32 % at 45 minutes after drug administration. A notable reduction in flow and availability of oxygenated blood to the uterus is observed; this uterine vascular resistance lasts for 30 minutes, decreases the partial pressure of oxygen and carbon dioxide, generating a stressful moment and physiological distress for the fetus, potentially increasing fetal morbidity and mortality (Hodgson *et al.,* 2002). On the other hand, it has been reported that administration of 0.2 mg kg^{-1} of xylazine in pregnant goats decreases maternal heart rate and blood pressure from five minutes up to 120 minutes after xylazine administration; meanwhile, a state of significant hypoxemia and respiratory acidosis occurs up to 60 minutes after anesthetic administration. Intrauterine pressure increases within two to five minutes and continues to rise for approximately 15 minutes, followed by a significant decrease in uterine arterial blood flow, a decrease in heart rate, and an increase in fetal blood pressure (Sakamoto *et al.,*1996).

Physiological effects of xylazine on male reproduction

Semen collection in males of high genetic value that are difficult to handle or unable to mate is achieved using different anesthetic protocols, depending on the species (Table 2).

Table 2. Effects of xylazine use in different anesthetic-reproductive protocols in males

Author	Specie	Dose/Anesthetic	Observed effects
Johnston, 1998	Horse	0.3 mg kg^{-1} of xylazine	Ejaculation occurred between two and twelve minutes.
Naoman, 2012	Donkey	0.3 mg kg^{-1} of xylazine	96.6 % ejaculation rate.
Íñiguez *et al.*, 2017	Creole-type bulls (or "Criollo bulls")	0.025 mg kg^{-1} of xylazine	No significant effect on seminal characteristics was reported.
Abril-Sánchez *et al.*, 2018	Male goats	Protocol 1 Without anesthesia Protocol 2 0.5 mg kg^{-1} of ketamine, 0.05 mg kg^{-1} of xylazine Protocol 3 0.5 mg kg^{-1} of ketamine 0.05 mg kg^{-1} of xylazine	Protocol 1 Sperm motility: 1.4 Number of motile sperm: 301.2×10^6 Live sperm: 346.8×10^6 Protocol 2 Sperm motility: 3.0 Number of motile sperm: 882.8×10^6 Live sperm: 933.8×10^6 Protocol 3 Sperm motility: 2.7 Number of motile sperm: 546.7×10^6 Live sperm: 650×10^6
Ulloa & Condo, 2019	Rams	Xylazine	With anesthetic Semen volume: 0.92 mL Sperm concentration: 901.2×10^6 spermatozoa/mL Live sperm: 63.7 % Post-thaw motility: 50 % Without anesthetic Semen volume: 0.65 mL Sperm concentration: 807.4×10^6 spermatozoa/mL Live sperm: 56.9 % Post-thaw motility: 43.7 %
Ulloa-Ramones & Ulloa-Ramones, 2020	Rams	0.05 mg kg^{-1} xylazine	Similar cortisol and testosterone concentrations were found in individuals with and without anesthetic. Significant differences were observed in the volume of semen collected.

			A 27 % increase was observed in breeding males that received the anesthetic.
Sousa *et al.*, 2016	Six-banded armadillos	Protocol 1 1 mg kg^{-1} of xylazine 7 mg kg^{-1} of ketamine 5 mg kg^{-1} of propofol Protocol 2 0.4 mg kg^{-1} of butorphanol 7 mg kg^{-1} of ketamine 5 mg kg^{-1} of propofol without anesthesia	Protocol 1 Sperm motility: 56 % Sperm concentration: 65 ×10^6 mL^{-1} No erection Protocol 2 Sperm motility: 60 % Sperm concentration: 6.7×10^6 mL^{-1} No erection Without anesthetic Sperm motility: 65 % Sperm concentration: 37.8 × 10^6 mL^{-1}

Table 2 shows that xylazine, used alone or in combination, has positive effects on semen collection in different animal species. Thus, it has been used in combination with drugs that suppress hyperactivity, as in horses, where after intravenous administration of 0.3 mg kg^{-1} of xylazine (a dose at which the animal remains standing and with continuous stimulation of females in estrus), ejaculation is achieved between two and twelve minutes (a period that varies depending on individual sensitivity), preceded by oral administration of a tricyclic antidepressant at 0.75 to 2.0 mg kg^{-1} (Imipramine; Johnston & De Luca, 1998). Similarly, using the same protocol in donkeys, 96.6 % ejaculated (Naoman & Ali, 2012). At the Wildlife Multiplication Center of the Federal Rural University of the Semi-Arid Region (UFERSA), Brazil, protocols for semen collection in sexually mature six-banded armadillos (*Euphractus sexcinctus* Linnaeus, 1758) aged three to five years were established. Treatments were: one without anesthetic and two with anesthetic. The first protocol consisted of intramuscular administration of 1 mg kg^{-1} xylazine and 7 mg kg^{-1} ketamine, followed by intravenous administration of 5 mg kg^{-1} propofol (Propovan, Cristalia, Fortaleza, Brazil); the second protocol consisted of intramuscular administration of 0.4 mg kg^{-1} butorphanol plus 7 mg kg^{-1} ketamine, followed by 5 mg kg^{-1} propofol. Using the xylazine-ketamine-propofol protocol, sperm motility was 56 %, which is considered low compared to 60 % with butorphanol/ketamine plus propofol and 65 % without any anesthesia; however, with the xylazine/ketamine plus propofol protocol, a higher sperm concentration of 65 × 10^6 mL^{-1} was obtained, compared to 6.7 × 10^6 mL^{-1} (butorphanol/ketamine plus propofol) and 37.8 × 10^6 mL^{-1} without any anesthetic; nevertheless, no penile erections or longer post-anesthesia recovery times were observed (Sousa *et al.*, 2016). In turn, Íñiguez *et al.* (2017) in the Cuenca Canton, Ecuador, studied the use of xylazine hydrochloride at a single intramuscular dose of

0.025 mg kg^{-1} body weight as a tranquilizer before semen collection by electroejaculation and transrectal massage in sexually mature Creole-type bulls that were sexually active and clinically healthy; they reported no significant effect on seminal characteristics. In the Department of Physiology, Faculty of Veterinary Medicine-Montevideo, Uruguay, semen quality was evaluated using the electroejaculation method and the stress factor in male goats through three different procedures: without anesthesia, sedation dose, and general anesthesia with 0.5 mg kg^{-1} ketamine plus 0.05 mg kg^{-1} xylazine administered intravenously in the jugular vein. They evaluated mass sperm motility on a scale from zero to five, reporting a score of 3.0 with sedation, followed by 2.7 with anesthesia, and 1.4 without treatment; likewise, higher numbers of motile sperm (882.8×10^6, 546.7×10^6, and 301.2×10^6) and live sperm (933.8×10^6, 650×10^6, and 346.8×10^6) were reported with sedation, anesthesia, and no treatment, respectively (Abril-Sánchez *et al.*, 2018). Similarly, Ulloa & Condo (2019) analyzed seminal characteristics, processing, cryopreservation, and post-thaw evaluation, with or without xylazine administration in difficult-to-handle rams using the stressful electroejaculator technique. They observed ejaculate volumes of 0.92 mL with xylazine and 0.65 mL without xylazine, sperm concentrations of 901.2×10^6 spermatozoa/mL and 807.4×10^6 spermatozoa/mL without treatment, live sperm of 63.7 % with xylazine application compared to 56.9 % without it, and post-thaw motility of 50 % with xylazine compared to 43.7 % without this anesthetic. Ulloa-Ramones & Ulloa-Ramones (2020) in Ecuador evaluated the intravenous administration of xylazine (0.05 mg kg^{-1}) in rams as a factor to reduce cortisol and testosterone levels during semen collection by electroejaculation, resulting in similar blood concentrations of cortisol and testosterone in all individuals. However, they observed significant differences in the volume of semen collected: breeding males that received xylazine ejaculated 27 % more semen volume compared to control breeding males.

CONCLUSIONS

Xylazine is a drug widely used for chemical restraint of farm and wild animals; however, its use must consider the potential sex-dependent effects. In pregnant females, it may induce physiological responses ranging from altered uterine blood flow to premature pregnancy termination. In contrast, in males, when used in semen collection programs employing the electro-ejaculation method, it can improve ejaculate volume; nevertheless, the sedation level or the physiological reproductive response desired in domestic and wild animals must be taken into account to determine the appropriate dose.

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Errata Erratum

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