ARTEROL®

FUMARATO DIHIDRATO DE EFORMOTEROL

TECHNICAL REPORT

AIR WITHOUT BLOOD

INTRODUCTION:

The following report is an update on our drug **ARTEROL®**, the world's first veterinary development of eformoterol (or formoterol) fumarate dihydrate, building on a 25-year track record.

Its active ingredient is a β2-adrenergic receptor (β2AR) agonist of the nervous system—receptors that are widely distributed throughout the body. The primary action of **ARTEROL®** is exerted on the respiratory system, inducing bronchodilatory and vasodilatory effects while increasing mucociliary clearance. It further inhibits endovascular inflammatory processes and activates mitochondrial biogenesis, which improves redox potential through the increased elimination of reactive oxygen species (ROS) and a subsequent increase in the energy source, adenosine triphosphate (ATP).

Below, we provide a brief description of each point, beginning with mitochondrial biogenesis. This more recently described action is fundamental, as it provides insight into how the drug meets the increased energy demand required to carry out its other functions.

ORIGINAL PHARMACEUTICAL RESEARCH

Laboratorio FUNDACION
CIENCIA Y TECNOLOGÍA FARMACÉUTICA

1 ARTEROL® ACTIVATES MITOCHONDRIAL BIOGENESIS

The mitochondrial proteome is encoded by nuclear DNA. The mitochondrion possesses its own DNA (mtDNA, which contains a limited number of genes that remain constant across the vertebrate lineage), which primarily encodes the proteins of the respiratory chain. This is the main producer of ATP (adenosine triphosphate), the primary high-energy storage molecule (Figure 1).

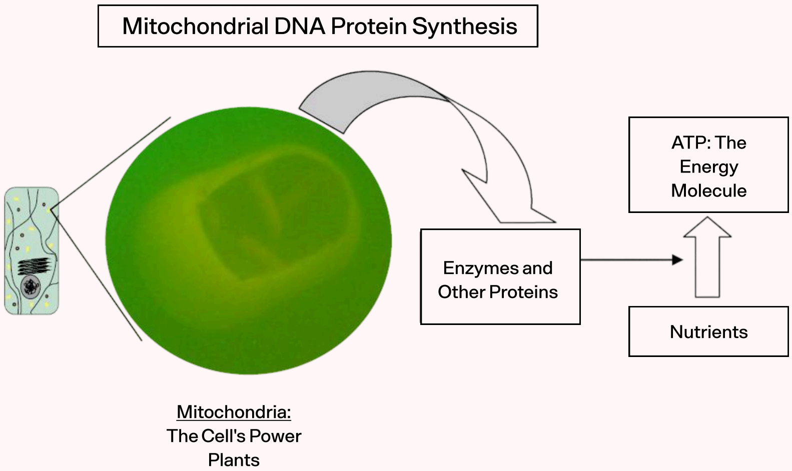


FIGURE 1

The physiological process known as mitochondrial biogenesis is the mechanism used by cells to:

1. Adapt to energy demands, expanding the total number and individual capacity of mitochondria when energy expenditure increases (e.g., during physical training).
2. Perform the crucial task of renewing damaged mitochondria (associated with pulmonary, neural, and renal diseases, among others). *(Shelbayeh OA et al; 2023).*

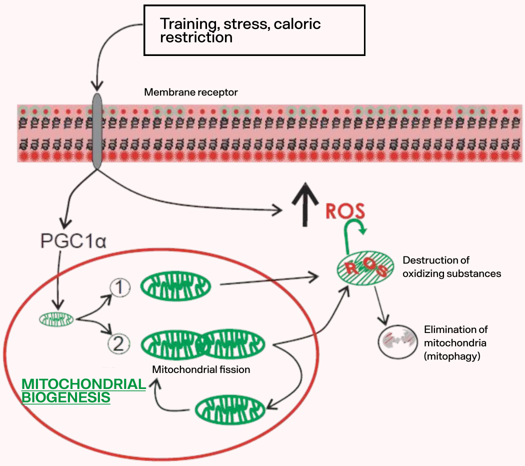


FIGURE 2

The induction of mitochondrial biogenesis generates new mitochondria, increases cellular respiration and ATP production, reduces pathological oxidative stress, and promotes cellular repair and regeneration. (Figure 2)

Mitochondria are vital to the physiology of airway smooth muscle due to their diverse, interconnected roles in calcium handling, redox regulation, and cellular bioenergetics *(Kotiadis, 2008; Wiley et al., 2016).*
Mitochondrial dysfunction is closely associated with airway diseases such as chronic bronchitis, chronic obstructive pulmonary disease (COPD), and exercise-induced pulmonary hemorrhage (EIPH).
In these pathological conditions, the increase in mitochondrial reactive oxygen species (ROS), altered bioenergetic profiles, and impaired calcium handling collectively contribute to changes in cellular signaling, gene expression, and, ultimately, alterations in contractile properties *(Suen et al., 2008; Wiley et al.,2016).*
This activity is nearly unique to eformoterol, the active ingredient in **ARTEROL®**, as other β2AR agonists, such as clenbuterol, do not produce this effect *(Wills et al., 2012).*
The induction of mitochondrial biogenesis was also observed in the recovery of spinal cord injuries in rats treated with eformoterol, highlighting the clinical significance of this pharmacological effect *(Scholpa et al., 2021).*

THE B2 RECEPTOR:
It is a G protein-coupled receptor (GPCR), also known as a seven-pass transmembrane domain receptor. This is because it consists of a protein chain that passes through the cell membrane seven times, leaving an initial terminal (NH2), three external loops, and three internal loops with a free terminal (COOH). Four of the internal loops and the free fraction together form the G PROTEIN. (Figure 3).

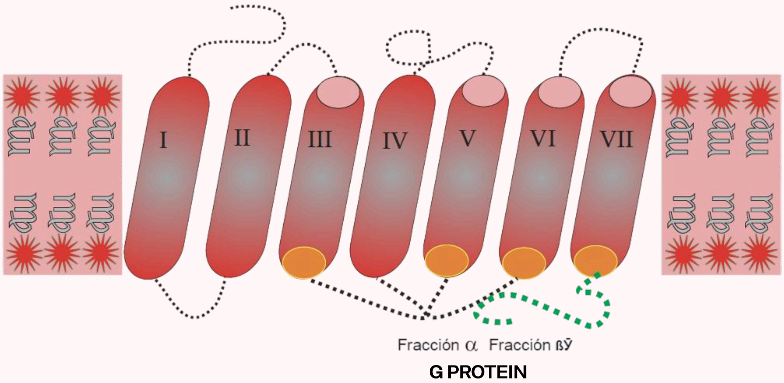


FIGURA 3

MECHANISM OF ACTION OF ARTEROL®:
he external loops are responsible for binding with the agonist (**ARTEROL®**), which triggers an inward conformational change. Upon activation, the internal loops bind to and activate the Gα subunit (the cAMP activation pathway) to produce responses such as bronchodilation and increased bronchial clearance.
Simultaneously, the intracellular COOH terminus, linked to the Gβγ subunit, activates the PI3K-Akt-eNOS3-sGC-PKG-p38-PGC-1α pathway (indicated by the green lines). This specific pathway, activated by eformoterol, is what induces mitochondrial biogenesis (Figura 4).

(AmpC: Cyclic adenosine monophosphate; PI3K: Phosphatidylinositol 3-kinase; Akt: Protein kinase B; NOS3: Nitric oxide synthase 3; sGC: Soluble guanudyl cyclase; PKG: Protein kinase G; PGC1α: Peroxisome proliferation-activated receptor gamma coactivator 1) *(Syrovatkina et al., 2016).*

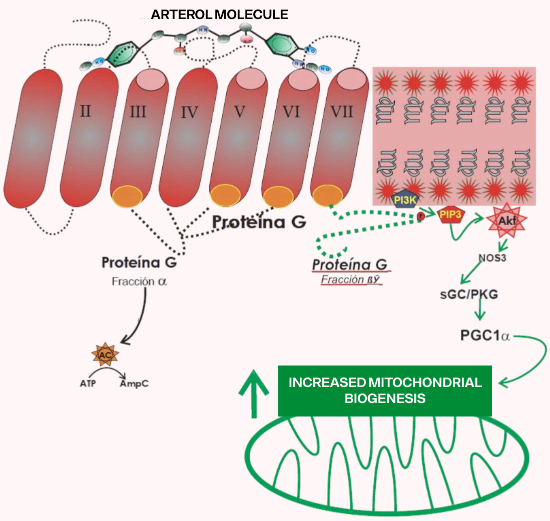


FIGURE 4

2 BRONCHODILATION:

The administration of **ARTEROL**® exerts a bronchodilatory effect by reducing transmural alveolar pressure, an action that improves the ventilatory process *(Barnes, 1993; Faulds et al., 1991; O´Donnell and Anderson, 1995; Verleden et al., 1993).*

At the molecular level, **ARTEROL**® inhibits bronchoconstriction induced by angiotensin II, adenosine, tachykinins, and histamine *(Nightingale et al.,1999; Ochsner, 1996; Verleden et al., 1993; O'Donnell and Anderson, 1995).* (Figura 5)

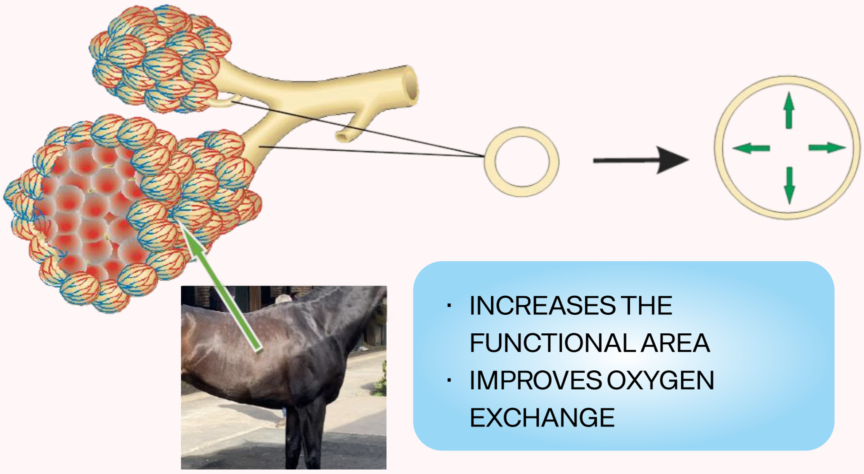


FIGURE 5

3 REDUCTION OF EXERCISE-INDUCED PULMONARY HEMORRHAGE (EIPH):

In an extensive clinical and necropsy study conducted in Hong Kong on Thoroughbred racehorses suffering from EIPH, it was confirmed that the hemorrhage was confined to the dorsocaudal region of the diaphragmatic lobe *(O'Calllahan et al., 1987; Pascoe, 1987)*. These findings led to the hypothesis of mechanical stress involving extremely negative inspiratory pressures and elevated capillary arterial pressures *(Birks et al., 1997)*, accompanied by secondary inflammatory changes.

In 2003, our team presented a hypothesis for EIPH based on a restrictive process affecting the lungs' and chest wall's ability to expand ("lung compliance"), exerted by the pressure of the saddle and, to a lesser extent, the jockey.

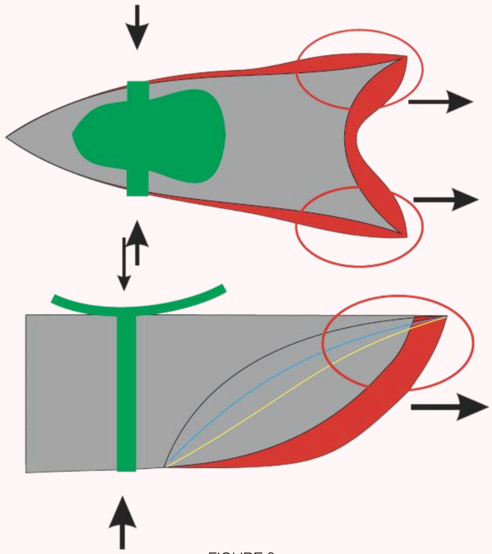


FIGURE 6

The primary effect was originally thought to be exerted on the apical and middle lobes. Consequently, ventilatory effort focuses on increasing negative intrathoracic pressure to compensate for this phenomenon by expanding the area of the diaphragmatic lobe.
This process leads to an increase in negative intra-alveolar pressure and a rise in positive intravascular pressure, ultimately resulting in capillary rupture and subsequent bleeding *(Ladaga et al., 2003).* (Figure 6)

Recently, this rise in arterial pressure was demonstrated by a massive release of red blood cells from the spleen to meet the increased O2 demand in the horse, causing a marked increase in blood volume (Figure 7). An increase in negative pressure during exertion was also observed *(Bayly WB et al., 2024).* All of these etiopathogenic factors could be associated with more susceptible genetic lines.

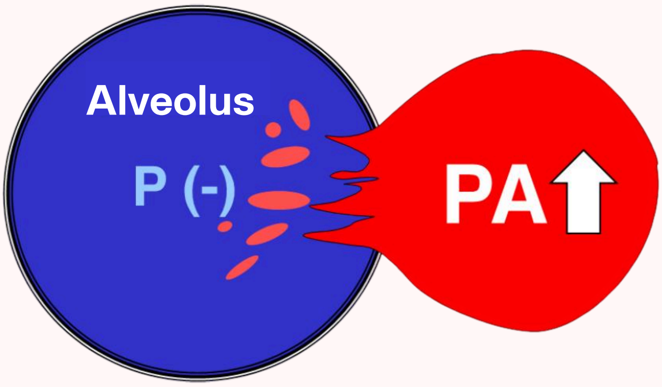


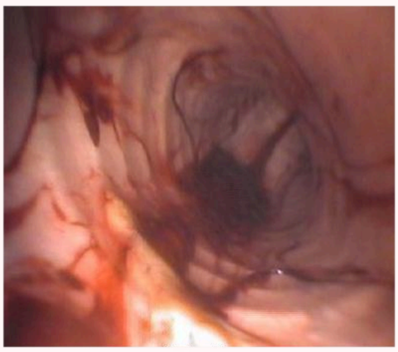
FIGURE 7

Having briefly outlined the causes of EIPH, let us examine the benefits provided by **ARTEROL®**

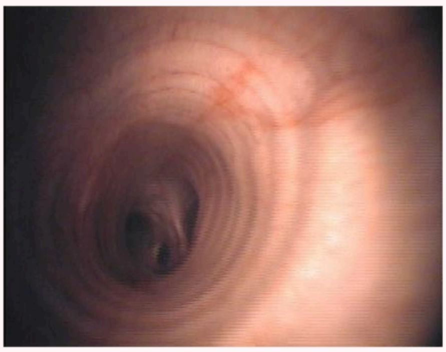
VASODILATORY EFFECT

Among other actions on the vascular system, Eformoterol includes a vasodilatory effect, resulting in a decrease in intravascular pressure *(Hoffman and Lefkowitz, 1982).*

WITHOUT TREATMENT



WITH TREATMENT[1]



(1) With treatment 0.08 mg/12 hs 5 days (Ladaga et al., 2003)

REDUCTION OF ALL ALVEOLAR VASCULAR INFLAMMATION FACTORS.

This action is carried out through the antagonism of several pathways that cause vascular alterations, namely:

1. Inhibition of the response induced by angiotensin, the discharge of tachykinins from sensory nerves in the airway (e.g., substance P) *(Verleden et al., 1993; Advenier et al., 1992)* and histamine release *(Nightingale, 1999; Ochsner, 1996).*
2. Inhibition of neutrophil and eosinophil adherence to the vascular endothelium, thereby reducing endothelial transudation *(Bowden et al., 1994).*
3. **ARTEROL®** decreases vascular permeability, which correlates with increases in intracellular cAMP levels *(Zink et al., 1995).*

The anti-edema effect results from a direct action on endothelial cells, inhibiting the formation of gaps at plasma transudation sites. This action is dose-dependent*(Baluk and McDonald, 1994).* (Figure 8)

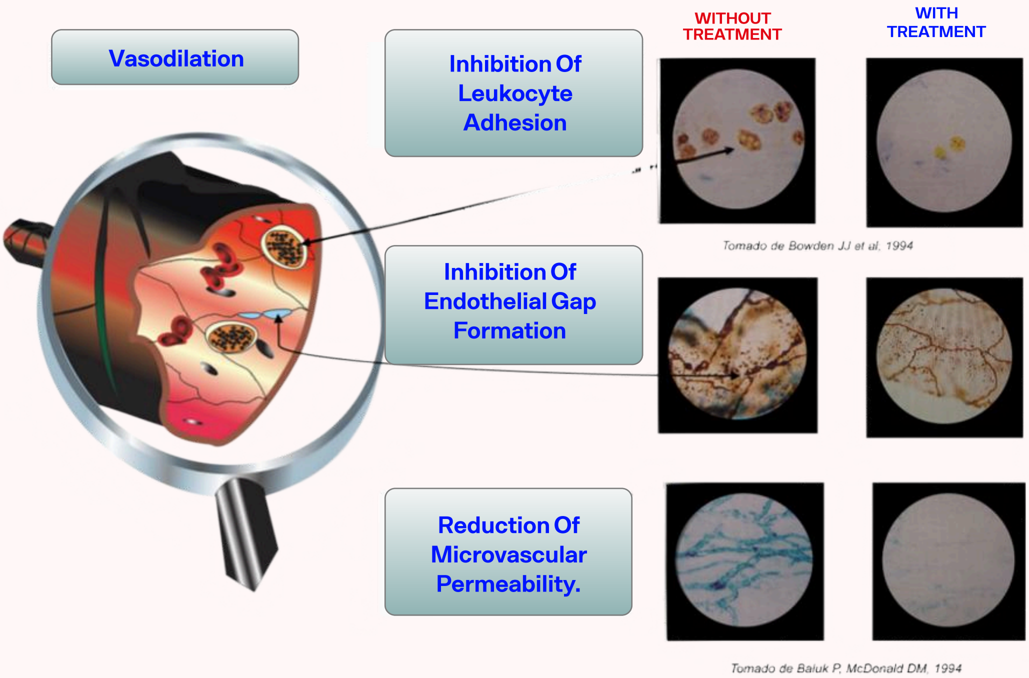
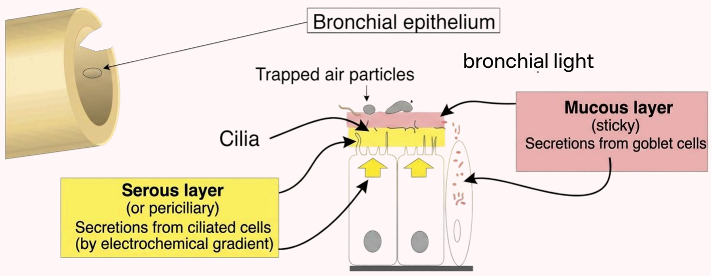


FIGURE 8

4 INCREASED BRONCHIAL SWEEP (Mucociliary Clearance):

The airways are lined with a dense thicket of hair-like structures, which are actually cellular extensions called cilia. These move in a coordinated fashion (utilizing energy) back and forth, displacing the mucous layer primarily toward the mouth (larynx). This activity is known as mucociliary clearance or "sweep," and it is essential for eliminating particles that enter during respiration. Figure 9 illustrates how this mechanism functions.



The normal mucus transport system depends largely on a proper balance (homeostasis) of ion and fluid transport within ciliated cells, mucin secretion, and ciliary beating. The fluid available to maintain mucus hydration within physiological parameters fluctuates between states of rest and activity.
Ciliary beating and mucus concentration generate neurogenic (purinergic) signals that coordinate the rate of Na+ absorption versus Cl− secretion, creating an electrochemical gradient *(Hill et al., 2022; Shah et al., 2023 Widdicombe et al., 2015).*

NORMAL BRONCHIAL SWEEP (Clearance)

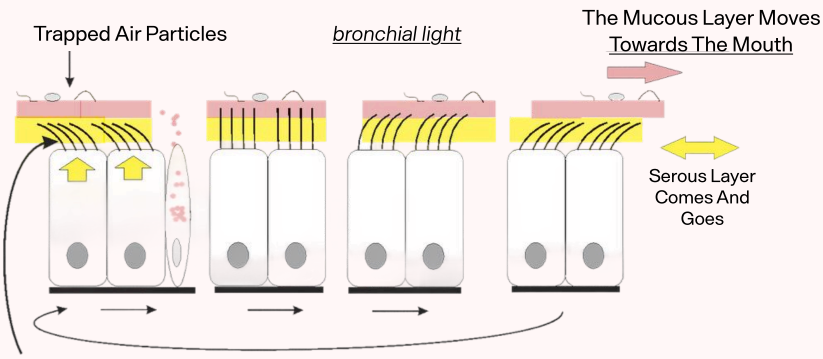


FIGURE 10 The cilia move forward carrying both layers and return exclusively through the serous layer (yellow) in an active process fueled by ATP consumption.

ABNORMAL BRONCHIAL SWEEP (Missing)

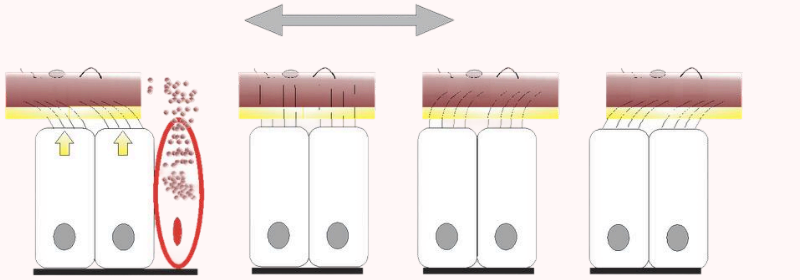


FIGURE 11 Under pathological conditions, glandular secretion is altered and mucus secretion increases; consequently, the cilia can no longer return through the serous layer, causing both layers to move back and forth ineffectively.

ARTEROL®
Thins the mucus.
Increases the ciliary stroke
(increased ATP production).

ARTEROL® is a powerful, long-acting ciliary stimulant that increases mucociliary clearance. Its effect on mucociliary clearance has been investigated both in vitro and in vivo using photoelectric techniques. These studies demonstrated an increase in ciliary beat frequency, showing it to be approximately 100 times more potent than terbutaline *(Lindberg et al., 1995).*

FREQUENTLY ASKED QUESTIONS FROM PHARMACOVIGILANCE:

Why is it administered exclusively via intramuscular injection at a single inoculation site?

The administration of β_2 -agonists via aerosol typically leads to a very rapid therapeutic response, generally within minutes. However, aerosol therapy depends on the delivery of the drug to the deep airways. This, in turn, depends on the size of the aerosol particles and respiratory parameters such as inspiratory flow rate, ratio, air volume, airway diameter *(Newhouse and Dolovich, 1986)*, and airway length (e.g., the neck of the animal), among others. In humans, successful aerosol therapy requires each patient to adapt their drug administration technique. Many patients, particularly children and the elderly, do not use optimal techniques due to inadequate instructions (for example, breath-holding time). *(Kelly, 1985; Newhouse and Dolovich, 1986).*

By contrast, the intramuscular route used for ARTEROL® provides a high degree of dose control, maximizes the dose-response curve, reduces cardiac side effects (Ladaga et al., 2006), and avoids the potential for tachyphylaxis seen in horses through aerosol administration, as well as the exacerbation of side effects seen via the intravenous route (Laboratorio Fundación, unpublished data).

As a highly selective and long-acting β_2 agonist, this route provides a sustained release with a peak observed 2 hours post-administration. This allows for optimal preparation prior to physical activity and potentially better mitigation of exercise-induced pulmonary hemorrhage (EIPH), along with localized sweating at the inoculation site a clear sign of response control (eee package leaflet).

Can ARTEROL® be used in cases of EIPH with laryngeal hemiplegia?

No, because this involves a mechanical obstruction. Under increased inspiratory effort, this condition can actually increase the severity of the hemorrhage.

Can ARTEROL® be used in competition?

First, it's important to understand that the effect of **ARTEROL®** is cumulative. Therefore, its use prior to moderate or intense physical activity during training in horses with EIPH or chronic bronchopulmonary involvement shows a favorable evolution as treatments are accumulated .

In general, the lowest recommended dose is sufficient (See package leaflet) *(Ladaga et al., 2003).*
Regarding the withdrawal period for competition, it is important to note that certain regulatory bodies do not operate based on LOQ (Limit of Quantitation), but rather on LOD (Limit of Detection), which creates uncertainty for the attending professional. This practice is inappropriate for professional practice and for approved drugs with established withdrawal periods *(García et al., 2011; Wasfi, 2012)*. Proper intervention by the attending veterinarian, alongside specific pathology diagnoses via medical prescription, promotes the protection of animal health and welfare. As a guide, the withdrawal period provided by the doping control center at the San Isidro Racecourse in Buenos Aires, Argentina, is set at 5 days.
Furthermore, it would be beneficial to apply the same regulatory concepts to equine athletes as those applied to human athletes. This refers to the following verbatim transcription:

"As of 2023, all beta-2 agonists are prohibited in and out of competition with certain exemptions; for example, formoterol is classified as a 'specific beta-2 agonist' and is permitted for inhalation in daily doses up to 54 µg (this dose is 5 times the therapeutic dose of **ARTEROL®** *in horses relative to body weight), when used with or without an authorization or objective indication for asthma and exercise-induced bronchoconstriction. Dosing rules are established to align with standard treatment guidelines; if an athlete complies with the specified dosing limits, they do not need to apply for one. These rules allow athletes with asthma and EIB to compete without fear of committing an anti-doping rule violation and reduce the administrative workload required for TUE applications" (Agencia Mundial Antidopaje. Lista de Prohibiciones 2023. Consultado el 3 de mayo de 2023. https://www.wada-ama.org/sites/default/files/2022-01/2022list_final_en_0.pdf).*

The time may come when these standards are applied to safeguard animal welfare and avoid the latent risks associated with the use of furosemide, such as sudden death during competition and the resulting risk of accidents for the jockey *(Ladaga et al., 2006; Carlson et al., 1999; Cooper et al., 1999).*

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