Comparación de dos combinaciones de anestésicos inyectables utilizados en laparotomía medial para recuperación de embriones en ovejas criollas colombianas

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Comparison of two injectable anesthetic combinations used to perform midline laparotomy for embryo recovery in Colombian creole ewes

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Abstract

In the countryside, the use of halogenated anesthetics is difficult, therefore the use of injectable agents is an essential tool in anesthetic practice. This study aimed to compare two multimodal injectable anesthesia protocols and to determine the appropriate protocol to perform a medial laparotomy and embryo recovery in sheep. 16 healthy adult creole sheep were used. Animals were randomized to receive xylazine 0.2 mg/kg PC IM and ketamine 10 mg/kg PC IV (XK group), or a continuous infusion of 5% solution of xylazine (50 mg), ketamine (500 mg), and guaifenesin (500 mL) at a rate of 2.2 mL/kg/h IV (XKG group). Heart and respiratory frequency, rumen motility, and body temperature were evaluated before anesthesia, after induction, and during recovery. Induction was assessed by muscle spasms, nystagmus, and limb movement. Anesthesia was evaluated based on time, mandibular relaxation, skin sensitivity, and reflexes. Recovery was evaluated on a scale for anesthetic agent (0-10). Cardiorespiratory parameters decreased below baseline after induction of anesthesia in both groups. Between the groups, there was a significant difference in decubitus time (XK: 9.06 ± 0.73 min; XKG: 7.81 ± 0.53 min) and recovery (XK: 53.13 ± 5.3 min; XKG: 98.38 ± 5.71 min). Changes in the cardiopulmonary system were similar in both anesthetic regimens, and they were within acceptable clinical range. It is concluded that, in short surgical procedures, xylazine-ketamine anesthesia provides rapid induction, maintenance of physiological parameters within optimum limits, and rapid recovery.

Keywords: cardiovascular system, guaifenesin, ketamine, xylazine.
rumen y temperatura corporal se evaluaron antes de la anestesia, después de la inducción y durante la recuperación. La inducción se evaluó según los espasmos musculares, nistagmo y movimiento de las extremidades. La anestesia se valora en función del tiempo, relajación mandibular, sensibilidad cutánea y reflejos. La recuperación se evaluó a una escala de agente anestésico (0-10). Los parámetros cardiorrespiratorios disminuyeron por debajo de los valores basales después de la inducción de anestesia en ambos grupos. Entre los grupos, hubo una diferencia significativa en el tiempo de decúbito (XK: 9,06 ± 0,73 min; XKG: 7,81 ± 0,53 min) y la recuperación (XK: 53,13 ± 5,3 min; XKG: 98,38 ± 5,71 min). Los cambios en el sistema cardiopulmonar fueron similares en ambos regímenes anestésicos, y estaban dentro de rangos clínicos aceptables. Se concluye que, en intervenciones quirúrgicas cortas, la anestesia xilacina-ketamina proporciona una rápida inducción, el mantenimiento de los parámetros fisiológicos dentro de los límites óptimos y una recuperación rápida.

Palavras chave: guaifenasina, ketamina, sistema cardiovascular, xilazina.

Comparação de duas combinações de anestésicos injetáveis utilizados na laparatomia medial para recuperação de embriões em ovelhas nativas colombianas

Resumo
O uso de anestésicos halogenetos é difícil em campo, razão pela qual o uso de agentes injetáveis é uma ferramenta essencial da prática anestésica. O objetivo do presente estudo foi comparar dois protocolos de anestésicos injetáveis multimodais e determinar o protocolo adequado para realizar uma laparatomia medial e recuperar embriões em ovelhas. Se utilizaram 16 ovelhas domésticas adultas e saudáveis. Os animais foram designados aleatoriamente ao azar para receber 0,2 mg/kg PC IM de xilazina e 10 mg/kg PC IV de cetamina (XK grupo) ou uma infusão contínua de solução ao 5% de xilazina (50 mg), cetamina (500 mg) e de guaifenesin (500 mL) a um índice de 2,2 mL/kg/h IV (grupo XKG). A frequência cardíaca e respiratória, motilidade do rumen e temperatura corporal foram avaliadas antes da anestesia, após a indução e durante a recuperação. A indução é avaliada se avaliou segundo os espasmos musculares, nistagmo e movimento das extremidades. A anestesia foi medida em função do tempo, relaxamento mandibular, sensibilidade cutânea e dos reflexos. A recuperação se avaliou com uma escala do agente anestésico (0-10). Os parâmetros cardiorrespiratórios diminuíram abaixo dos valores basais após indução do anestesia em ambos os grupos. Entre os grupos, houve uma diferença significativa no tempo de decúbito (XK: 9,06 ± 0,73 min; XKG: 7,81 ± 0,53 min) e a recuperação (XK: 53,13 ± 5,3 min; XKG: 98,38 ± 5,71 min). As mudanças no sistema cardiopulmonar foram similares em ambos os regimes anestésicos, e estavam dentro de rangos clínicos aceitáveis. Conclui-se que, em intervenções cirúrgicas curtas, a anestesia xilazina-cetamina proporciona uma rápida indução, a manutenção dos parâmetros fisiológicos dentro dos limites ótimos e uma recuperação rápida.

Palavras chave: guaifenesin, cetamina, sistema cardiovascular, xilazina.
INTRODUCTION

Intravenous anesthesia advantages include rapid onset of action independent of ventilation status, reduction of adverse effects of other anesthetic drugs if used in balanced anesthesia protocols, allowing for provision of continuous analgesia if needed, smoother recovery from anesthesia, low costs (considering that the minimum requirement is a needle and a syringe), and reduction of the hazards of occupational health and atmospheric pollution (1). Surgical procedures in small ruminants can be performed under intravenous anesthesia. Xylazine and ketamine combinations have been popular for anesthetizing small ruminants since 1970. Although rapid onset and short duration of action are the advantages of a single bolus injection of xylazine and ketamine, repeated dosing or continuous infusion often results in a prolonged recovery (2). Injectable general anesthesia using drug combinations such as ketamine and xylazine with or without guaifenesin are common in certain surgical procedures (3).

Xylazine induces dose-dependent sedation and central nervous depression in sheep (4). Alpha-2 adrenergic receptors are potent sedative drugs that provide muscle relaxation and analgesia. A range of xylazine doses alters respiratory mechanics and gas exchange, causing tachypnea, increased airway pressures and respiratory resistance, decreased lung compliance, pulmonary edema, and hypoxemia with or without hypercapnia (5), produced by vasoconstriction, which follows peripheral α2B-receptor stimulation caused by high plasma α2-agonist concentrations (6). In sheep, it has a short elimination half-life and it is rapidly cleared from plasma after intramuscular (IM) and intravenous (IV) administration (7). The effects of xylazine on the digestive tract are variable and include reduced rumen motility and ruminal tympany (8). In adult sheep, recumbency occurred 15 minutes after the administration of 0.3 mg/kg BW of IM xylazine and lasted 55 minutes in adult rams (9). In contrast, IV injection of xylazine (0.3 mg/kg BW) in adult ewes caused recumbency within 3 minutes, which lasted for 54 minutes (10). After the administration of 0.4 mg/kg BW IV xylazine, adult sheep were recumbent for 41 minutes, followed by 34 minutes of head drooping (11).

The α2-adrenergic agonists are often combined with ketamine for anesthesia induction in pregnant sheep (12). Ketamine is commonly used in small ruminants for induction and maintenance of anesthesia. It is a dissociative anesthetic with analgesic effects, which provides mild cardiovascular stimulation and largely maintains the swallowing and cough reflexes (13). Apnea is not uncommon, especially after rapid IV injection, and significant salivation can also be observed (14). When used alone, ketamine provides poor muscle relaxation and the peripheral reflexes are maintained. Therefore, it is strongly recommended to administer ketamine in conjunction with a α2-adrenoceptor agonist to improve muscle relaxation and sedation (15). The combination of ketamine and α2 adrenergic agonists enhances the degree of analgesia and prolongs anesthesia, but respiratory depression may be severe and the recovery delayed when large doses are used (16). Ketamine possesses most of the characteristics required for suitability for continuous intravenous anesthesia, lending itself well for field anesthesia (17).

Guaifenesin is not an anesthetic but a muscle relaxant that disrupts nerve impulse transmission at the level of the internuncial neurons of the spinal cord, brain stem, and subcortical areas of the brain, with no effect on diaphragmatic function, and mild, if any, analgesic and sedative effects (18). Cardiovascular and respiratory depression is minimal. Dilution in 5% solutions avoids potential hemolysis and, in case of perivascular administration, tissue necrosis (19). It is not often used alone in small ruminants, but it can be used in combination with xylazine or ketamine (20) to induce and maintain short-term anesthesia. It can also be used as a constant-rate infusion, being part of a solution of xylazine (50 mg), ketamine (500 mg), and guaifenesin (500 mL of a 5% solution) (21), often referred to as “triple drip”. Anesthesia is induced in 5 to 10 minutes by the rapid administration of 0.5 to 2 mL/kg BW of the above-mentioned mixture and maintained by infusion at 2 to 2.6 mL/kg/hour. A constant level of anesthesia is produced, and recovery is smooth but not rapid (22).

Therefore, the objective of this study was to evaluate the clinical usefulness and anesthetic effect of xylazine-ket-
amine (XK) and to compare these effects with those of xylazine-ketamine-guaifenesin (XKG) in order to perform exploratory laparotomy and allow the flushing of the uterine horns in ewes.

**Materials and Methods**

**Animals**

Sixteen healthy, adult Colombian creole ewes aged 95.06 ± 9.94 months and weighing 26 ± 7 kg were used in this study. All animals were subjected to anthelmintic prophylaxis two months before surgery. Prior to the study, the health of the animals was evaluated using a complete blood count, liver and renal biochemical profile, and fecal parasitologic examination. The ewes were housed in groups (eight animals) per pen in a facility at the Veterinary Clinic of the University of Nariño with free access to food and water. Before the study, food and water were withheld for 24 hours and the hair over the right jugular vein was clipped. This study was reviewed and approved by the Animal Ethics Committee of the Veterinary program of the Animal Science Faculty at University of Nariño.

**Anesthesia Protocols**

A sheep was removed from its place, weighed and taken to the preparation room. Jugular groove was cleaned and treated with 1% cetrimide. A teflon intravenous catheter was placed into the jugular vein after infiltration of the skin with 1 mL lidocaine via a 25 gauge needle. The catheter was secured in place and flushed with 5 mL of heparinized saline (10 IU/mL). Anesthesia was induced in animals of group XK (n=8) using 0.2 mg/kg BW xylazine intramuscularly (IM) and, after 5-7 minutes, 10 mg/kg BW ketamine was administered intravenously (IV). Ewes of the XKG group were anesthetized with a constant-rate infusion of a solution of xylazine (50 mg), ketamine (500 mg), and guaifenesin (500 ml of a 5% solution) and the maintenance with an infusion at 2.2 ml/kg/hour intravenously.

**Physiological Parameters**

Cardiopulmonary data were collected in the following order: Heart rate (HR, bpm) was measured using transthoracic auscultation with a stethoscope in the region of the fourth left intercostal space for 1 minute; respiratory rate (fR, rpm) was assessed by observing the movements of the thorax for 1 minute; rectal temperature (RT, °C) was measured with a clinical mercury-in-glass thermometer inserted into the rectum for 1 minute; variables were measured before drug administration (baseline, T0) and 15 minutes after drug administration in four time points: T15, T30, T60, and T120 minutes.

**Anesthesia Evaluation**

Clinical signs relating to the quality of induction including muscle twitching of the face, neck and whole body along with the presence or absence of nystagmus, leg movement and paddling were recorded. Anesthesia was evaluated recording time, jaw relax, skin sensitivity (response to pin pricks) and reflexes (palpebral and gag).

The quality of recovery was scored on a visual analogue scale for each anesthetic agent used. The scale ranged from zero, indicating an excitable recovery with numerous unsuccessful attempts to stand and obvious distress, to 10, indicating a fast, smooth recovery with rapid return to normal behavior.

**Surgery**

Animals were solid fasting for twenty-four hours before surgery, and liquid fasting for 12 hours. The surgery technique for uterine flushing and embryo recovery was the same used for the two groups. The ewes were placed in spinal recumbence, head downwards on an inclined stretcher. The surgical area was shaved and disinfected. A midline laparotomy of 5-7 cm and 3 cm in front of the udder was carried out.
Analysis of Data

All data are presented as mean ($x$) and standard error (SE). Sedation scores were compared by analysis of variance (ANOVA). Changes over time and differences between groups were explored by ANOVA for repeated measures for the time points followed by Bonferroni-adjusted t-tests for effects over time where appropriate. Significance level was set at $p<0.05$. The results were analyzed using descriptive statistics with the Statistical Package for Social Sciences (SPSS) 19.0 (23).

RESULTS

The combinations produced satisfactory anaesthesia and no major adverse events were seen during any phase of the anesthetic induction or recovery. Table 1 shows the time lapse for recumbency, surgery and recovery of ewes receiving multimodal anesthesia xylazine (50 mg), ketamine (500 mg) and guaifenesin via IV by infusion at a rate of 2.2 ml/kg/hour and the combination xylazine 0.2 mg/kg BW IM and ketamine 10 mg/kg BW IV.

Ewes of the xylazine-ketamine group showed a slight regurgitation, expressed ataxia with head drooping and a sternal recumbency after administration of xylazine 0.2 mg/kg BW IM. Following the intravenous application of ketamine, sheep were in lateral recumbency in 9.06 ± 0.73 min. The animals had diminished response of pinpricks. We did not observe any muscle twitching of the face and neck, nystagmus or paddling.

After a rapid administration (0.8 ml/kg) of the XKG solution via IV by infusion at a rate of 2.2 ml/kg/hour, ewes showed salivation and a poor response of pinpricks. Time to recumbency was significantly ($p<0.05$) faster (7.81 ± 0.53 min) compared to xylazine-ketamine.

Table 1. Time lapse expressed by average ± standard (SD) deviation for recumbency, surgery and standing ewes after administration of two injectable anesthesia combinations

<table>
<thead>
<tr>
<th>Multimodal anesthesia</th>
<th>Average Time to recumbency (minutes)</th>
<th>Time in surgery (minutes)</th>
<th>Time to stand (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine/xylazine</td>
<td>9.06 ± 0.73*</td>
<td>36.88 ± 1.81</td>
<td>53.13 ± 5.30*</td>
</tr>
<tr>
<td>Xylazine+ketamine+guaifenesin</td>
<td>7.81 ± 0.53</td>
<td>37.25 ± 2.38</td>
<td>98.38 ± 5.71</td>
</tr>
</tbody>
</table>

* Significantly different from that of sheep receiving xylazine-ketamine-guaifenesin ($p<0.05$).
After surgery, the sheep in the xylazine-ketamine group took a sternal recumbency and were standing up in 53.13 ± 5.3 min. Meanwhile, at the end of the laparotomy, the administration of the XKG solution was suspended and recovery to standing occurred in 98.38 ± 5.71 min (Table 1). The recovery of XK group was significantly (p<0.05) faster compared with xylazine-ketamine-guaifenesin.

The effects before, during and after surgery under anesthesia with xylazine/ketamine and xylazine-ketamine-guaifenesin combinations on the clinical parameters (heart rate, respiration rate, body temperature, and rumen motility) of ewes are presented in Table 2.

Table 2. Mean clinical parameters of Colombian creole ewes (n=16) during laparotomy for embryo recovery under two injectable anesthesia procedures, XK (n=8) and XKG (n=8)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Time points (minutes)</th>
<th>Before surgery</th>
<th>During surgery</th>
<th>After surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>T0 T15 T30 T60 T120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>XK</td>
<td>81.00 ± 2.10</td>
<td>71.67 ± 1.91*</td>
<td>77.50 ± 1.52#</td>
<td>65.67 ± 2.42*</td>
</tr>
<tr>
<td></td>
<td>XKG</td>
<td>82.00 ± 2.93</td>
<td>71.67 ± 2.94*</td>
<td>67.00 ± 2.10*</td>
<td>62.67 ± 2.42*</td>
</tr>
<tr>
<td>fR (rpm)</td>
<td>XK</td>
<td>27.17 ± 2.04</td>
<td>26.00 ± 1.26#</td>
<td>20.33 ± 2.34*</td>
<td>20.50 ± 2.17*</td>
</tr>
<tr>
<td></td>
<td>XKG</td>
<td>29.00 ± 2.10</td>
<td>23.00 ± 2.10*</td>
<td>22.33 ± 1.51*</td>
<td>22.33 ± 1.51*</td>
</tr>
<tr>
<td>RT (°C)</td>
<td>XK</td>
<td>38.27 ± 0.18</td>
<td>38.12 ± 0.19</td>
<td>38.07 ± 0.22</td>
<td>38.15 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>XKG</td>
<td>38.27 ± 0.18</td>
<td>38.08 ± 0.23</td>
<td>38.12 ± 0.19</td>
<td>38.05 ± 0.25</td>
</tr>
<tr>
<td>Rm</td>
<td>XK</td>
<td>1.55 ± 0.52</td>
<td>1.50 ± 0.55</td>
<td>1.50 ± 0.55</td>
<td>1.50 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>XKG</td>
<td>1.67 ± 0.52</td>
<td>1.33 ± 0.52</td>
<td>1.50 ± 0.55</td>
<td>1.67 ± 0.52</td>
</tr>
</tbody>
</table>

Results are mean ± standard deviation
# Significantly different from that of sheep receiving xylazine-ketamine-guaifenesin (p<0.05).
* Significantly different from baseline (time-0) values (p<0.05).
XK; xylazine + ketamine
XKG; xylazine + ketamine + guaifenesin
HR; Heart rate; bpm; beats per minute
fR; respiratory rate; rpm; respiration per minute
RT; rectal temperature
Rm; Rumen motility
Both groups presented heart and respiratory rates that were within the physiological limits during the surgical procedure. Throughout the procedures, there were no significant differences in corporal temperature and rumen motility between the groups and among the treatments. These parameters remained within the normal limits for each group.

Heart rate decreased at all time points in every treatment compared to the baseline. There was a significant difference in heart rates between the groups (p<0.05) at T30, T60 and T120. In XK group assessments, there was a statistically significant difference in heart rate at T15 (77.5 ± 1.52 bpm) and at T60 (65.67 ± 1.51 bpm) (p<0.05) compared to the baseline values (81 ± 2.1 bpm). Ewes receiving the XKG combination presented statistically significant differences in heart rate at four time points—T15 (71.67 ± 2.94 bpm), T30 (67 ± 2.1 bpm), T60 (62.67 ± 2.42 bpm), and T120 (70.67 ± 2.07 bpm)—compared to baseline values (82 ± 4.73 bpm). Heart rate increased during the exteriorization and manipulation of the uterine horns for flushing and embryo recovery.

As to respiratory rate, there was a statistically significant difference between the XK and XKG groups (p<0.05) at T15 and T120. Compared to baseline values (27.17 ± 2.04 rpm) in the xylazine-ketamine group, we observed a significant difference (p<0.05) in the respiratory rate at T30 (20.33 ± 2.34 rpm) and T60 (20.5 ± 2.17 rpm), while in the xylazine-ketamine-guaifenesin group, the respiratory rate decreased significantly at T15 (23 ± 2.1 rpm), T30 (22.33 ± 1.51 rpm), T60 (22.33 ± 2.34 rpm), and T120 (22 ± 1.79 rpm), compared to the baseline rate (29 ± 2.1 rpm).

**Discussion**

Xylazine, ketamine and guaifenesin were chosen for anesthesia in ewes because they are inexpensive and available drugs in Colombia. Although injectable anesthetics are available and commonly used, their safe use by veterinarians and scientists require experience and accurate calculation of the dose in order to prevent or minimize undesirable effects (24). For surgical embryo transfer, the practitioner must choose the appropriate anesthetic agents or a combination of agents to be safe and fast during the surgical procedure in sheep.

Between the two multimodal protocols, respiratory rate showed a significant difference at T15 and T120. In the XKG group, there was a significant depression in the respiratory rate in the induction at T15, T30, T60 and T120, while the difference was at T15 and T60 in XK group. After T60, however, respiratory rates mildly increased. This may have occurred partly because ketamine induces a mild respiratory inhibition, which usually appears at an increased rate (25). Ketamine has desired effects such as maintenance and stimulation of respiration, bronchodilation, maintenance of functional residual capacity, and achievement of equivalent minute ventilation rates both in spontaneously breathing individuals and in those that are wide awake (26). Hobbs et al. (27) reported this pattern of breathing in other small and laboratory animals. Sedation with XK and XKG treatment initially resulted in lateral recumbency, which may have limited lung expansion and resulted in decreased ventilation (28).

Both protocols presented a significant decrease (p<0.05) of 15% in heart rate (11 ± 1 bpm) at T15, which are values similar to those reported by Hughan et al. (29). Between T30 and T60, heart rate showed a significant difference in the XK group compared to the XKG group. This may be caused by xylazine administration, which causes dose-dependent cardiovascular depression and bradycardia by enhancing vagal tone and baroreceptor reflexes (30). The Reports (31) of intravenous administration of xylazine at 0.4 mg/kg BW, decreases heart rate by 30% and a bolus of 0.5 mg/kg BW causes an immediate decrease in heart rate by 25% and in cardiac output by 37%, followed by a sustained bradycardia for 60 minutes (32). In contrast, an intramuscular administration of xylazine at a dose of 0.05 mg/kg induces only minor cardiovascular changes (33). Xylazine possesses hypotensive and hypoxic effects by depressing cardiovascular and respiratory activities. In contrast to most anesthetic drugs, ketamine has been shown to have incremental effects on heart rate, blood pressure and respiratory rate, due to the
increase in sympathetic activation (34). Lin et al. (35) reported a transient bradycardia followed by a period of stabilization during administration of ketamine. In this study, heart rate increased in both anesthetics protocols during manipulation of the uterus and the flushing in surgery. Singh et al. (36) described the autonomic response, such as tachycardia and hypertension, due to the manipulation of organs during surgical procedures.

Although cyclical contractions of the reticulum and rumen are inhibited by administration of xylazine due to sympathetic blockade and reduction in norepinephrine release (37), no significant differences between rumen motility measured before and after anesthesia with xylazine-ketamine or xylazine-ketamine-guaifenesin were observed.

Regarding body temperature, there were no significant differences between rectal temperatures measured before and after anesthesia. In the present study, the decrease in rectal temperature of sheep identified after xylazine administration was not clinically significant. The decrease in body temperature caused by depression of the thermoregulatory center (38) and reduced muscular activity (39) may be the result of administering $\alpha_2$-agonists. Nevertheless, Zuhair et al. (40) reported a significant decrease in the body temperature of sheep after 60 minutes of anesthesia with a combination of xylazine-ketamine. The results indicated a stable body temperature in all the ewes.

In this study, induction with xylazine-ketamine was significantly higher in time and smoother compared to the xylazine-ketamine-guaifenesin protocol. There were no time significant differences between the two groups during surgery, although recovery was significantly faster in the xylazine-ketamine group than the xylazine-ketamine-guaifenesin group. After standing, the return of muscle strength appeared to be more complete, as the sheep in this group appeared to be less atactic and they were able to walk sooner than those anesthetized with xylazine-ketamine-guaifenesin. A rapid recovery is generally considered desirable, if it is accompanied by a shorter period of anesthesia. The longer recovery time in XKG ewes may be attributed to slower drug elimination from the plasma and to the guaifenesin muscle relaxant effect. Guaifenesin’s mechanism of action is to disrupt nerve impulse transmission at the level of the internuncial neurons of the spinal cord, brain stem, and subcortical areas of the brain (41).

When compared to xylazine-ketamine anesthesia, xylazine-ketamine-guaifenesin induced comparable anesthetic effects in healthy sheep. Changes in the cardiopulmonary system were similar in both anesthetic regimens and these changes were within acceptable clinical ranges. The time it took for the sheep to stand was significantly shorter for xylazine-ketamine than the combination of xylazine-ketamine-guaifenesin. We conclude that, in short surgical interventions involving midline laparotomy, xylazine-ketamine anesthesia is preferred over xylazine-ketamine-guaifenesin, as it achieves a rapid anesthetic induction, maintenance of physiological parameters within optimal limits and a faster recovery.

References


