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Evaluating The impact of preemptive Flunixin meglumine on Tiva protocols During Donkey Castration

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Abstract This clinical study aimed to evaluate the effect of adding Flunixin meglumine to a total intravenous anesthesia (TIVA) protocol involving ketamine, xylazine, and medetomidine in donkeys for castration.Ten healthy adult donkeys were randomly divided into two equal groups (n=5 each) and subjected to castration. Group A (Flunixin group) received Flunixin IV at 1.1 mg/kg one hour prior to anesthesia induction involving ketamine (2.2 mg/kg IV), xylazine (0.5 mg/kg IV), and medetomidine (0.1 µg/kg IV). TIVA maintenance was achieved via continuous IV infusion of saline supplemented with ketamine, xylazine, and medetomidine. Group B (Traditional group) received acepromazine as a sedative, followed by xylazine (1.1 mg/kg IV) and ketamine (2.2 mg/kg IV), with repeated doses for maintenance. Physiological and hematological parameters were monitored at baseline and at 15, 30, 45, and 60 minutes post-induction. Flunixin addition led to remarkably stable respiratory and heart rates, a progressive drop in body temperature, and a moderate increase in blood sugar in Group A compared to Group B. Packed cell volume and hemoglobin values showed no significant differences between the two groups. Castration with preemptive Flunixin enhances anesthesia quality, provides better physiological stability, and reduces the need for repeated maintenance doses, benefiting both animal welfare and anesthetic efficacy in donkeys.

Keywords: Castration, Donkeys, Flunixin, Medetomidine, Ketamine, Preemptive analgesia, Total intravenous anesthesia, Xylazine.

INTRODUCTION

Pain is a disagreeable sensory and emotional sensation related to tissue injury, real or probable (1). Flunixin meglumine is a non-steroidal anti-inflammatory drug (NSAID) with potent anti-inflammatory, anti-pyretic, and analgesic effects primarily through inhibition of cyclooxygenase enzymes (COX-1 and COX-2), thereby reducing prostaglandins and thromboxanes. In animals it is commonly used for mastitis, fever, lameness, and endotoxemia(3).

Castration inevitably causes some level of pain, requiring proper analgesic treatment (4). Flunixin meglumine (FM) is a nonselective NSAID that has long-lasting analgesic properties due to its accumulation and slow release in inflamed tissues (6). It is used after musculoskeletal injuries (7), abdominal operations (8), and is considered the NSAID of choice after castration of equines(4). NSAIDs are organic acids that inhibit cyclooxygenase, decreasing prostaglandins and inhibiting inflammatory processes (9,10).

The α2-adrenoceptor agonists (xylazine, detomidine, medetomidine) are widely used in veterinary medicine for sedative, analgesic and muscle relaxation properties (15). Ketamine is a dissociative anesthetic that maintains hemodynamic stability and reduces pain (16), and has been used in equine medicine since, the mid-1970s(17). In later years its use extended to various TIVA protocols (15).

Despite the widespread use of TIVA protocols in equine practice, limited information is available regarding the adjunctive use of flunixin meglumine during donkey castration. Therefore, this study aimed to evaluate the effects of flunixin administration on anesthetic quality, physiological stability, and hematological parameters in donkeys undergoing castration under TIVA protocols.

MATERIALS AND MTHODS

This study was performed under ethical approval No. P.G.1399. To reduce animal numbers (3Rs principle), the control group used for comparison was shared with a parallel study on diclofenac (Approval No. P.G.730) and its data have been reported in a parallel study (manuscript submitted for publication).

Ten healthy adult donkeys (*Equus asinus*), aged 3 years and weighing 97 ± 18 kg, were randomly divided into two equal groups (n=5): Group A (flunixin group) and Group B (Traditional group). Donkeys in both groups underwent castration.

Surgical Operation Technique:

Castration in donkeys is a common surgical procedure performed to control reproduction, reduce aggressive behavior, and improve handling. After proper sedation and surgical preparation, an incision is made over the scrotum, the testicles are exteriorized, and the spermatic cords are ligated and transected. Adequate analgesia and anesthesia are essential to minimize pain, stress, and postoperative complications.

Anesthetic Protocols:

Group A (Flunixin + TIVA Protocol):

Animals in Group A received flunixin (IV) at 1.1 mg/kg(20). Sixty minutes later, anesthesia was induced with ketamine 2.2 mg/kg IV(20), xylazine 0.5 mg/kg IV, and medetomidine 0.1 mg/kg IV. TIVA maintenance(14), was achieved via continuous IV infusion at 32 mL every 15 minutes (100 mL saline supplemented with ketamine ~1.32 mg/kg/h, xylazine ~0.21 mg/kg/h, medetomidine ~33 µg/kg/h).

Group B (Traditional Induction Protocol):

Animals in Group B received acepromazine(22) as a sedative. Fifteen minutes later: xylazine 1.1 mg/kg IV and ketamine 2.2 mg/kg IV(20). Repeated ketamine-xylazine doses were required to maintain adequate anesthesia.

Monitoring and Parameters:

Physiological and hematological parameters (HR, RR, rectal temperature, PCV, Hb, blood sugar, CRT) were monitored at baseline and at 15, 30, 45, and 60 minutes post-induction.

Scoring of recovery quality (40)

Score	General Definition
1	Successfully standing from the first attempt
2	Successfully standing from the second attempt
3	Animals remains calm during more than two attempts to stand
4	Risk of injury during several attempts to stand
5	Animal injured in recovery period

RESULT

Induction, Surgical, and Recovery Times

A statistically significant difference was observed in the induction time between the two groups (P≤0.05). Group A exhibited a more rapid onset with mean induction time of 1.00 ± 0.00 min, while Group B required 9.00 ± 6.20 min. No significant difference was observed in surgical period (43.20 ± 14.16 vs 30.80 ± 20.46 min) or recovery time (15.40 ± 7.40 vs 24.60 ± 19.26 min).

Table 1. Effect of group in Induction, Surgical Period and Recovery

Group	Induction Mean±SD (min)	Surgical Period Mean±SD (min)	Recovery Mean±SD (min)
A: flunixin-TIVA	1.00 ±0.00 b	43.20 ±14.16	15.40 ±7.40
B: traditional protocol	9.00 ±6.20 a	30.80 ±20.46	24.60 ±19.26
T-test	5.398 *	15.667 NS	11.288 NS

*Means with different letters in same column differed significantly. * (P≤0.05).*

Quality of Anesthetic Recovery

Group A (Flunixin-TIVA) recovered significantly better. 60% achieved score 1 (standing on first attempt) and 20% scored 2 (good). No Group A animals had a poor recovery (scores 3–5). In contrast, 100% of Group B animals experienced a problematic emergence: 60% scored 3 and 40% scored 4. Statistical analysis confirmed a significant difference between groups (P<0.05).

Table 2. Effect of group and period in Recovery score

Group	Score 1 No.(%)	Score 2 No.(%)	Score 3 No.(%)	Score 4 No.(%)	Score 5 No.(%)	P-value
A: flunixin-TIVA	3/5 (60%)	1/5 (20%)	0/5 (0%)	0/5 (0%)	1/5 (20%)	0.0398 *
B: traditional protocol	0/5 (0%)	0/5 (0%)	3/5 (60%)	2/5 (40%)	0/5 (0%)	0.0427 *
P-value	0.0451 *	0.348 NS	0.0451 *	0.088 NS	0.348 NS	---

** (P≤0.05), NS: Non-Significant.*

Physiological Parameters

Respiratory Rate (RR)

In Group A, the respiratory rate remained remarkably stable throughout (baseline 27.80 ± 4.92 breaths/min, no significant changes over 60 min). Group B displayed considerable post-induction instability, rising significantly from 14.80 ± 4.76 to33.80 ± 11.90 breaths/min at 30 min (P<0.05), remaining elevated thereafter.

Table 3. Effect of group and period in Respiratory rate — RR (breath/min)

Group	0 min	15 min	30 min	45 min	60 min
A: flunixin-TIVA	27.80±4.92 Aa	30.00±10.00 Aa	25.60±6.61 Ba	28.00±8.72 Aa	28.20±7.39 Aa
B: traditional protocol	14.80±4.76 Bc	26.00±12.10 Ab	33.80±11.90 Aa	28.00±12.74 Aab	27.20±12.21 Aab
LSD: 6.922 *					

Capital letters (A,B): significant difference between groups. Lowercase (a,b,c): significant difference between time points. * (P≤0.05).

Heart Rate (HR)

Group A maintained cardiovascular stability with no significant changes over 60 min (baseline 46.00 ± 3.31 pulse/min). Group B displayed transient tachycardia post-induction, rising from 38.60 ±6.46 to 46.40 ± 12.83 pulse/min

at 30 min (P<0.05), returning to baseline at 60 min. No significant between-group differences were found at any time point.

Table 4. Effect of group and period in Heart rate — HR (pulse/min**)**

Group	0 min	15 min	30 min	45 min	60 min
A: flunixin-TIVA	46.00±3.31 Aa	44.40±10.59 Aa	41.60±4.56 Aa	41.80±9.54 Aa	42.40±5.68 Aa
B: traditional protocol	38.60±6.46 Ab	46.20±12.61 Aa	46.40±12.83 Aa	45.40±17.42 Aab	38.40±11.71 Ab
LSD: 6.741 *					

*Capital letters (A): significant difference between groups. Lowercase (a,b): significant difference between time points. * (P≤0.05).*

Rectal Temperature

Group A showed a significant progressive decline in temperature from 38.20 ± 0.88 °C (baseline) to 36.50 ± 0.82 °C at 60 min (P<0.001). Group B remained stable throughout (baseline 36.68 ± 1.14 °C, no significant changes). Despite these contrasting patterns, no significant between-group differences were found at any time point

Table 5. Effect of group and period in rectal body Temperature (°C)

Group	0 min	15 min	30 min	45 min	60 min
A: flunixin-TIVA	38.20±0.88 Aa	37.56±0.81 Aab	37.10±0.62 Ab	36.66±0.56 Ab	36.50±0.82 Ab
B: traditional protocol	36.68±1.14 Aa	36.64±0.92 Aa	36.16±0.83 Aa	35.98±0.84 Aa	35.88±0.76 Aa
LSD: 1.066 *					

*Capital letters (A): significant difference between groups. Lowercase (a,b): significant difference between time points. * (P≤0.05).*

Capillary Refill Time (CRT)

No group × time interaction or between-group differences in CRT were observed at any time point (P>0.05). Values were stable and normal (≤2 seconds) in all animals in both groups.

Table 6. Effect of group and period in Capillary refill time — CRT (sec)

Group	0 min	15 min	30 min	45 min	60 min
A: flunixin-TIVA	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
B: traditional protocol	2.00±0.00	1.80±0.44	1.80±0.44	1.80±0.44	2.00±0.00
LSD: 0.406 NS					

NS: Non-Significant.

Hematological Parameters

Hemoglobin (Hb)

Group A exhibited a significant progressive decrease in Hb from 9.20 ± 1.10 g/dl (baseline) to 7.64 ± 0.72 g/dl at 45 min (P<0.05). Group B maintained stable Hb throughout (baseline 9.32 ± 1.26 g/dl). Group A Hb was significantly lower than Group B at all post-induction time points (P<0.05).

Table 7. Effect of group and period in Hemoglobin — Hb (g/dl)

Group	0 min	15 min	30 min	45 min	60 min
A: flunixin-TIVA	9.20±1.10 Aa	8.04±0.56 Bab	7.78±0.70 Bb	7.64±0.72 Bb	8.14±0.27 Bab
B: traditional protocol	9.32±1.26 Aa	9.56±2.11 Aa	9.84±1.99 Aa	9.86±1.96 Aa	10.04±1.94 Aa
LSD: 1.416 *					

*Capital letters (A,B): significant difference between groups. Lowercase (a,b): significant difference between time points. * (P≤0.05)*

Packed Cell Volume (PCV)

PCV findings mirrored Hb. No significant between-group difference at baseline or 15 min. Starting from 30 min and persisting to 60 min, Group A PCV was significantly lower than Group B (P<0.05). Within-group analysis showed no significant changes from baseline for either group across time points.

Table 8. Effect of group and period in Packed Cell Volume — PCV (%)

Group	0 min	15 min	30 min	45 min	60 min
A: flunixin-TIVA	30.78±3.56 Aa	27.30±1.55 Aa	26.24±2.18 Ba	26.04±2.28 Ba	26.58±2.04 Ba
B: traditional protocol	31.10±7.24 Aa	30.28±5.81 Aa	31.50±7.39 Aa	31.40±7.40 Aa	33.10±7.84 Aa
LSD: 5.095 *					

*Capital letters (A,B): significant difference between groups. Lowercase (a): no significant difference between time points. * (P≤0.05).*

Biochemical Parameters — Blood Sugar

Only Group A developed progressive hyperglycemia, with blood glucose increasing significantly from 75.00 ± 19.27 mg/dl (baseline) to 139.60 ± 23.22 mg/dl at 60 min (P<0.05). Group B remained stable near baseline (79.60 ± 9.55 mg/dl). Group A blood sugar was significantly higher than Group B from 30 min onwards (P<0.05).

Table 9. Effect of group and period in Blood Sugar (mg/dl)

Group	0 min	15 min	30 min	45 min	60 min
A: flunixin-TIVA	75.00±19.27 Ad	94.60±43.63 Acd	113.00±36.84 Bbc	133.00±30.23 Aab	139.60±23.22 Aa
B: traditional protocol	79.60±9.55 Aa	87.00±20.13 Aa	96.80±26.25 Aa	94.40±26.14 Ba	96.60±31.22 Ba
LSD:					

Group	0 min	15 min	30 min	45 min	60 min
23.037 *					

*Capital letters (A,B): significant difference between groups. Lowercase (a,b,c,d): significant difference between time points. * (P≤0.05).*

DISCUSSION

Quality of Anesthetic Recovery

The markedly better recovery quality in Group A was attributable to two key factors. First, the TIVA technique avoids the peaks and troughs in drug concentration characteristic of repeated bolus administration (37) , preventing abrupt uncontrollable awakening. Second, preemptive flunixin provided sustained analgesia extending into the postoperative period (38), reducing pain, fear and panic during emergence. This is consistent with earlier research in horses (23) and donkeys (24).

In contrast, 100% of Group B animals experienced problematic recovery. The significant increases in HR and RR after induction in Group B reflect pronounced physiological stress and probable nociceptive stimulation, leading to ataxia, repeated standing attempts, and in some cases self-inflicted injuries (2).

Hematological and Biochemical Effects

The progressive reduction of Hb and PCV in Group A is a well-documented effect of alpha-2 adrenergic agonists (xylazine and medetomidine) mediated via splenic sequestration of erythrocytes (25). This was greater in Group A due to continuous infusion of two potent alpha-2 agonists, causing prolonged splenic relaxation (26,27) . Therefore, this TIVA protocol should be avoided in hypovolemic animals or those actively bleeding (25).

The sustained hyperglycemia in Group A results from continuous alpha-2 agonist infusion inhibiting insulin secretion from pancreatic β-cells (27,40) . Group B's stable glucose reflects a lesser degree of alpha-2 agonist-mediated suppression with single-bolus dosing (32).

Role of Preemptive Analgesia

Preemptive flunixin meglumine (1.1 mg/kg IV) effectively blunted afferent pain signals from the castration stimulus, evidenced by the stable HR and RR in Group A contrasting sharply with tachycardia and tachypnea in Group B. This analgesic mechanism also reduced the total anesthetic requirement, as a stable plane was maintained by CRI in Group A while Group B required repeated bolus doses. These results correlate with studies showing benefit of flunixin post-castration (30).

Analysis of Anesthetic Protocols

TIVA maintained produced constant plasma levels of ketamine, xylazine, and medetomidine, keeping a stable anesthetic plane (2). The combination of ketamine with alpha-2 agonists provides a rapidly acting, analgesic, muscle-relaxant protocol (17), and the addition of medetomidine improved sedative and analgesic properties (31,32). The traditional protocol showed inherent limitations for prolonged procedures, with significantly longer induction time (9.00 ± 6.20 min) and dangerous recovery outcomes in 100% of animals, emphasizing the need for more controlled anesthesia techniques (33).

CONCLUSION

A multimodal TIVA protocol (ketamine-xylazine-medetomidine CRI) with preemptive flunixin meglumine provides significantly better anesthetic quality, physiological stability, and recovery outcomes compared to the traditional protocol for donkey castration. Clinicians should be aware of the transient reduction in PCV and Hb and the hyperglycemic effect associated with continuous alpha-2 agonist infusion.

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Conflict of Interest

The authors declare no conflict of interest.

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