Submitted: 18 February, 2025 Accepted: 25 April, 2025 Published: 08 October, 2025 DOI:10.22514/sv.2025.142

Open Access
Signa Vitae

ORIGINAL RESEARCH

Circadian rhythms on the median effective dose (ED_{50}) of ciprofol in hysteroscopy: a prospective, double-blind, dose-response study

Xinpei Zhang[1,†], Yanhong Zhou[1,†], Wan Lei[1,2], Qian Wang[1,3], Yingjie Yang[1], Xinzhong Chen[1], Lili Xu[1,*]

[1]Department of Anesthesia, Women's Hospital, Zhejiang University School of Medicine, 310006 Hangzhou, Zhejiang, China
[2]Department of Anesthesiology, Hangzhou Linping District Maternal & Child Health Care Hospital, 311199 Hangzhou, Zhejiang, China
[3]Department of Anesthesia, Zhejiang Hospital, 310030 Hangzhou, Zhejiang, China

***Correspondence**
xulili@zju.edu.cn
(Lili Xu)

† These authors contributed equally.

Abstract

Background: Recent studies indicate that the pharmacokinetics and pharmacodynamics of sedative-hypnotics, such as propofol, may be influenced by circadian rhythms and the timing of surgery. Ciprofol, a short-acting intravenous sedative derived from propofol, is frequently utilized for sedation during non-tracheal intubation, general anesthesia, and in intensive care settings. This study aimed to evaluate circadian rhythm's effect on the median effective dose (ED_{50}) of ciprofol sedation during hysteroscopy. **Methods**: A total of 290 female patients undergoing hysteroscopy were randomly assigned to either a daytime group (8:00–18:00) or a nighttime group (20:00–8:00). Each group received one of four doses of ciprofol (0.3, 0.4, 0.5, 0.6 mg/kg) or 2.0 mg/kg propofol, followed by continuous infusion. As required, a bolus was administered. ED_{50} was determined through probit regression analysis and induction requirements, emergence times, duration of stay in the Post-Anesthesia Care Unit (PACU), pain levels assessed using a numeric rating scale, Ramsay sedation scale scores, and any intraoperative or postoperative adverse events were recorded. **Results**: The ED_{50} of ciprofol was determined to be 0.29 mg/kg for the nighttime group and 0.36 mg/kg for the daytime group. The nighttime group exhibited lower induction requirements ($p = 0.031$), longer emergence times (8.0 *vs.* 6.0 minutes; $p < 0.001$), and extended PACU stays (30.0 *vs.* 28.0 minutes; $p = 0.040$). Furthermore, Ramsay sedation scores were lower in the nighttime group ($p = 0.002$). **Conclusions**: For women undergoing hysteroscopic procedures, nighttime surgery significantly decreased the ED_{50} of ciprofol needed to suppress the response to cervical dilation and lowered the induction requirements compared to daytime surgery. Additionally, nighttime surgery was, however, associated with longer emergence times, extended PACU stays, and lower postoperative Ramsay sedation scale scores. **Clinical Trial Registration**: We registered with the Chinese Clinical Trial Registration Center (ChiCTR2400087340, 25 July 2024).

Keywords

Ciprofol; Circadian rhythm; Propofol; Cervical dilation; Hysteroscopy

1. Introduction

Hysteroscopy is a widely utilized surgical procedure for diagnosing and treating various gynecological conditions, including uterine polyps and adhesions [1] (Sutton, 2006). During hysteroscopic surgery, several sedative and analgesic drugs are often employed for total intravenous anesthesia (TIVA) to alleviate adverse reactions caused by cervical dilation, including body movement, chills, and contraction pain. In recent years, propofol has become the preferred sedative agent for outpatient hysteroscopic procedures owing to its rapid onset, quick recovery, and effective sedation. However, its administration is frequently associated with several adverse events, such as injection site pain, respiratory depression, hypotension, and agitation during rapid intravenous infusion. Ciprofol, a novel 2,6-disubstituted phenol derivative, has gained recognition as a viable clinical alternative to propofol for adult sedation, particularly in gastrointestinal endoscopies. This is due to its reduced incidence of injection pain, minimal impact on the respiratory and circulatory systems, and enhanced safety profile [2, 3]. The median effective dose (ED_{50}) of ciprofol in preventing the hysteroscopy dilation response was 0.444 mg/kg (95% Confidence Interval (CI), 0.385–0.503 mg/kg) for outpatient hysteroscopy, with a potency ratio of ciprofol to propofol observed as 1.0:4.5 (95% CI, 1:3.9–1:5.1) [4].

The circadian rhythm describes the biological changes that occur in a 24-hour cycle, influencing various human physiological functions, such as learning, memory, mood, and

work efficiency [5]. This rhythm significantly affects the pharmacokinetics and pharmacodynamics of many commonly used drugs. As a result, it is vital to adjust both the dosage and timing of drug administration in accordance with these rhythms to enhance therapeutic effectiveness [6]. A randomized controlled trial revealed that morning surgeries conducted under general anesthesia, which required higher doses of propofol and remifentanil, were linked to reduced postoperative sleep disturbances, improved pain relief, and lower occurrences of nausea, vomiting, and dizziness compared to evening surgeries [7]. A recent observational study noted that patients receiving propofol target-controlled infusion (TCI) with Narcotrend monitoring at night showed lower Narcotrend indices, mean arterial pressure (MAP), and heart rate (HR). Conversely, those receiving propofol TCI guided by Bispectral Index (BIS) required lower propofol concentrations at night [8].

At present, there is no published research examining the effects of circadian rhythms on the dosage or concentration of ciprofol. We hypothesize that circadian rhythms may influence the ciprofol dosage required to attenuate the response to cervical dilation. Our prospective, double-blinded, randomized study aims to determine how the timing of surgery affects the ciprofol dosage needed to achieve a 50% reduction in the cervical dilation response (ED_{50}). Furthermore, we evaluated the induction dose, emergence time, postoperative pain relief, and sedation level during outpatient hysteroscopic surgery.

2. Methods

2.1 Subjects

The Research Ethics Committee at the Women's Hospital, Zhejiang University School of Medicine, approved our trial protocol on 24 June 2024 (IRB-20240195-R). We then registered the trial on 25 July 2024, with the Chinese Clinical Trial Registry, which participates in the World Health Organization International Clinical Trials Registry Platform (Identifier: ChiCTR2400087340). This prospective, single-blind, randomized study was conducted at the Women's Hospital, Zhejiang University School of Medicine in Hangzhou, Zhejiang Province, China from 01 August 2024 to 05 December 2024. All participants provided written informed consent.

We recruited and screened 290 women classified as American Society of Anesthesiologists (ASA) physical status I–II, aged 18 to 40, who were scheduled to undergo hysteroscopic surgery. Patients with body mass index (BMI) $\geq$25 kg/m^{-2}, pregnancy, and a history of cardiopulmonary disease, severe neuropsychiatric diseases, renal or liver insufficiency, and obstructive sleep apnea were excluded from the study. Additionally, patients with a history of alcohol addiction, psychotropic medication use, egg/soy allergy, diabetes, risk factors for gastroesophageal reflux, sedative-hypnotics allergy, or contraindications were excluded.

2.2 Randomization and blinding

Random numbers were generated using SPSS version 27.0 (IBM Corp., Armonk, NY, USA) by a trial coordinator (LBX) in a 1:1 ratio. These numbers were sealed in sequentially numbered envelopes and securely stored at the research site until the conclusion of the study. Patients were randomly assigned to either the daytime group (8:00–18:00) or the nighttime group (20:00–8:00), with each group randomized to receive one of the following dosages: 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg ciprofol or 2.0 mg/kg propofol. Throughout the trial, the trial coordinator (LBX) opened the envelopes according to the order of patient recruitment prior to the administration of anesthesia. A senior pharmacist (NJW) prepared the study drugs based on group allocations, ensuring that all medications maintained a consistent appearance. Importantly, all investigators involved in data collection and follow-up (XLL, ZYH, LSX), the patients, and the entire healthcare team were unaware of the group assignments.

2.3 Study protocol

All patients were monitored non-invasively for blood pressure, electrocardiogram (ECG), pulse oximetry (SpO_2), end-expiratory carbon dioxide partial pressure ($EtCO_2$), and spectral entropy (SE) to assess the depth of anesthesia using the CARESCAPE Monitor B650 (GE Healthcare, Helsinki, Finland) upon entering the operating room. Additionally, all patients received supplementary oxygen at a rate of 5 L/min through a Venturi mask.

Following the disinfection of the skin and vaginal area, intravenous administration of the appropriate dose of ciprofol or propofol was given, preceded by 0.15 μg/kg of sufentanil. Once the patient SE reached $<$60 and lost consciousness (LOC), defined as the loss of response to verbal commands and eyelash reflexes, the gynecologist commenced cervical dilation using a probe. If a participant demonstrated a positive response to the insertion (*e.g.*, SE $>$60, body movement, coughing, eye opening), a bolus dose of 0.2 mg/kg ciprofol or 0.5 mg/kg propofol was administered every four minutes. Should the responses to cervical dilation remain unimpeded after three doses of 0.2 mg/kg ciprofol, an additional dose of 0.1 μg/kg sufentanil was administered every ten minutes. The responses to cervical dilation (*i.e.*, whether there was loss of response) were recorded for each patient. A probit regression model was employed to calculate the ED_{50} and the 95% effective dose (ED_{95}) of ciprofol in inhibiting responses to cervical dilation when combined with a low dose of sufentanil. Throughout the procedure, a continuous intravenous infusion of 1.5 mg/kg/h ciprofol or 6 mg/kg/h propofol was administered to maintain an SE between 40 and 60. After recovery, patients were transferred to the Post-Anesthesia Care Unit (PACU).

2.4 Data collection

Intraoperative data included the number of doses administered for ciprofol or propofol, the induction requirements for each medication, total surgery time (defined as the time from speculum insertion to the removal of the hysteroscope), and the emergence time from anesthesia. The emergence time was specifically determined as the interval between the cessation of ciprofol or propofol and the spontaneous opening of the patient's eyes. Additional intraoperative data included evaluations of postoperative pain intensity and sedation level. Postoperative pain intensity was assessed using the Numeric

Rating Scale (NRS, where 0–10 points represented different degrees of pain, 0 = no pain, 10 = severe pain), and sedation level was evaluated using the Ramsay Sedation Scale: 1 = restlessness; 2 = completely awake, quiet, and cooperative; 3 = drowsy but responsive to verbal commands; 4 = light sleep but responsive to touch or pain; 5 = asleep but slow to respond to touch or pain; 6 = deeply asleep and unresponsive. These assessments were made at 1, 6, 12, and 24 hours after surgery.

Mean blood pressure (MBP), heart rate (HR), and SpO_2 were measured every 5 minutes during the intraoperative period. Postoperative data included the administration of Non-steroidal Anti-inflammatory Drugs (NSAIDs), opioids, and other painkillers, along with overall patient satisfaction scores, Modified Aldrete scores, duration of PACU stay, length of hospital stay, and postoperative complications. All adverse events were meticulously documented and managed according to standard clinical protocols. Bradycardia was defined as an HR less than 50 beats per minute, which would be treated with 0.5 mg of intravenous atropine. Hypotension was defined as systolic blood pressure $<$90 mmHg or a decrease of $>$30% from baseline, and was treated with 4 μg of intravenous norepinephrine. Desaturation was defined as SpO_2 less than 95% and was managed with jaw-lift or pressure-assisted ventilation with a mask. The incidence of nausea, vomiting, and shivering was also documented and managed according to routine clinical protocols.

The primary outcome of this study was the success rate of the procedure, defined as the patient remaining still during cervical dilation, achieving adequate sedation (as indicated by an SE $<$60), and not requiring rescue doses of medication [9]. The doses of ciprofol required to inhibit the response to cervical dilation in 50% (ED_{50}) and 95% (ED_{95}) of patients, combined with sufentanil, were determined using probit regression analysis. Secondary outcomes included the number of rescue doses of ciprofol or propofol administered. Additionally, we assessed various other outcomes, such as the induction requirements of ciprofol, emergence time from anesthesia, length of PACU stay, postoperative numeric rating scale score of pain and Ramsay sedation scale score, postoperative data (use of NSAIDs, opioids, and other painkillers, overall satisfaction score, Modified Aldrete score and length of hospital stay after surgery), intraoperative and postoperative adverse events (tachycardia, bradycardia, hypertension, hypotension, desaturation, nausea and vomiting, shivering and injection pain).

2.5 Statistical analysis

A pilot study was conducted involving fifty patients who were randomly assigned to five groups, with ten patients in each group. The proportions of patients with successful anesthesia for cervical dilation were 0.7, 0.3, 0.5, 0.7 and 0.8 in patients who received 2.0 mg/kg propofol and the ciprofol at doses of 0.3, 0.4, 0.5 and 0.6 mg/kg, respectively. Using PASS 2021 (NCSS, LLC, Kaysville, UT, USA), we performed a chi-square test for multiple proportions (α = 0.05, power = 0.90) to calculate the required sample size. The analysis incorporated an effect size of Cramer's V = 0.184, derived from the pilot proportions, indicating moderate differences between dose groups. The initial calculation determined 23 patients per group. To account for potential dropouts or loss to follow-up, we increased the sample size by 20%, resulting in a final enrollment target of 29 patients per group.

The Kolmogorov-Smirnov test was employed to evaluate the normal distribution of continuous variables. Variables that exhibited a normal distribution were presented as mean $\pm$ standard deviation (SD) and differences between or among groups analyzed using an independent samples *t*-test or one-way Analysis of Variance (ANOVA). Conversely, variables that did not follow a normal distribution were presented as the median and quartile difference (IQR) and compared using the Mann-Whitney U test between groups. Categorical variables were expressed as counts of patients (%) and compared using the Chi-square test. For each group, probit regression analysis was conducted to assess the number of ineffective and effective responses at each dose, allowing for the estimation of the ED_{50} and ED_{95} for ciprofol and computation of the relative mean potency with a 95% CI [10, 11]. Probit regression was performed with dose transformed via base-10 logarithm. The model assumes linearity between log10(dose) and probit-transformed response probabilities, independence of observations, and normally distributed errors. ED_{50}/ED_{95} values with 95% CIs were derived using the delta method [12]. Model fit was confirmed by Pearson's goodness-of-fit test ($p > 0.90$) and residual analysis. Additionally, Kaplan-Meier log-rank survival analysis was performed to compare the cumulative probability of the emergence time after anesthesia discontinuation. The induction requirement of ciprofol (mg/kg) was calculated by dividing the total ciprofol consumption during induction by the body weight of the subjects.

Statistical analysis was conducted using IBM SPSS for Windows version 22.0 (IBM Corp, Armonk, NY, USA), while graphical representations were generated using GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego, CA, USA). Two side *p*-values $<$ 0.05 were considered to be statistically significant.

3. Results

3.1 Patient characteristics

A total of 290 female patients undergoing hysteroscopy were randomly assigned to either the daytime group (8:00–18:00) and the nighttime group (20:00–8:00). Each group was further randomized into different ciprofol groups at the dose of 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg or 2.0 mg/kg propofol group (n = 29 each) and included in the final analysis as shown in Consort Flow Diagram (Fig. 1). The baseline demographic and surgical characteristics were comparable across the two main groups, as shown in Table 1.

3.2 Primary outcome

The effective number of patients in the nighttime group was significantly greater than that in the daytime group (p = 0.022; Table 2; Fig. 2). The ED_{50} of ciprofol in the nighttime group was determined to be 0.29 mg/kg (95% CI, 0.23 to 0.32), in contrast to 0.36 mg/kg (95% CI, 0.31 to 0.41) in the daytime group (Table 2; Fig. 3). Moreover, the ED_{95} values of ciprofol

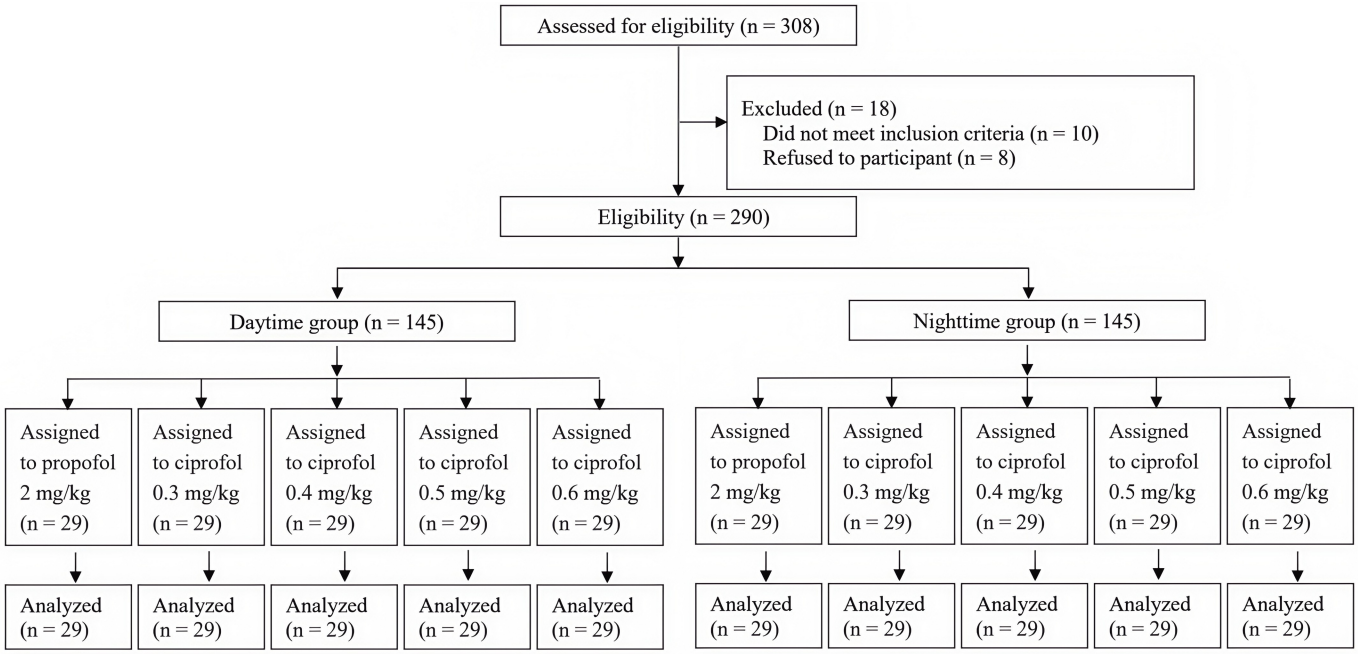

FIGURE 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram defining patient assessment and enrollment numbers in the study.

TABLE 1. Patient characteristics.

	Daytime Ciprofol Group (n = 116)	Nighttime Ciprofol Group (n = 116)	*p*-value
Age (yr)	31.5 ± 4.8	30.5 ± 5.9	0.131
Height (cm)	160.2 ± 5.8	160.1 ± 6.5	0.873
Weight (kg)	55.7 ± 6.5	55.8 ± 6.3	0.955
BMI (kg/m^2)	21.7 ± 2.1	21.8 ± 2.0	0.840
Type of hysteroscopic surgery			
Polypectomy	78 (67.2)	75 (64.7)	0.782
Lysis of adhesion	38 (32.8)	41 (35.3)	
Surgery duration (min)	23.0 ± 2.1	22.2 ± 2.2	0.854

Data are presented as mean ± SD or N (%).
BMI: Body mass index.

were 0.66 mg/kg (95% CI, 0.55 to 0.89) in the nighttime group and 0.82 mg/kg (95% CI, 0.67 to 1.21) in the daytime group (Table 2). The estimate of the relative median potency for ciprofol in the nighttime group compared to the daytime group was 0.80 (95% CI, 0.62 to 0.96). The results of 95% CIs did not contain 1, demonstrating a significant difference between the two groups. Age, height, weight, operative time, and hysteroscopy type demonstrated negligible confounding effects on outcomes, supported by non-significant associations in multivariable regression ($p > 0.05$) and minimal effect estimate changes (<5%) after adjustment.

3.3 Secondary outcome

There was no statistically significant difference in the number of patients requiring either one or two rescue doses of ciprofol between the daytime and nighttime groups ($p > 0.05$; Table 2). Furthermore, no patients required three rescue doses of ciprofol.

3.4 Additional outcomes

The induction requirements of ciprofol were lower in patients in the nighttime ciprofol group (0.50 [0.40, 0.60] mg/kg) compared to those in the daytime ciprofol group (0.50 [0.43, 0.60] mg/kg; $p = 0.031$). The emergence time (8.0 [7.0 to 9.0] min) and the duration of PACU stay (30.0 [27.0 to 31.0] min) were longer in patients in the nighttime ciprofol group than those in the daytime ciprofol group (6.0 [5.0 to 7.0] min; $p < 0.001$; Fig. 4, 28.0 [27.0 to 30.0] min; $p = 0.040$), respectively. No statistically significant differences were observed in the incidence of intraoperative and postoperative complications or side effects between the daytime and nighttime groups ($p > 0.05$; Table 2).

TABLE 2. Efficacy outcomes.

	Daytime Ciprofol Group (n = 116)	Nighttime Ciprofol Group (n = 116)	*p*-value
Primary outcome			
Effective number of patients (%)	73 (62.9)	89 (76.7)	**0.022**
ED_{50}[a]	0.36 (0.31, 0.41)	0.29 (0.23, 0.32)	-
ED_{95}[a]	0.82 (0.67, 1.21)	0.66 (0.55, 0.89)	-
Secondary outcomes			
One rescue dose of ciprofol	36 (31.0)	25 (21.6)	0.068
Two rescue doses of ciprofol	4 (3.4)	3 (2.6)	0.701
Three rescue doses of ciprofol	0 (0.0)	0 (0.0)	>0.999
Other outcomes			
Induction requirements of ciprofol (mg/kg)	0.50 (0.43, 0.60)	0.50 (0.40, 0.60)	**0.031**
Total requirements of ciprofol (mg/kg)	1.01 (0.87, 1.21)	0.98 (0.84, 1.14)	0.187
Total requirements of ciprofol (mg/kg/min)	0.05 (0.04, 0.06)	0.04 (0.04, 0.05)	0.203
Time to anesthesia emergence (min)	6.0 (5.0, 7.0)	8.0 (7.0, 9.0)	<**0.001**
Length of PACU stay (min)	28.0 (27.0, 30.0)	30.0 (27.0, 31.0)	**0.040**
Intraoperative adverse events[b]	55 (47.4)	64 (55.2)	0.237
Postoperative adverse events[b]	14 (12.1)	19 (16.4)	0.347

Data are presented as median (interquartile range) or N (%) or mean (95% CI). p values in bold indicate < 0.05.
[a]*: Probit regression analysis.*
Data are presented as mean ± SD or N (%) or median (interquartile range).
[b]*: Included hypertension, hypotension, bradycardia, tachycardia, desaturation, injection pain, nausea and vomiting, and shivering.*
ED: effective dose; PACU: post-anesthesia care unit.

3.5 Postoperative numeric rating scale score of pain and ramsay sedation scale score

The numeric rating scale score of pain measures at times, 1 h, 6 h, 12 h, and 1 d after surgery showed no statistically significant difference between the daytime and nighttime groups ($p > 0.05$; Table 3). In contrast, the Ramsay sedation scale score at 1 h post-surgery was notably higher in the nighttime ciprofol group (2 [2 to 2]) compared to the daytime ciprofol group (2 [2 to 2]; $p = 0.002$; Fig. 5). There were no statistically significant differences in the Ramsay sedation scale scores at 6 h, 12 h, and 1 d after surgery ($p > 0.05$; Table 3).

3.6 Postoperative data and adverse events

There was no statistically significant difference in the use of NSAIDs, opioids, and other painkillers between the daytime and nighttime groups. Additionally, there were no notable differences in the overall satisfaction scores, Modified Aldrete scores, lengths of PACU stay and hospital stay after surgery, and postoperative complications between the daytime and nighttime groups ($p > 0.05$; Table 4). The incidences of intraoperative and postoperative side effects, including bradycardia, tachycardia, hypertension, hypotension, desaturation, nausea and vomiting, shivering, and injection pain, were similar for both the daytime group and the nighttime group ($p > 0.05$; Table 4).

3.7 Subgroup analysis

Baseline demographic and surgical characteristics were comparable across all groups, as shown in **Supplementary Table 1**. Patients receiving ciprofol at doses of 0.4 mg/kg, 0.5 mg/kg, and 0.6 mg/kg demonstrated a higher adequate number compared to those administered 0.3 mg/kg ciprofol in both daytime and nighttime groups ($p < 0.001$; $p = 0.020$; **Supplementary Table 2**). In the daytime group, fewer patients in the higher ciprofol dose groups required a rescue dose of medication ($p = 0.005$), while no statistically significant difference was noted in the nighttime group ($p > 0.05$). Emergence times were shorter for patients receiving 0.3 mg/kg ciprofol compared to those on any other dose of ciprofol or propofol in the daytime group ($p < 0.001$), but no such differences were observed in the nighttime group ($p > 0.05$). The incidence of intraoperative adverse events was lower for patients receiving 0.3 mg/kg and 0.4 mg/kg ciprofol compared to those receiving 2.0 mg/kg propofol in the daytime group ($p = 0.045$). In the nighttime group, the incidence was also lower for those receiving 0.3 mg/kg ciprofol ($p = 0.029$; **Supplementary Table 2**). Postoperative adverse events occurred less frequently in patients treated with 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, and 0.6 mg/kg ciprofol as opposed to those receiving 2.0 mg/kg propofol in both groups ($p < 0.001$; $p = 0.003$). Ramsay sedation scale scores measured one hour after surgery, were lower for patients receiving ciprofol in both the daytime and nighttime groups ($p < 0.001$; **Supplementary Table 3**).

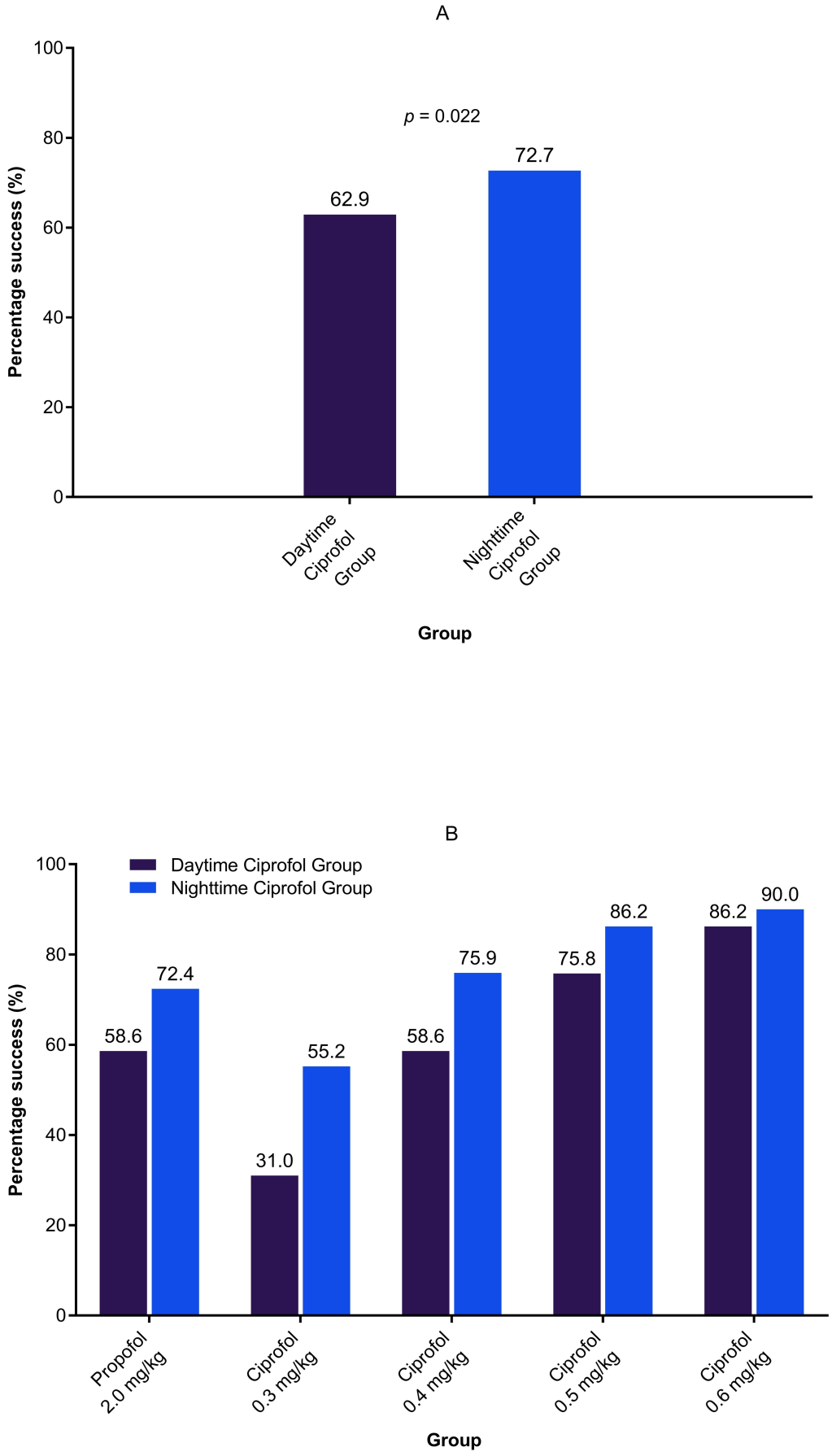

FIGURE 2. Success rates of ciprofol and propofol in daytime and nighttime groups. (A) Success rate of ciprofol in the daytime and nighttime groups. (B) Success rate of propofol and ciprofol at different doses in the daytime and nighttime groups.

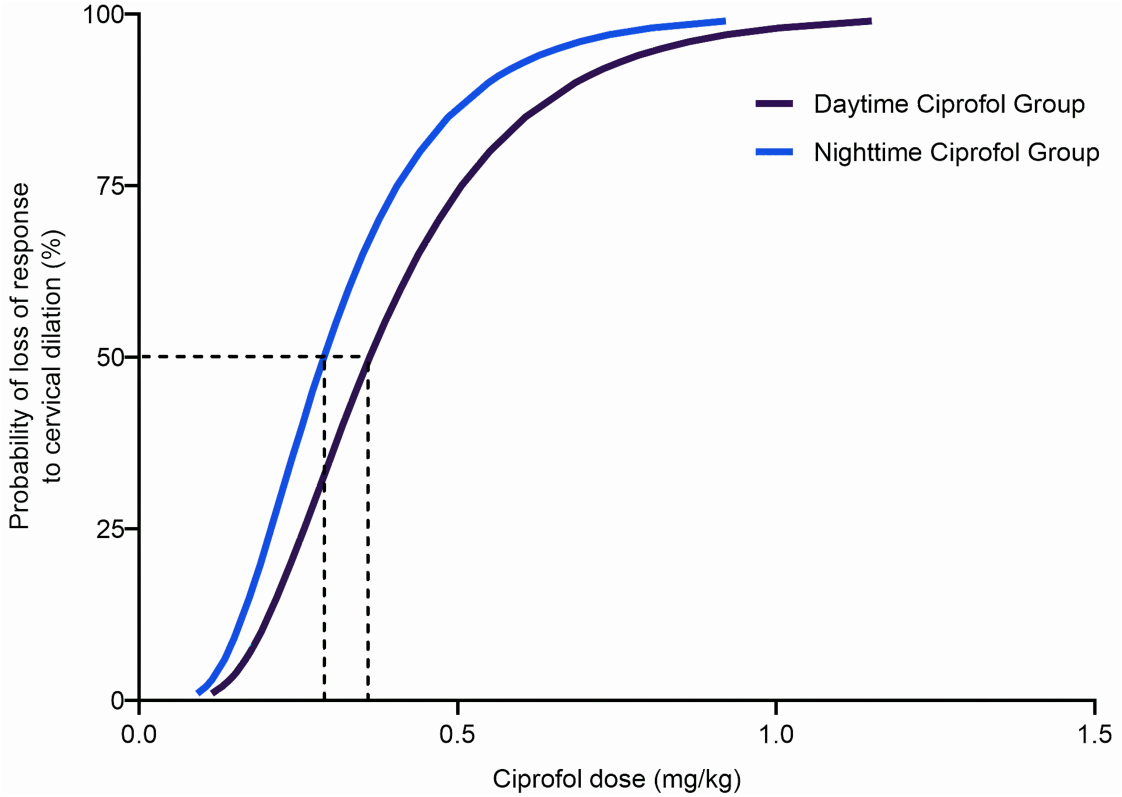

FIGURE 3. Probit dose-response curves for ciprofol inhibition of cervical dilation response. Solid lines represent fitted probit models for daytime (navy blue) and nighttime (sky blue) surgical cohorts. Dashed vertical lines mark the estimated ED_{50} values: 0.36 mg/kg (95% CI 0.31–0.41) for daytime *vs.* 0.29 mg/kg (95% CI 0.23–0.32) for nighttime administration.

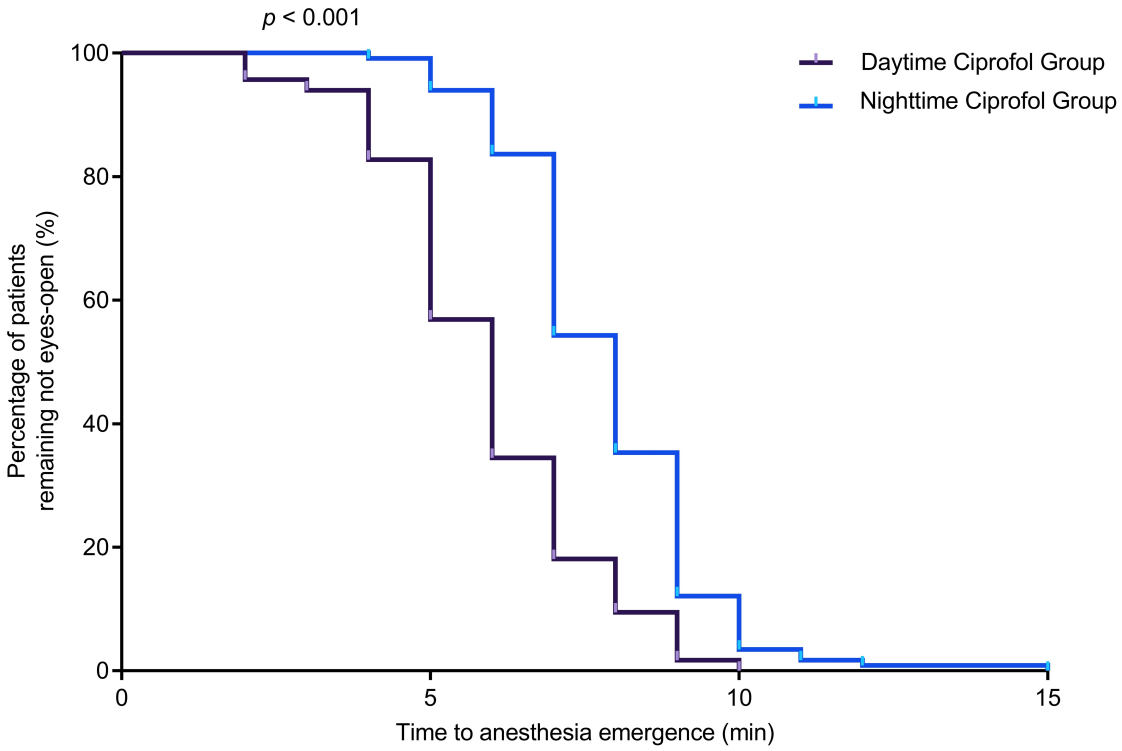

FIGURE 4. Cumulative percentages of patients remaining unconscious after discontinuation of ciprofol in daytime and nighttime group (area within different colors lines). Vertical axes represent percentage of non-recovered patients; horizontal axes show time since drug discontinuation (minutes). Median emergence time was 6.0 [5.0 to 7.0] min for daytime *vs.* 8.0 [7.0 to 9.0] min for nighttime groups (log-rank test $\chi^2 = 28.4$, $df = 1$, $p < 0.001$), using the Kaplan-Meier survival analysis.

TABLE 3. Postoperative numeric rating scale score of pain and Ramsay sedation scale score.

	Daytime Ciprofol Group (n = 116)	Nighttime Ciprofol Group (n = 116)	*p*-value
Numeric rating scale of pain, median (IQR), point[a]			
1 h after surgery	1 (1, 2)	1 (1, 2)	0.539
6 h after surgery	0 (0, 1)	1 (0, 1)	0.518
12 h after surgery	0 (0, 1)	0 (0, 1)	0.051
1 d after surgery	0 (0, 0)	0 (0, 0)	0.301
Ramsay sedation scale, median (IQR), point[b]			
1 h after surgery	2 (2, 2)	2 (2, 2)	**0.002**
6 h after surgery	2 (2, 2)	2 (2, 2)	0.291
12 h after surgery	2 (2, 2)	2 (2, 2)	>0.999
1 d after surgery	2 (2, 2)	2 (2, 2)	>0.999

Data are median (interquartile range). p values in bold indicate < 0.05.
[a]*: Score ranges from 0 to 10, where 0 = no pain and 10 = the worst pain.*
[b]*: Ramsay sedation score ranges from 1 (restlessness) to 6 (deeply asleep and does not respond) and 2 indicates completely awake, quiet and cooperative.*

Furthermore, the incidence of injection pain was significantly lower in the ciprofol groups compared to propofol ($p < 0.001$; **Supplementary Table 4**).

4. Discussion

In this study, we initially explored the impact of circadian rhythms on the dosage of ciprofol required to alleviate the response to cervical dilation during hysteroscopy. Our objective was to establish the ED_{50}, ED_{95}, and the appropriate dosage of ciprofol. Our findings indicated that women undergoing nighttime hysteroscopic surgery needed significantly lower ED_{50}values of ciprofol to inhibit the response to cervical dilation compared to those who underwent surgery during the daytime. Furthermore, these women experienced reduced induction requirements, prolonged postoperative emergence times, extended stays in the PACU, and more profound sedation in the early postoperative phase.

A previous clinical trial examined the potential impact of circadian rhythms on prolonged propofol infusion in patients in the intensive care unit. The findings indicated no significant differences in propofol pharmacokinetics or BIS scores related to circadian rhythms [13]. However, the use of dexmedetomidine during nighttime surgeries was associated with a notable reduction in the total doses of both dexmedetomidine and remifentanil compared to daytime surgeries in patients undergoing laparoscopic abdominal procedures [14]. Meanwhile, The CNS-depressant effects of ketamine-midazolam in rats demonstrate circadian variation, with significantly prolonged duration during the light (rest) phase, driven by time-dependent differences in Gamma-Aminobutyric Acid-ergic (GABAergic) receptor sensitivity, N-Methyl-D-Aspartate (NMDA) receptor activity, and metabolic processes [15]. More recently, a single-center clinical study with a small sample size demonstrated that nighttime surgeries not only reduced the dosage of propofol but also decreased propofol concentrations in TCI during selective laparoscopic abdominal surgeries under general anesthesia [7, 8].

Our data showed that patients undergoing night surgeries require lower ED_{50}, ED_{95}, and induction doses of ciprofol in combination with sufentanil compared to those undergoing daytime surgeries. However, the total amount of ciprofol administered was not significantly different between the two groups, indicating that the light-dark cycle may affect the induction dose of sedatives by altering their metabolism and clearance. Furthermore, the overall requirement of ciprofol could be influenced by the preset doses for each group and the duration of the operation. Given the limited sample size of this study, large-scale clinical trials are necessary to validate these preliminary findings.

Biological rhythms have been demonstrated in various animal studies to influence the pharmacology and effects of anesthetic hypnotics such as propofol, sodium pentobarbital, ketamine, and midazolam. These agents exhibit their maximum duration of action when administered during the animal's rest period, which aligns with nighttime in humans [15–18]. A noteworthy example of the significance of circadian rhythms in biological systems and their practical relevance to pharmacology involves methyleugenol. When administered intraperitoneally at a dose of 200 mg/kg, methyleugenol significantly reduced sleep duration (measured as the time between the loss and recovery of the righting reflex) when injected at 20:00, 24:00 and 04:00, in contrast to animals anesthetized at 08:00, 12:00 and 16:00 [19]. The period of the day or night referred to in Sato's and Challet's studies [16, 20], were adjusted and narrowed to a shorter time frame, in order to avoid the potential influence of the time boundary between day and night.

Notably, patients undergoing nighttime surgery exhibited a lower ciprofol ED_{50} (0.29 *vs.* 0.36 mg/kg) but prolonged emergence times (8.0 *vs.* 6.0 min) and extended PACU stays (30.0 *vs.* 28.0 min), despite comparable postoperative pain scores (NRS; Table 3). The dissociation between reduced dosing requirements and delayed recovery may reflect circadian-driven pharmacokinetic dynamics. Specifically, the signifi-

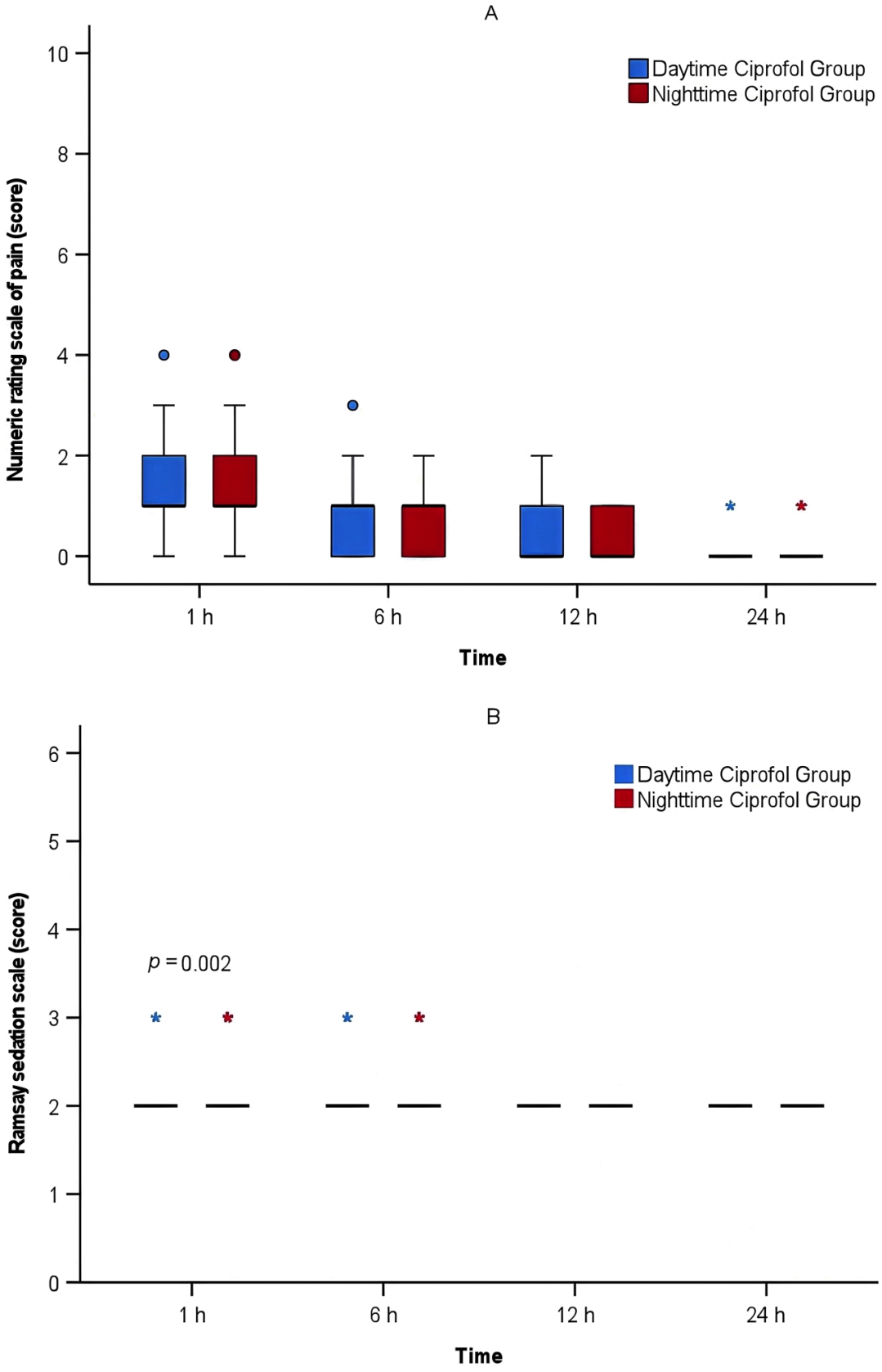

FIGURE 5. Numeric rating scale of pain (A) and the Ramsay sedation scale (B) on postoperative hours 1, 6, 12 and 24. Numeric Rating Scale (NRS) of Pain Box-and-whisker plots compare postoperative pain intensity (0–10 scale) at 1/6/12/24 h. Blue boxes represent daytime group, red boxes nighttime group. Horizontal line marks median. No significant intergroup differences detected at any timepoints (Mann-Whitney U test $p > 0.05$). Outliers (beyond whiskers) plotted as individual dots. Ramsay Sedation Scale Bar graphs show sedation levels (1–6 scale) with asterisk indicating significant circadian variation at 1 h (*$p = 0.002$ by Mann-Whitney U test). Nighttime group (red) exhibited higher sedation scores (2 [2 to 2]) compared to daytime (blue, 2 [2 to 2]), suggesting prolonged residual sedation.

TABLE 4. Intraoperative and postoperative data and side effects.

	Daytime Ciprofol Group (n = 116)	Nighttime Ciprofol Group (n = 116)	*p*-value
Postoperative data			
Use of NSAIDs	0 (0.0)	0 (0.0)	>0.999
Use of opioids	0 (0.0)	0 (0.0)	>0.999
Use of other painkillers	0 (0.0)	0 (0.0)	>0.999
Overall satisfaction score (point)[a]	10 (10, 10)	10 (10, 10)	0.641
Modified Aldrete score	10 (10, 10)	10 (10, 10)	0.301
Length of hospital stay after surgery (d)	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	0.757
Postoperative complications[b]	0 (0.0)	0 (0.0)	>0.999
Intraoperative adverse events			
Tachycardia[c]	5 (4.3)	4 (3.4)	0.734
Bradycardia[d]	3(2.6)	4 (3.4)	0.701
Hypertension[e]	0 (0.0)	0 (0.0)	>0.999
Hypotension[f]	22 (19.0)	32 (28.6)	0.120
Desaturation[g]	19 (16.4)	24 (20.7)	0.398
Nausea and vomiting	0 (0.0)	0 (0.0)	>0.999
Shivering	0 (0.0)	0 (0.0)	>0.999
Injection pain	1 (0.9)	0 (0.0)	0.316
Postoperative adverse events			
Tachycardia[c]	0 (0.0)	0 (0.0)	>0.999
Bradycardia[d]	0 (0.0)	0 (0.0)	>0.999
Hypertension[e]	0 (0.0)	0 (0.0)	>0.999
Hypotension[f]	0 (0.0)	0 (0.0)	>0.999
Desaturation[g]	0 (0.0)	0 (0.0)	>0.999
Nausea and vomiting	10 (8.6)	15 (12.9)	0.290
Shivering	3 (2.6)	4 (3.4)	0.701

Data are presented as median (interquartile range) or N (%).
NSAIDs: Nonsteroidal Anti-inflammatory Drugs.
[a]*: Scale range from 0 to 10 (0 represents the worst satisfaction and 10 represents the best satisfaction).*
[b]*: Generally defined as new-onset medical conditions that were harmful to patients' recovery and required medical intervention, i.e., grade II or higher on the Clavien-Dindo classification.*
[c]*: Defined as heart rate >100 beats per minute or an increase of >20% from baseline.*
[d]*: Defined as heart rate <60 beats per minute or a decrease of >20% from baseline.*
[e]*: Defined as systolic blood pressure >160 mmHg or an increase of >30% from baseline.*
[f]*: Defined as systolic blood pressure <90 mmHg or a decrease of >30% from baseline.*
[g]*: Defined as pulse oxygen saturation less than 90% or a decrease of more than 5% (absolute value) from baseline.*

cantly lower Ramsay Sedation Scale (RSS) scores at 1 h postoperatively (median 2 *vs.* 2; $p = 0.002$) in the nighttime group suggests accelerated ciprofol clearance, potentially mediated by hepatic Cytochrome P450 (CYP450) enzyme fluctuations regulated by circadian rhythms [6]. While rodent studies demonstrate similar diurnal variations in propofol metabolism [8], our findings underscore the critical interplay between circadian biology and newer sedatives like ciprofol [21], advocating for time-of-day adjustments in dosing to optimize both anesthetic efficacy and recovery efficiency.

Additionally, we observed no statistically significant differences in the incidence of adverse events (either individual or collective) during or after surgery between the two groups. This finding suggests that ciprofol is safe and applicable for hysteroscopic sedation both during the day and at night. Specifically, all four doses of ciprofol significantly reduced the incidence of injection pain and the total number of postoperative adverse events compared to 2.0 mg/kg propofol in both the daytime and nighttime groups. One possible explanation for these results is that ciprofol's potency is approximately five times greater than that of propofol. Consequently, only 20% of a ciprofol dose is required to achieve the same anesthetic effect as propofol, as demonstrated in previous clinical trials [22–24]. This leads to faster clearance, quicker patient recovery, and

lower concentrations in the aqueous phase [25, 26]. Overall, ciprofol offers notable advantages in intraoperative comfort and postoperative safety compared to propofol. However, further investigation is necessary to determine whether the combination of sufentanil and ciprofol provides a superior safety profile compared to sufentanil and propofol.

Entropy monitoring (SE <60) was selected to assess sedation depth based on its validated ability to quantify Electroencephalogram (EEG) signal irregularity and correlate with hypnotic effects across diverse anesthetic agents, including propofol and benzodiazepines [27]. Although direct evidence for ciprofol remains unavailable, its pharmacological profile as a structural isomer of propofol—both acting through enhanced Gamma-Aminobutyric Acid Type A Receptor (GABAA) receptor agonism—supports the extrapolation of SE thresholds established in propofol studies [28]. Crucially, entropy indices demonstrate minimal agent-specific variability in sedation monitoring, with an SE <60 consistently defining moderate-to-deep sedation thresholds independent of drug class [29]. This mechanistic and clinical consistency justifies applying an SE <60 as a generalizable biomarker for ciprofol-induced sedation, pending further pharmacokinetic validation.

The 20% reduction in ciprofol ED_{50} during nighttime procedures (0.29 *vs.* 0.36 mg/kg) supports tailored dosing strategies: initiating with 0.3 mg/kg for nighttime hysteroscopy versus 0.4 mg/kg for daytime cases. This adjustment balances efficacy with reduced risks of overdosing (*e.g.*, hypotension/respiratory depression), particularly relevant for high-volume nighttime surgical settings. Clinicians should anticipate prolonged emergence (8.0 *vs.* 6.0 min) and allocate PACU resources accordingly to manage delayed recovery.

The strengths of this study include consistent stimulation during cervical dilation and a relatively homogeneous patient population. However, several notable limitations exist. First, certain populations were excluded from the study, such as individuals with ASA physical status classification II–IV, obesity, the elderly, and postmenopausal women. Therefore, the dose-response relationship of ciprofol in these populations remains unclear and warrants further exploration in future studies. Second, there is currently no TCI pump suitable for ciprofol, which limited our ability to accurately measure plasma and effect-site concentrations of ciprofol or perform precise dose adjustments.

This absence of appropriate technology could have affected the robustness of our findings. Lastly, while we examined the effect of circadian rhythms on the average effective dose of ciprofol for inhibiting cervical dilation during hysteroscopy, we did not assess how these rhythms affect opioids like sufentanil. This consideration should be addressed in future investigations.

5. Conclusions

This prospective, double-blind study demonstrates that Women who underwent night hysteroscopic surgery exhibited significantly lower ED50 values of ciprofol for inhibiting the response to cervical dilation and required lower induction dosages compared to those who had daytime hysteroscopic procedures. However, this increased sensitivity was accompanied by prolonged emergence times and extended PACU stays, alongside lower Ramsay sedation scale scores in the early postoperative period. These findings highlight the importance of considering circadian rhythms in anesthetic practice to optimize dosing strategies and recovery outcomes. Future studies should investigate the underlying mechanisms of ciprofol's chronopharmacology and explore personalized dosing regimens based on surgical timing to improve patient care in ambulatory settings.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper, and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

XPZ—designed and performed the study; collected and analyzed the data; and wrote the manuscript. LLX—helped in designing the study and writing the manuscript. YHZ and QW—helped in performing the study and collecting the data. WL and YJY—helped in analyzing the data. XZC—helped in revised the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Research Ethics Committee at the Women's Hospital, Zhejiang University School of Medicine, approved our trial protocol on 24 June 2024 (IRB-20240195-R). All participants provided written informed consent.

ACKNOWLEDGMENT

The authors thank the colleagues in the Department of Anesthesiology, Women's Hospital, Zhejiang University School of Medicine, Hangzhou, China.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at https://oss.signavitae.com/mre-signavitae/article/1972929454819688448/attachment/Supplementary%20material.docx.

REFERENCES

[1] Riemma G, Vitale SG, Manchanda R, Rathore A, Török P, De Angelis C, *et al*. The role of hysteroscopy in reproductive surgery: today and tomorrow. Journal of Gynecology Obstetrics and Human Reproduction. 2022; 51: 102350.

[2] Chen W, Xu Y, Zeng Y, Xing G. A meta-analysis and systematic review based on perioperative management of elderly patients: is ciprofol an alternative to propofol? European Journal of Clinical Pharmacology. 2025; 81: 111–121.

[3] Ortegal GH, Barbosa EC, Faria PC, Couto JV, Silva GC, Souza MH, *et al*. Ciprofol versus propofol for adult sedation in gastrointestinal endoscopic procedures: a systematic review and meta-analysis. Minerva Anestesiologica. 2024; 90: 1013–1021.

[4] Jin L, Jiao CC, Chen XP, Sun LH, Zhang Y, Cheng XZ, *et al*. The potency-ratio of ciprofol and propofol under procedural sedation and anesthesia for outpatient hysteroscopy during cervical dilation: a study using up-and-down sequential allocation method. BMC Anesthesiology. 2024; 24: 426.

[5] Reppert SM, Weaver DR. Coordination of circadian timing in mammals. Nature. 2002; 418: 935–941.

[6] Ruan W, Yuan X, Eltzschig HK. Circadian rhythm as a therapeutic target. Nature Reviews Drug Discovery. 2021; 20: 287–307.

[7] Song B, Li Y, Teng X, Li X, Yang Y, Zhu J. Comparison of morning and evening operation under general anesthesia on intraoperative anesthetic requirement, postoperative sleep quality, and pain: a randomized controlled trial. Nature and Science of Sleep. 2020; 12: 467–475.

[8] Shen JH, Ye M, Chen Q, Chen Y, Zhao HL, Khan A, *et al*. Effects of circadian rhythm on Narcotrend index and target-controlled infusion concentration of propofol anesthesia. BMC Anesthesiology. 2021; 21: 215.

[9] Tan H, Lou AF, Wu JE, Chen XZ, Qian XW. Determination of the 50% and 95% effective dose of remimazolam combined with propofol for intravenous sedation during day-surgery hysteroscopy. Drug Design, Development and Therapy. 2023; 17: 1753–1761.

[10] Pace NL, Stylianou MP. Advances in and limitations of up-and-down methodology: a précis of clinical use, study design, and dose estimation in anesthesia research. Anesthesiology. 2007; 107: 144–152.

[11] Meister GC, D'Angelo R, Owen M, Nelson KE, Gaver R. A comparison of epidural analgesia with 0.125% ropivacaine with fentanyl versus 0.125% bupivacaine with fentanyl during labor. Anesthesia & Analgesia. 2000; 90: 632–637.

[12] Wang QQ, Yu SC, Qi X, Hu YH, Zheng WJ, Shi JX, *et al.* Overview of logistic regression model analysis and application. Zhonghua Chinese Journal of Preventive Medicine. 2019; 53: 955–960. (In Chinese)

[13] Bienert A, Kusza K, Wawrzyniak K, Grześkowiak E, Kokot ZJ, Matysiak J, *et al.* Assessing circadian rhythms in propofol PK and PD during prolonged infusion in ICU patients. Journal of Pharmacokinetics and Pharmacodynamics. 2010; 37: 289–304.

[14] Song B, Li Y, Teng X, Li X, Yang Y, Zhu J. The effect of intraoperative use of dexmedetomidine during the daytime operation *vs.* the nighttime operation on postoperative sleep quality and pain under general anesthesia. Nature and Science of Sleep. 2019; 11: 207–215.

[15] Rebuelto M, Ambros L, Waxman S, Montoya L. Chronobiological study of the pharmacological response of rats to combination ketamine-midazolam. Chronobiology International. 2004; 21: 591–600.

[16] Sato Y, Kobayashi E, Hakamata Y, Kobahashi M, Wainai T, Murayama T, *et al*. Chronopharmacological studies of ketamine in normal and NMDA epsilon1 receptor knockout mice. British Journal of Anaesthesia. 2004; 92: 859–864.

[17] Rebuelto M, Ambros L, Montoya L, Bonafine R. Treatment-time-dependent difference of ketamine pharmacological response and toxicity in rats. Chronobiology International. 2002; 19: 937–945.

[18] Chassard D, Duflo F, de Queiroz Siqueira M, Allaouchiche B, Boselli E. Chronobiology and anaesthesia. Current Opinion in Anesthesiology. 2007; 20: 186–190.

[19] Dong D, Yang D, Lin L, Wang S, Wu B. Circadian rhythm in pharmacokinetics and its relevance to chronotherapy. Biochemical Pharmacology. 2020; 178: 114045.

[20] Challet E, Gourmelen S, Pevet P, Oberling P, Pain L. Reciprocal relationships between general (Propofol) anesthesia and circadian time in rats. Neuropsychopharmacology. 2007; 32: 728–735.

[21] Warltier David C, Chassard D, Bruguerolle B. Chronobiology and anesthesia. Anesthesiology. 2004; 100: 413–427.

[22] Teng Y, Ou M, Wang X, Zhang W, Liu X, Liang Y, *et al*. Efficacy and safety of ciprofol for the sedation/anesthesia in patients undergoing colonoscopy: phase IIa and IIb multi-center clinical trials. European Journal of Pharmaceutical Sciences. 2021; 164: 105904.

[23] Teng Y, Ou MC, Wang X, Zhang WS, Liu X, Liang Y, *et al*. Pharmacokinetic and pharmacodynamic properties of ciprofol emulsion in Chinese subjects: a single center, open-label, single-arm dose-escalation phase 1 study. American Journal of Translational Research. 2021; 13: 13791–13802.

[24] Hu C, Ou X, Teng Y, Shu S, Wang Y, Zhu X, *et al*. Sedation effects produced by a ciprofol initial infusion or bolus dose followed by continuous maintenance infusion in healthy subjects: a phase 1 trial. Advances in Therapy. 2021; 38: 5484–5500.

[25] Zhong J, Zhang J, Fan Y, Zhu M, Zhao X, Zuo Z, *et al*. Efficacy and safety of ciprofol for procedural sedation and anesthesia in non-operating room settings. Journal of Clinical Anesthesia. 2023; 85: 111047.

[26] Zhou J, Wang L, Zhong Z, Yuan L, Huang J, Zou P, *et al*. Pharmacological mechanism and clinical application of ciprofol. Frontiers in Pharmacology. 2025; 16: 1572112.

[27] Dinu AR, Rogobete AF, Popovici SE, Bedreag OH, Papurica M, Dumbuleu CM, *et al*. Impact of general anesthesia guided by state entropy (SE) and response entropy (RE) on perioperative stability in elective laparoscopic cholecystectomy patients—a prospective observational randomized monocentric study. Entropy. 2020; 22: 356.

[28] Vakkuri A, Yli-Hankala A, Talja P, Mustola S, Tolvanen-Laakso H, Sampson T, *et al*. Time-frequency balanced spectral entropy as a measure of anesthetic drug effect in central nervous system during sevoflurane, propofol, and thiopental anesthesia. Acta Anaesthesiologica Scandinavica. 2004; 48: 145–153.

[29] Schmidt GN, Bischoff P, Standl T, Hellstern A, Teuber O, Schulte Esch J. Comparative evaluation of the Datex-Ohmeda S/5 Entropy Module and the bispectral index monitor during propofol-remifentanil anesthesia. Anesthesiology. 2004; 101: 1283–1290.

How to cite this article: Xinpei Zhang, Yanhong Zhou, Wan Lei, Qian Wang, Yingjie Yang, Xinzhong Chen, *et al*. Circadian rhythms on the median effective dose (ED_{50}) of ciprofol in hysteroscopy: a prospective, double-blind, dose-response study. Signa Vitae. 2025; 21(10): 28-39. doi: 10.22514/sv.2025.142.