A 50-50% mixture of nitrous oxide-oxygen in transrectal ultrasound-guided prostate biopsy: A randomized and prospective clinical trial

Gabriel da Silva Cazarim¹*, Nubia Vercosa²*, Leonel Carneiro¹‡, Rachel Pastor¹‡, Elizabeth Fernandes Vaz da Silva¹‡, Louis Barrucand³‡, Ismar Lima Cavalcanti¹‡

¹ Department of Anesthesiology, Federal Fluminense University, University hospital Antonio Pedro, Niterói, Rio de janeiro, Brazil, ² Department of Anesthesiology, Federal University of Rio de Janeiro, University hospital Clementino Fraga Fihlo, Rio de Janeiro, Rio de Janeiro, Brazil, ³ Department of Statistic, Federal University of Rio de Janeiro, University hospital Clementino Fraga Fihlo, Rio de Janeiro, Rio de Janeiro, Brazil

* These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
* gabriel_cazarim@hotmail.com

Abstract

Introduction
Transrectal ultrasound-guided biopsy (TUSPB) is the standard method of diagnosis for prostate cancer, and although it is well tolerated by some patients, it presents a discomfort rate of 65 to 90%, which may be associated with pain. For convenience, it is agreed that a method of analgesia and sedation is necessary. For this purpose, this study aimed to evaluate the impact of inhalation of a 50–50% N₂O-O₂ gas mixture on pain intensity in these patients.

Material and methods
Randomized, double-blinded clinical trial, conducted at Antônio Pedro University Hospital (Hospital Universitário Antônio Pedro), Niterói, RJ, Brazil, containing two groups of 42 patients: a control (C) group, which received 100% oxygen inhalation, and a nitrous oxide (NO) group, which received inhalation of the 50–50% N₂O-O₂ mixture, self-administered during TUSPB. The pain intensity and degree of satisfaction were evaluated through a visual analogue scale (VAS), as was the frequency of adverse events.

Results
Eighty-four patients were included in the study, with 42 in each group. The mean pain intensity was lower in the NO group than in the C group [2.52 (0–10) vs 5.95 (0–10), p < 0.001], and the degree of satisfaction was higher in the NO group than in the C group (8.14 vs. 4.69,
p < 0.001). The adverse effects were somnolence, dizziness, nausea, vomiting, discomfort and euphoria without differences between the groups.

**Conclusion**

The 50–50% N₂O-O₂ mixture was effective in reducing pain intensity and increasing the degree of satisfaction in TUSPB, with tolerable side effects.

**Introduction**

Transrectal ultrasound-guided prostate biopsy (TUSPB) is the standard method used for early diagnosis of cancer when associated with prostate-specific antigen (PSA) plasma levels[1]. Although well tolerated by many patients, between 65 and 90% of men undergoing TUSPB complain of discomfort[2] associated with pain. Several methods of analgesia and/or sedation have been proposed, including periprostatic[2,3,4] or intraprostatic[5] nerve block, topical anesthesia with lidocaine[6] or EMLA[7] at the puncture site, and general anesthesia with propofol and remifentanil[8].

The inhalation of 50–50% of nitrous oxide (N₂O)-oxygen (O₂) by the self-administration valve proposed in the present study is a good alternative to the routinely used methods in TUSPB since it is a safe, cost-effective technique that promotes analgesia on demand without the need of an anesthesiologist [9,10].

Nitrous oxide can be self-administered for analgesia in various procedures, such as intra-articular injection of drugs[11], vascular access puncture[12], sigmoidoscopy[13], colonoscopy[14], ophthalmologic procedures[15] and prostate biopsy[16]. Nitrous oxide has been used in emergencies, accident care and patient transport in ambulances [17].

Considering that pain is an event that has a socio-cultural influence[18], and to date, no study with these characteristics has been performed in the Brazilian population, this clinical trial is justified.

The hypothesis of the present study is that the inhalation of 50–50% N₂O-O₂, per self-administration valve, will be able to reduce pain in patients undergoing TUSPB.

The objective of the study was to evaluate the pain intensity in patients submitted to TUSPB. The secondary objectives were to determine the frequency of adverse events and the degree of satisfaction of these patients with the treatment proposed in the research.

**Material and methods**

This prospective and randomized clinical trial was performed after approval by the Research Ethics Committee of the Antônio Pedro University Hospital (Hospital Universitário Antônio Pedro) of the Fluminense Federal University (Universidade Federal Fluminense–UFF) on 02/05/2015 (Presentation Certificate for Ethical Assessment (CAAE) n. 39144914.8.0000.5243) and was registered in the Clinical Trials on 14/09/2016 (NCT: 02899182), and the authors confirm that all ongoing and related trials for this intervention are registered.

A total of 84 men aged 18 years old or older who were ASA I to III and underwent elective or outpatient transrectal ultrasound-guided prostate biopsy were recruited, from May 2015 to November 2016.

The following exclusion criteria were adopted: patients who had participated in another study in the last month, those using psychoactive drugs, those known to be hypersensitive to
any study medication, patients with severe diseases in organs such as the kidneys, liver, lungs, heart and brain, patients with impossibility to report the intensity of pain and those unable to inhale the gas mixture through the self-administering device.

An informed consent form was signed by each of the volunteer participants, who were advised of the risks and benefits of the research. The patients were divided into two groups according to a sequence of random numbers generated electronically through the program GraphPad Prism. Forty-two patients were allocated to the O₂ group (C) and 42 to the N₂O oxide group (NO). Group C received topical anesthesia in the anal canal (lidocaine hydrochloride jelly 2%—CRISTÁLIA Produtos Químicos e Farmacêuticos, Itapira, SP, Brazil) plus 100% oxygen inhalation under a facemask. In turn, the NO group received topical anal anesthesia (lidocaine hydrochloride jelly 2% gel—CRISTÁLIA Produtos Químicos e Farmacêuticos, Itapira, SP, Brazil) plus inhalation of the 50–50% N₂O-O₂ gas mixture (LIVOPAN®, Linde Gases, Rio de Janeiro, RJ, Brazil) through a self-administration valve.

Patients were monitored in the procedure room using a noninvasive blood pressure device, electrocardioscope and pulse oximeter. The values of systolic blood pressure, diastolic blood pressure, heart rate and peripheral oxygen saturation were recorded immediately before and after the procedure.

The biopsies were performed by the same radiologist, with the patient in the left lateral decubitus position. A GE Logic S6 device (GE Healthcare, Waukesha, WI, USA) and a GE E8C RS probe were used to perform the transrectal ultrasonographies. The biopsies were obtained in an average of 10 punctures with a Gallini 18G x 25 cm needle.

An anesthesiologist followed the examinations without intervening in the analgesia proposed by randomization. Ten minutes after the examination was completed, an investigator who was not involved in the procedure presented and explained the 10 cm visual analog scale (VAS) to the patients, to evaluate their pain intensity (0 to 10) during the procedure and the level of satisfaction (0 to 10) with the administered treatment. The occurrence rates of nausea, vomiting, dizziness, hemodynamic changes, laughter crisis and somnolence during the examination were also evaluated.

The sample size was calculated based on the primary outcome, using the results of the study by Pita et al.⁹ as a parameter. Faced with a reduction of 30% in pain intensity when the 50–50% N₂O-O₂ mixture was used for analgesia, a sample of 42 patients in each group was required to detect such a difference, with respective probabilities of type-1 and type-2 error of 0.05 (α) and 0.2 (β) by the two-tailed test (study power of 80%).

Values were expressed as numbers of patients, means, medians, interquartile ranges and 95% confidence intervals. The Shapiro-Wilk test showed that all parameters had a normal distribution, which allowed the use of a parametric Student’s t-test in the comparison of the NO group with the control group to calculate the two-tailed p-probability. A value of p < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software v.19.0 (IBM, New York, USA).

**Results**

We selected 84 eligible patients. (Fig 1)

There were no significant differences between the groups in relation to age, weight and height (Table 1).

Regarding pain during the procedure, the main evaluated outcome, 13 patients in the C group (30.9%) and only 3 patients (7.1%) in the NO group classified it as intense pain (odds ratio [OR] 0.172, 95% confidence interval [CI], two-tailed p = 0.012). Twenty-seven patients (64%) in the NO group and 8 patients (19%) in the C group classified pain as mild (OR 7.65,
95% CI, two-tailed p = 0.0001). Some patients classified the pain as moderate, including 21 patients in the C group (50%) and 12 patients (28.5%) in the NO group (OR 0.4, 95% CI, two-tailed p = 0.074).

**Table 1. Demographic data for the nitrous oxide (NO) and control (C) groups.**

| Variables     | n  | Min | 25%  | Median | 75%  | Max | Mean | SD   | 95% CI          | Lower | Upper | 2-tailed p-value |
|---------------|----|-----|------|--------|------|-----|------|------|-----------------|-------|-------|-----------------|
| Age (year)    | NO | 42  | 53   | 63     | 70   | 76.5| 86   | 69.45| 8.42 | 66.83, 72.08    |       |       | 0.0760          |
|               | C  | 42  | 52   | 62     | 65.5 | 72  | 83   | 66.38| 7.19 | 64.14, 68.62    |       |       |                 |
| Weight (kg)   | NO | 42  | 46   | 64.5   | 70   | 77.5| 106  | 70.5 | 12.06| 66.74, 74.26    |       |       | 0.3453          |
|               | C  | 42  | 50   | 65     | 73   | 80  | 115  | 73.05| 12.53| 69.14, 76.95    |       |       |                 |
| Height (m)    | NO | 42  | 1.58 | 1.65   | 1.695| 1.72| 1.8  | 1.688| 0.059| 1.67, 1.71      |       |       | 0.9558          |
|               | C  | 42  | 1.5  | 1.65   | 1.7  | 1.725| 1.8  | 1.689| 0.0588| 1.671, 1.71     |       |       |                 |

Two-tailed p-value: Unpaired Student’s t-test; NO: Nitrous oxide; C: Control; kg: Kilograms; m: Meters; N: Number of patients.
The mean pain scores of the two groups were 5.95 (min = 0; max = 10) in the C group and 2.52 (min = 0; max = 10) in the NO group (Table 2) with a two-tailed p value of 0.0001. Satisfaction indexes also differed between the two groups, with means of 8.14 (min = 1, max = 10) in the NO group and 4.69 (min = 0; max = 8) in the C group (Table 2) and a two-tailed p value of 0.0001.

In the C group, the frequencies of adverse events were as follows: 4 (9.5%) patients with somnolence; 3 (7.1%) with dizziness, 3 (7.1%) with euphoria and, finally, 5 (11.9%) with discomfort. In the NO group, the adverse events were: 4 (9.5%) patients experienced somnolence; dizziness 4 (9.5%); vomiting 1 (2.3%); euphoria 5 (11.9%); discomfort 2 (4.7%) (Table 3).

There were no differences in systolic blood pressure, diastolic blood pressure, heart rate and peripheral oxygen saturation between the two studied groups either before or immediately after the procedure (Table 4).

**Discussion**

The fixed gas mixture of 50% oxygen and 50% nitrous oxide has been safely used as an option for sedation and analgesia for various diagnostic or therapeutic procedures[11–17,19].

The pharmacological underpinnings for such indications are widely known[20,21]. Historical studies on potency have shown that 30% nitrous oxide is equivalent to 10–15 mg of morphine[22,23].

This is the first study performed in the Brazilian population using the fixed gas mixture of oxygen and nitrous oxide at 50% through a self-administration valve (Livopan) in a transrectal ultrasound-guided prostate biopsy.

Five to 95% of patients report pain or discomfort during transrectal prostate biopsy. The penetration of the prostatic capsule by the needle is the main cause of pain during the biopsy, and the degree of major discomfort occurs at the time of the introduction of the transducer [24]. Regarding intensity, a large number of patients report moderate and intense pain during

| Variables | n | Min | Max | Mean | SD | 95% CI | 2-tailed p-value |
|-----------|---|-----|-----|------|----|--------|-----------------|
| Pain | NO | 42 | 0 | 10 | 2.52 | 1.62 | 3.42 | <0.0001 |
| C | 42 | 0 | 4 | 10 | 5.95 | 5.12 | 6.79 | <0.0001 |
| Contentedness | NO | 42 | 0 | 9 | 8.14 | 8.1 | 8.9 | <0.0001 |
| C | 42 | 0 | 2.5 | 9 | 4.69 | 3.9 | 5.5 |<0.0001 |

VAS: Visual Analog Scale, N: Number of patients, two tailed P value: Unpaired Student’s t-test

https://doi.org/10.1371/journal.pone.0195574.t002

Table 3. Adverse events.

| Adverse events | Nitrous oxide n(%) | Control n(%) | P value (*) |
|---------------|-------------------|--------------|-------------|
| Somnolence | 4 (9.5) | 4 (9.5) | 0.8182 |
| Dizziness | 4 (9.5) | 3 (7.1) | 0.1182 |
| Nausea | 0 | 0 | 0.9501 |
| Vomiting | 1 (2.3) | 0 | 0.5617 |
| Euphoria (laughter crisis) | 5 (11.9) | 3 (7.1) | 0.2314 |
| Discomfort | 2 (4.7) | 5 (11.9) | 0.1182 |

(*) p-value (two tailed): Mann-Whitney nonparametric analysis for independent samples

https://doi.org/10.1371/journal.pone.0195574.t003
The innervation of the prostate originates in the lower hypogastric plexus, formed by sacral fibers S2 to S4 [26].

The present study demonstrated that, in the Brazilian population, the administration of the 50–50% N₂-O₂ mixture through a self-administration valve was able to significantly reduce pain intensity in transrectal ultrasound-guided prostate biopsy.

In this study, the mean pain intensity of the patients in the NO group was 2.52, while the mean pain intensity of those in the C group was 5.95 (p < 0.0001). A similar result was found by Masood et al. [16], who found mean pain intensities (Entonox®) of 2.2 (± 1.52) in the nitrous oxide group and of 5.73 (± 1.62) in the control group (p < 0.001). McIntyre et al. [27] also achieved a significant reduction in pain scores of the nitrous oxide group (median 1.1) compared with the control (air) group (median 3.4) (p < 0.001). A different result was observed by Spie et al. [28], who did not find a significant difference, although pain tended to be lower in the nitrous oxide group (mean 2.9 in the nitrous oxide group and 3.5 in the control group, p = 0.10).

Similar to that found in the present study, Manikandan et al. [29] also found a mean pain intensity of 2.2 (± 1.59) when they used a mixture of oxygen and 50% nitrous oxide (Entonox®). However, the control group (no analgesia before prostate biopsy) had an average of 2.9 (± 1.59). Notably, even without any kind of analgesia, the control group of the study by Manikandan et al. [29], presented a low mean pain intensity compared with those in the present study and in the study by Masood et al. [16] This difference between the results of the control group of the present study and those of the study by Manikandan et al. [29] can be explained by differences in the timing of the assessment; in the study method of Manikandan et al. [29], the pain intensity assessment was performed immediately before and after the procedure, while in the present study, it was performed 10 minutes after the test.

Table 4. Systolic (SAP) and diastolic (DAP) arterial pressures, heart beat frequency (HBF) and peripheral oxygen saturation (SpO₂) before (B) and after (A) prostate biopsy.

| Variables | n | Min | Percentile |
|-----------|---|-----|------------|
|           |   |     | 25% | Median | 75% | Max | Mean | SD | C. I. (95%) | Lower | Upper | 2 tailed P value |
| B PAS (mm Hg) NO | 42 | 110 | 120 | 146 | 158.5 | 199 | 144.9 | 23.12 | 137.7 | 152.1 | 0.6648 |
|          | 42 | 79  | 129.5 | 143 | 160 | 252 | 147.3 | 27.38 | 138.7 | 155.8 |   |
| PAD (mm, Hg) NO | 42 | 63  | 73 | 80 | 88 | 109 | 81.64 | 12.09 | 77.87 | 85.41 | 0.3777 |
|          | 42 | 53  | 74.5 | 84 | 91.5 | 119 | 84.12 | 13.45 | 79.93 | 88.31 |   |
| FC (bpm) NO | 42 | 40  | 63.5 | 76 | 86.5 | 116 | 75.74 | 17.2 | 70.38 | 81.1 | 0.7375 |
|          | 42 | 47  | 65 | 75 | 90 | 124 | 76.98 | 16.53 | 71.83 | 82.13 |   |
| SpO₂ (%) NO | 42 | 94  | 97 | 97 | 98 | 99 | 97.2 | 0.98 | 96.89 | 97.5 | 0.3920 |
|          | 42 | 95  | 97 | 97 | 98 | 99 | 97.38 | 0.99 | 97.07 | 97.69 |   |
| A PAS (mm Hg) NO | 42 | 104 | 130 | 144 | 166.5 | 249 | 148.8 | 28.98 | 139.8 | 157.8 | 0.5999 |
|          | 42 | 73  | 128 | 144 | 160.5 | 246 | 145.5 | 28.63 | 136.6 | 154.4 |   |
| PAD (mm, Hg) NO | 42 | 53  | 71.5 | 81.5 | 93 | 150 | 83.6 | 17.26 | 78.22 | 88.97 | 0.7908 |
|          | 42 | 31  | 72.5 | 83 | 92 | 122 | 82.64 | 15.49 | 77.82 | 87.47 |   |
| FC (bpm) NO | 42 | 45  | 68 | 84.5 | 94 | 127 | 82.17 | 16.19 | 77.12 | 87.21 | 0.3209 |
|          | 42 | 46  | 64 | 75.5 | 89 | 120 | 78.45 | 17.86 | 72.89 | 84.02 |   |
| SpO₂ (%) NO | 42 | 93  | 97 | 97 | 98 | 99 | 97.44 | 1.097 | 97.09 | 97.79 | 0.5418 |
|          | 42 | 95  | 97 | 97.5 | 98 | 98 | 97.31 | 0.81 | 97.06 | 97.56 |   |

NO: Nitrous oxide; C: Control; bpm: Beats per minute; two-tailed p-value: Unpaired Student’s t-test; N: Number of patients.

https://doi.org/10.1371/journal.pone.0195574.t004
Spie et al. [28] found lower pain intensity values in the control group (mean of 3.5) than those observed in the present study (mean 5.95) and the study by Masood et al.[16] (mean 5.73).

McIntyre et al.[27] also obtained median values for pain intensity of 3.4, similar to those of the study by Spie et al.[28] It should be noted that in the study by Spie et al.[28], the present study and the study by Masood et al.[16], intrarectal lidocaine gel was used in the control group, while in the study by Manikandan et al [29], no analgesia was used. This difference between the pain scores in the control groups could be explained by the possible differences between prostate size, number of punctures during biopsy, type of needle or technique employed [27]. It could also be explained by sociocultural differences among participants, who were French in the study by Spie et al.[28], British in the study by Masood et al.[16] and Manikandan et al.[29] and Brazilian in the present study. This hypothesis is justified in that the interindividual variation in pain intensity in response to an identical procedure, injury or noxious condition has been widely described. Sensitivity to pain is influenced by genetic factors, epigenetic factors, personal history and psychological factors. In addition, personal beliefs, pain representation and personal cultural experience can affect the intensity and expression of pain [18].

The present study demonstrated that patients in the nitrous oxide group had a significantly higher degree of satisfaction than those in the control group, with means of 8.14 (NO) vs 4.69 (C) (p < 0.0001). We have not found information regarding patient satisfaction with the type of analgesia received in previously published studies on nitrous oxide in transrectal ultrasound-guided prostate biopsy, which makes this result unprecedented. Ball et al.[30] studied the degree of satisfaction of patients with continuous or intermittent use of nitrous oxide and obtained a high satisfaction rate, with means of 9.9 (± 0.4) in the continuous group and 9.7 (± 0.9) in the intermittent group (p = 0.23). Maleskar et al.[31] found a high degree of patient satisfaction (median of 9.4) when they received nitrous oxide for analgesia. We emphasize that in both the studies by Ball et al.[30] and by Maleskar et al.[31], the procedure studied was colonoscopy, different from that of the present study.

The frequency of adverse events was relatively low in our study. This result is similar to that found by McIntyre et al.[27], who demonstrated the absence of adverse effects, respiratory problems and prolonged drowsiness when nitrous oxide was used for prostate biopsy. Spie et al.[28], unlike the study by McIntyre et al.[27] and the present study, found higher frequencies of adverse effects. We could justify such differences for methodological reasons, especially the way data was collected, which in the research of Spie et al.[28] was performed immediately after the end of the procedure, and the patients responded to a questionnaire listing the adverse effects: anxiety, euphoria, excitement, restlessness, memory, changes in environmental and sensory perceptions, whereas in curent study, spontaneous reporting was utilized (assessing somnolence, nausea, vomiting, laughter crisis/euphoria, dizziness, discomfort) 10 minutes after the end of the procedure.

The present study has some limitations. The participants were all residents of the state of Rio de Janeiro and, due to the continental characteristics of the brazilian territory, there are regional differences in pain intensity even in Brazil, which limits the extrapolation for the entire brazilian population. The assessment of pain intensity alone is not able to determine the multidimensionalities that are part of the nature of pain. Another limitation of the study was due to late prostate biopsy being performed only in men, the results can not be extrapolated to women and to other diagnostic or therapeutic procedures.

In the current study, there were no significant differences in the values of systolic blood pressure, diastolic blood pressure, heart rate and peripheral O2 saturation between the two groups at the studied moments. These results point to the hemodynamic stability presented by
patients in the nitrous oxide group. The same results were found by Massod et al.[16], who did not find significant differences in heart rate between nitrous oxide and control groups, although the mean value for heart rate was lower than that in the nitrous oxide group, which could suggest better analgesia. Massod et al.[16] also found no significant difference in oxygen saturation, a result similar in our study.

Conclusion
The present study concluded that pain intensity was significantly reduced in patients who inhaled N₂O:O₂ (50–50%) by means of a self-administered valve for transrectal ultrasound-guided prostate biopsy. The frequency of adverse effects and systolic and diastolic blood pressures, heart rate and peripheral oxygen saturation values were similar between those who inhaled and those who did not inhale nitrous oxide. The level of patient satisfaction was significantly higher among those who used the N₂O:O₂ mixture than among those who used oxygen alone.

Supporting information
S1 File. TCLE.
(DOCX)
S2 File. TCLE ingles.
(DOCX)
S3 File. Questionnaire—Original version.
(DOCX)
S4 File. Questionnaire—English.
(DOCX)
S5 File. CONSORT Plos One.
(DOC)
S6 File. Study protocol—Original version.
(DOCX)
S7 File. Study protocol—English.
(DOCX)

Author Contributions
Conceptualization: Ismar Lima Cavalcanti.
Formal analysis: Gabriel da Silva Cazarim, Louis Barrucand.
Investigation: Gabriel da Silva Cazarim.
Methodology: Gabriel da Silva Cazarim, Leonel Carneiro, Rachel Pastor, Elizabeth Fernandes Vaz da Silva, Louis Barrucand, Ismar Lima Cavalcanti.
Project administration: Gabriel da Silva Cazarim, Nubia Verçosa, Ismar Lima Cavalcanti.
Resources: Gabriel da Silva Cazarim, Leonel Carneiro, Rachel Pastor.
Supervision: Gabriel da Silva Cazarim, Nubia Verçosa, Ismar Lima Cavalcanti.
Validation: Gabriel da Silva Cazarim.
References

1. Uno H, Nakano M, Ehara H, Degushi T. Indications for 14-core transrectal ultrasound-guided prostate biopsy. Urology. 2008; 71(1):23–27. https://doi.org/10.1016/j.urology.2007.09.020 PMID: 18242358

2. Inal G, Yazici S, Adnan O, Ozturk B, Kosan M, Cetinkaya M. Effect of periprostatic nerve blockade before transrectal ultrasound-guided prostate biopsy on patient comfort: A randomized placebo controlled study. Int J Urol. 2004; 11:148–151. PMID: 15009362

3. Ozveri H, Cevik I, Dillioglugil O, Akdas A. Transrectal periprostatic lidocaine injection anesthesia for transrectal prostate biopsy: a prospective study. Prostate Cancer and Prostatic Dis. 2003; 6(4):311–314.

4. Autorino R, de Sio M, di Lorenzo G, Damiano R, Perdona S, Cindolo L, et al. How to decrease pain during transrectal ultrasound guided prostate biopsy: a look at the literature. J Urol. 2005; 174(6):2091–2097. https://doi.org/10.1097/01.ju.0000181212.51025.06 PMID: 16280735

5. Bingqian L, Peihuan L, Yudong W, Jinxing W, Zhiyong W. Intraprostatic local anesthesia with periprostatic nerve block for transrectal ultrasound guided prostate biopsy. J Urol 2009; 182(2):479–483. https://doi.org/10.1016/j.juro.2009.04.029 PMID: 19524987

6. Kubo Y, Kawakami S, Numan N, Takazawa R, Fujii Y, Masuda H, et al. Simple and effective local anesthesia for transperineal extended prostate biopsy: Application to three-dimensional 26-core biopsy. Int J Urol. 2009; 16: 420–423. https://doi.org/10.1111/j.1442-2042.2009.02269.x PMID: 19416405

7. Basar H, Basar M, Ozan S, Akpinar S, Basar H, Batislam E. Local anesthesia in transrectal ultrasound-guided prostate biopsy: EMLA cream as a new alternative technique. Scand J Urol and Nephrol. 2005; 39: 130–134.

8. Kang SG, Tae BS, Min SH, Ko YH, Kang SH, Lee JG, et al. Efficacy and cost analysis of transrectal ultrasound-guided prostate biopsy under monitored anesthesia. Asian J Androl. 2011; 13(5): 724–727. https://doi.org/10.1038/ajand.2011.16 PMID: 21623389

9. Pita CP, Pazmiño S, Vallejo M, Salazar-Pousada DS, Hidalgo L, Pérez-López FR, et al; Research Group for the Birth Humanization Project of Enrique C. Sotomayor Hospital. Inhaled intrapartum analgesia using a 50–50% mixture of nitrous oxide–oxygen in a low-income hospital setting. Arch Gynecol Obstet. 2012; 283(3):627–631.

10. Emmanouil DE; Quock RM. Advances in understanding the actions of nitrous oxide. Anesth Prog. 2007; 54(1): 9–18. https://doi.org/10.2344/0003-3006(2007)54[9:AIUATAO]2.0.CO; 2 PMID: 17352529

11. Uziel Y, Chapnick G, Rothshild M, Tauber T, Press J, Harel L, et al. Nitrous oxide sedation for intra-articular injection in juvenile idiopathic arthritis. Pediatr Rheumatol Online J. 2008; 15(6):1–4.

12. Gerhardt RT, King KM, Wiegert RS. Inhaled nitrous oxide versus placebo as an analgesic and anxiolytic adjunct to peripheral intravenous cannulation. Am J Emerg Med. 2001; 19(6): 492–494. https://doi.org/10.1053/ajem.2001.25780 PMID: 11593469

13. Harding TA, Gibson JA. The use of inhaled nitrous oxide for flexible sigmoidoscopy: a placebo-controlled trial. Endoscopy. 2000; 32(6): 457–460. https://doi.org/10.1055/s-2000-652 PMID: 10863911

14. Forbes GM, Collins BJ. Nitrous oxide for colonoscopy: a randomized controlled study. Gastrointestinal Endoscopy. 2000; 51(3): 271–277. PMID: 10699770

15. Cook HL, Newsom RS, Mensah E, Saeed M, James D, Flytche TJ. Entonox as an analgesic agent during panretinalphotocoagulation. Br J Ophthalmo. 2002; 86(10): 1107–1108.

16. Masood J, Shah N, Lanes T, Andrews H, Simpson P, Barua JM. Nitrous oxide (entonox) inhalation and tolerance of transrectal ultrasound guided prostate biopsy: a double-blind randomized controlled study. J Urol. 2002, 168(1):116–120. PMID: 12050003

17. Baskett PJ. Use of Entonox in the ambulance service. Br Med J. 1970; 4(2): 41–43.

18. International Association for Study of Pain (IASP) Blogs.2011 out 1 [cited 26 july 2017]in : [https://www. iasppain.org/files/Content/ContentFolders/GlobalYearAgainstPain2/AcutePainFactSheets/1-Problem_ Portuguese.pdf, ]

19. Young A, Ismail M, Papapetrou AG, Barua JM, Calléry JG, Masood J. Entonox® inhalation to reduce pain in common diagnostic and therapeutic outpatient urological procedures: a review of the evidence. Ann R Coll Surg Engl. 2012; 94(1):8–11 https://doi.org/10.1308/003588412X13171221499702 PMID: 22524905
20. Boulland P, Favier J-C, Villevielle T, Allanic L, Plancade D, Nadaud J, et al. Premixed 50% nitrous oxide and oxygen: theoretical recalls and practical modalities. Annales Françaises d’Anesthésie et de Réanimation. 2005; 24 (10):1305–1312. https://doi.org/10.1016/j.anfar.2005.05.018 PMID: 16099128

21. Pellat JM, Hodaj H, Kaddour A, Long JÁ, Payen JF, Jacquot C, et al. Le MEOPA (Kalinox®) Mélange Équimolaire Oxygène et Protoxyde d’Azote dans le traitement de la douleur. Douleurs 2004; 5(5): 275–281.

22. Chapman WP, Arrowood JG, Beecher HK. The analgesic effects of low concentrations of nitrous oxide compared in man with morphine sulphate. J Clin Invest 1943; 22(6):871–875. https://doi.org/10.1172/JCI101461 PMID: 16695072

23. Parbrook GD, Rees GA, Robertson GS. Relief of post-operative pain: comparison of a 25 per cent nitrous-oxide and oxygen mixture with morphine. Br Med J 1964; 2(5407):480–482. PMID: 14161988

24. Nash PA, Bruce JE, Indudhara R, Shinorara K. Transrectal ultrasound guided prostatic nerve blockade eases systematic needle biopsy of the prostate. J Urol. 1996; 155(2):607–609. PMID: 8558671

25. De Sio M, D’armiento M, Di Lorenzo G, Damiano R, Perdonà S, De Placido S, et al. The need to reduce patient discomfort during transrectal ultrasonography-guided prostate biopsy: what do we know? BJU Int. 2005; 96(7):977–983. https://doi.org/10.1111/j.1464-410X.2005.05736.x PMID: 16225512

26. Hollabaugh JR RS, Dmochowski RR, Steiner RS. Neuroanatomy of the male rhabdosphincter. Urology. 1997; 49(3): 426–434. https://doi.org/10.1016/S0090-4295(96)00497-9 PMID: 9123709

27. McIntyre IG, Dixon A, Pentelides ML. Entonox analgesia for prostate biopsy. Prostate Cancer Prostatic Dis. 2003; 6(3):235–238. https://doi.org/10.1038/sj.pcan.4500670 PMID: 12970727

28. Spie R, Watfa J, Dubruille T, Michel F. Value of nitrous oxide-oxygen mixture (Entonox) in transrectal prostate biopsies. Prog Urol. 2008; 18(6):358–363. https://doi.org/10.1016/j.purol.2008.03.028 PMID: 18558324

29. Manikandan R, Srirangan SJ, Brown SC. Nitrous oxide vs periprostatic nerve block with 1% lidocaine during transrectal ultrasound guided biopsy of the prostate: a prospective, randomized, controlled trial. J Urology. 2003; 170:1881–1883.

30. Ball AJ, Din S, Donnelly M, Riley SA. A randomized controlled trial comparing continuous and as-required nitrous oxide use during screening colonoscopy. Eur J Gastroenterol Hepatol. 2015; 27(3): 271–278. https://doi.org/10.1097/MEG.000000000000281 PMID: 25629571

31. Maleskar S, Balaji P, Gardiner A, Culbert B, Monson JRT, Duthie GS. Randomized controlled trial of patient-controlled sedation for colonoscopy: Entonox vs modified patient-maintained target-controlled propofol. Colorectal Dis. 2010; 13(1): 48–57.