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Blind Endotracheal Intubation in Neonatal Rabbits

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TITLE:

Blind Endotracheal Intubation in Neonatal Rabbits

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KEYWORDS:

Rabbit, endotracheal intubation, newborn, rabbit airway, anesthesia, ventilation

SUMMARY:

We describe a technique of endotracheal intubation in newborn rabbits after esophageal catheterization with a gastric tube.

ABSTRACT:

The newborn rabbit is a useful animal model for various pathologies and procedures. Airway management of the rabbit is complex due to its anatomical characteristics, which is further complicated in the case of the newborn. Of the different methods of advanced airway management, endotracheal intubation is less aggressive than tracheostomy, and is more feasible than supraglottic management given the lack of supraglottic devices of such a small size. As direct glottis visualization is very difficult in animals this size, this blind intubation model is presented as an effective alternative, especially for experiments requiring prolonged anesthesia. Using this method, we performed blind intubations with a 90% success rate.

INTRODUCTION:

The peculiar anatomy of the rabbit's respiratory system makes endotracheal intubation complicated. The oral cavity is deep and narrow with a limited range of mouth opening and a

relatively large tongue, making direct visualization of the larynx difficult even with the use of a laryngoscope^{1,2}. Furthermore, intubation of the newborn rabbit, which is 10-20 times smaller than the adult, is even more of a challenging procedure. In addition to these anatomical characteristics, rabbits are prone to laryngospasm secondary to manipulation of the airway.

Several intubation techniques have been described in the literature, such as laryngoscope tracheal intubation, blind intubation, otoscope intubation, retrograde intubation, endoscope intubation, tracheostomy and supraglottic device ventilation³⁻⁹. All these techniques have been carried out successfully in adult rabbits; however, there are few publications regarding advanced airway management in the newborn rabbit¹⁰. One study describes performing tracheostomies in animals of this age to assess breathing mechanics and lung volumes, though this is an aggressive and irreversible technique¹¹. This method for endotracheal intubation has proven to be an effective and low-cost method of airway management in newborn rabbits.

PROTOCOL:

The intubation procedure outlined below was approved by the Barcelona University Institutional Animal Care and Use Committee (CEEA 180/19)

1. Experimental preparation

1.1. Obtain the following materials: 2 mm diameter polyvinyl chloride (PVC) endotracheal tube, 6 French (Fr) polyurethane feeding tube, flat and wide tip dissection forceps and sterile lubricant (**Figure 1**).

1.2. During the intubation and surgery processes, maintain sterile conditions.

2. Anesthesia

2.1. Anesthetize the animal with 5 mg/kg xylazine and 25 mg/kg ketamine applied together intramuscularly in the *vastus lateralis* of the femoral quadriceps. Alpha-2 agonists such as dexmedetomidine can also be used instead of xylazine.

2.2. Allow 3-5 minutes for the medication to reach effect.

2.3. Readminister the same dose until deep anesthesia is achieved (negative pedal, palpebral and corneal reflexes). Ideally, the rabbit will maintain spontaneous breathing until intubation.

3. Endotracheal intubation

3.1. Place the rabbit on a thermal blanket at 37 °C. Place the animal in dorsal recumbency with the head in hyperextension. Align the head as straight and midline as possible. A four-centimeter roll made of sterile gauze placed in the dorsal region of the neck may be helpful to achieve at least 45° of extension (**Figure 2**).

3.2. Use flat and wide tip dissection forceps to open the oral cavity approximately one centimeter wide. Use forceps with a blunt and wide end to avoid mouth trauma.

3.3. Measure the distance between mouth and the region of stomach (first rib). Introduce a lubricated 6 French (Fr) polyurethane gastric tube to the pre-measured mark (**Figure 3-4**).

3.4. Move the feeding tube to the left and fix with adhesive tape to the surgical field.

3.5. Introduce the 2 mm diameter PVC endotracheal tube, covered with sterile lubricant to the 3 cm mark.

3.5.1. If there are any difficulties in advancing the tube, move it completely and reattempt until a smooth glide is achieved. The risk of trauma to the larynx and epiglottis is significant with multiple intubation attempts. Fix the tube with adhesive tape to the surgical field (**Figure 5-6**).

3.6. Connect the endotracheal tube to the ventilator with following settings: Tidal volume - 10 mL/kg; respiratory rate: 40 breaths/min; FiO₂ 100%, PEEP 6.

3.7. Confirm the correct position of the tube by the detection of CO₂ in exhaled air (ETCO₂), direct visualization of thoracic expansion and fogging of the tube. Monitoring of the animal by pulse oximetry and lung auscultation can also be helpful (**Figure 7**).

3.8. At the conclusion of the experiment, use a standardized euthanasia protocol that consists of intravenous administration of pentobarbital 120-240 mg/kg. Repeat doses until breath cessation, reflex absence and asystole are achieved.

NOTE: In the surgical experiments, the animal is alive during the ventilation process and finally is euthanized.

REPRESENTATIVE RESULTS:

In the laboratory, this technique is carried out by medical personnel with experience in experimentation with New Zealand rabbits and expertise in pediatric airway management. The procedure has been performed by one investigator in 10 animals with a global success rate of 90%. Previously, another researcher had practiced optimizing both the position of the animal and the correct tube fixation length. None of the animals were intubated in esophagus. Only one attempt (10%) failed due to the extremely small size of the animal (**Table 1**). Once blind intubation has been practiced, success is achieved on the first attempt in most cases, even if the procedure is performed by personnel not previously trained in rabbit airway management. We achieved a 100% intubation success rate in one or two attempts in animals weighing equal to or greater than 120 g.

FIGURE AND TABLE LEGENDS:

Figure 1: Materials needed for the experiment: Dissection forceps, feeding tube, endotracheal

tube, sterile lubricant.

Figure 2: Position of the animal before intubation: Dorsal recumbency and mild hyperextension.

Figure 3: Mouth opening with dissection forceps.

Figure 4: The feeding tube is introduced into the stomach.

Figure 5-6: The endotracheal tube is introduced 3 cm blindly and fixed, along with the feeding tube, to the surgical field.

Figure 6: The fixed endotracheal tube and feeding tube.

Figure 7: The animal is connected to the ventilator.

Table 1: Animal sample features and endotracheal intubation attempts.

DISCUSSION:

Rabbits are widely used in animal experimentation due to their availability, high reproduction rate with short intergenerational times, and easy handling². In our center, we use 5-7-day old New Zealand rabbits (*Oryctolagus cuniculus*) as a neonatal model to study the neurologic repercussions of extracorporeal surgery on the developing brain. As a result, we utilize procedures that require deep anesthesia and must use endotracheal intubation for periods of time up to an hour.

In the adult rabbit, several airway management techniques have been described, usually in a surgical context. When we tried to extrapolate these techniques to the rabbit kits, several problems arose. The rabbits weigh between 110 and 210 grams and have a maximum mouth opening range of 1 cm. These characteristics make direct visualization of the larynx difficult and it is complicated to find endoscopic material small enough. Another option described in the literature is to place an otoscope in the oral cavity and use it as a guide to introduce the PVC tube⁷. In newborn rabbits, both the presence of the tongue and the small size of the mouth make direct visualization of the glottis difficult. In addition, there are no supraglottic devices of adequate dimensions for newborns.

Due to the circumstances mentioned above, there are two fundamental mechanisms to secure the airway of the very small rabbit: tracheostomy and blind intubation. Tracheostomy is an invasive approach that has been used in both adult and newborn rabbits^{10,12}. It has the advantage of direct tracheal visualization, allowing the proceduralist to identify the exact location of the endotracheal tube. However, there is a high risk of mechanical complications and infection of the incision area, in addition to a decreased rate of survival of the animals once the procedures are completed¹³. Endoscopic/fiberoptic intubation is a method that would allow for direct visualization without surgery⁵. Another option to prevent laryngeal trauma is the use of a tight-

fitting face mask, which has some additional drawbacks such as suboptimal ventilation and gastric distension.

In the laboratory, blind intubation is used to manage newborn rabbit airways. This method has been described in the adult rabbit as a feasible technique with high success index⁶. In the initial experiments, we tried to perform this technique without occlusion of the digestive tract. The result was a high rate of introduction of the endotracheal tube into the esophagus. With the insertion of the gastric tube prior to intubation, however, successful intubation was achieved in 90% of cases. Regarding the size of the endotracheal tube, we initially tried to use peripheral venous catheters up to 14 gauge in diameter, observing difficulties in both insertion (greater rigidity) and ventilation (diameter too small and difficulty connecting to the ventilator). In our experience, the use of 2 mm internal diameter endotracheal tubes introduced to a depth no more than 3 cm has been the most effective in ventilating small rabbits, shown by correct gasometric and ETCO₂ values. While intubation in adult rabbits generally requires hyperextension of the head to essentially a 90° angle to the horizontal surface of the table, we were able to perform the procedure with only a mild head extension.

One of the important elements to be considered when performing this procedure is the friable nature of the newborn rabbit trachea. Keeping this in mind, we introduce the pre-lubricated tube slowly and carefully to avoid tracheal perforation. We recommend not inserting the tube further than 3 cm, as this was found to be the optimal length during practice attempts, and halting the intubation if the proceduralist encounters any resistance; in this situation, it is preferable to remove the tube and start over. We experienced one case of likely tracheal perforation in the smallest animal in the cohort.

The main limitation of this technique is the difficulty in detecting the correct position of the endotracheal tube once introduced. In our experience, the advancement of the device without resistance, the detection of appropriate ETCO₂, the fogging of the tube and visualization of thoracic expansion during ventilation, as well as the maintenance of vital signs have been sufficient to corroborate the suitability of the procedure in all cases. In some of the experimental subjects, arterial gasometries were performed during ventilation showing adequate levels of carbon dioxide and oxygen concentration. In addition, though histopathology analysis was not performed, previously published literature has described mucosal injury to the trachea in the absence of clinical changes secondary to intubation¹⁴.

In conclusion, the model of intubation in newborn rabbits we have described is a rapid technique that uses basic supplies and allows for effective ventilation. We believe this technique can be incorporated in the armamentarium of airway management of neonatal rabbits.

ACKNOWLEDGMENTS:

This work was supported by grants from the *Instituto de Salud Carlos III* [PFIS2017-0224], “Beca Ruza” from the *Sociedad Española de Cuidados Intensivos Pediátricos* and the Vanderbilt Medical Scholars Program.

The sponsors were not involved in the collection, analysis, or interpretation of the data.

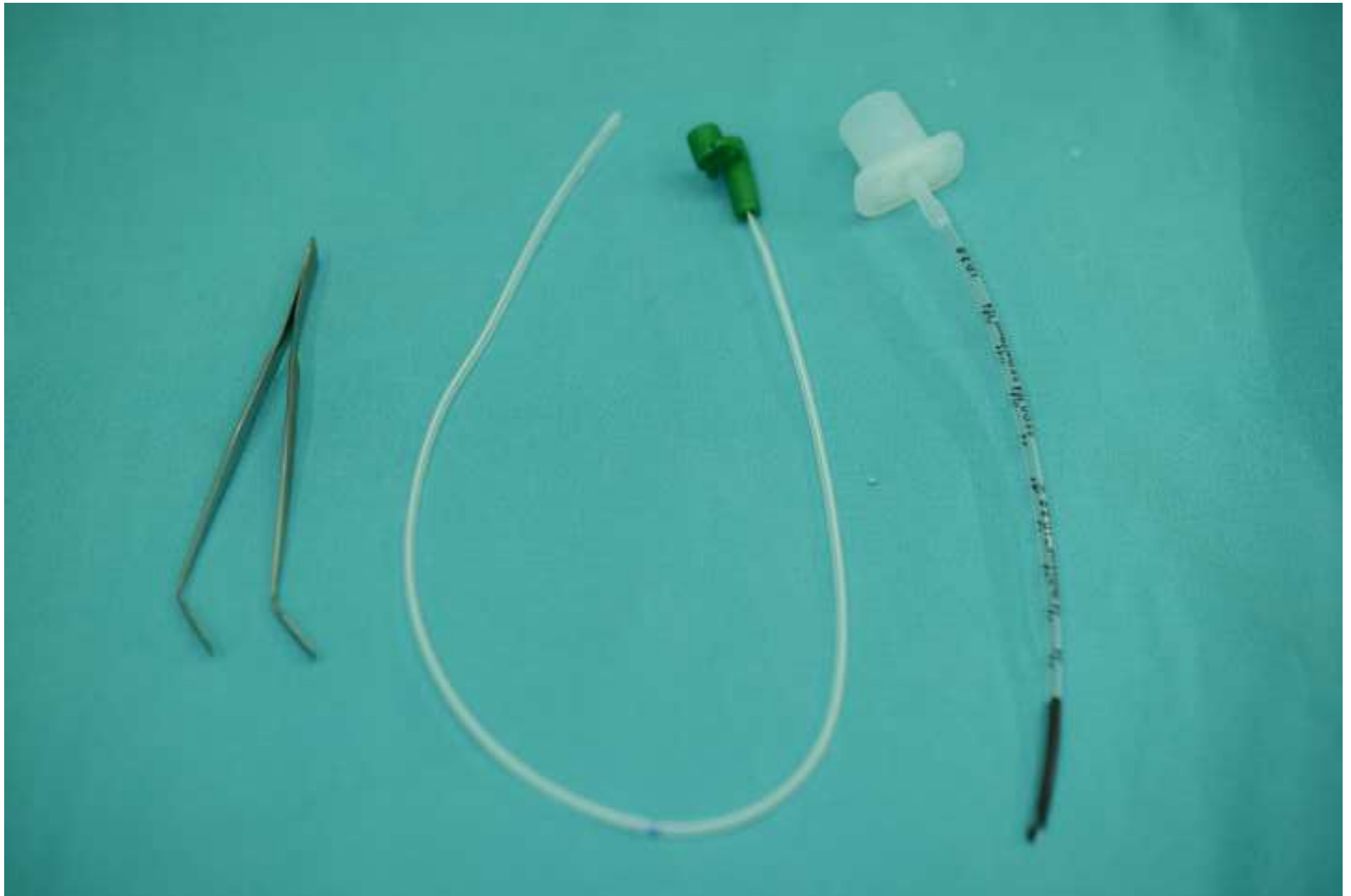
We would like to thank Isabel Salas for the help provided within the animal laboratory and the Mr. Carles Fábrega and Ms. Gemma Fernandez-Asensio from the Audio-Visual Technical Team from Sant Joan de Déu Hospital for their technical support with the video recording and production.

DISCLOSURES:

The authors have nothing to disclose.

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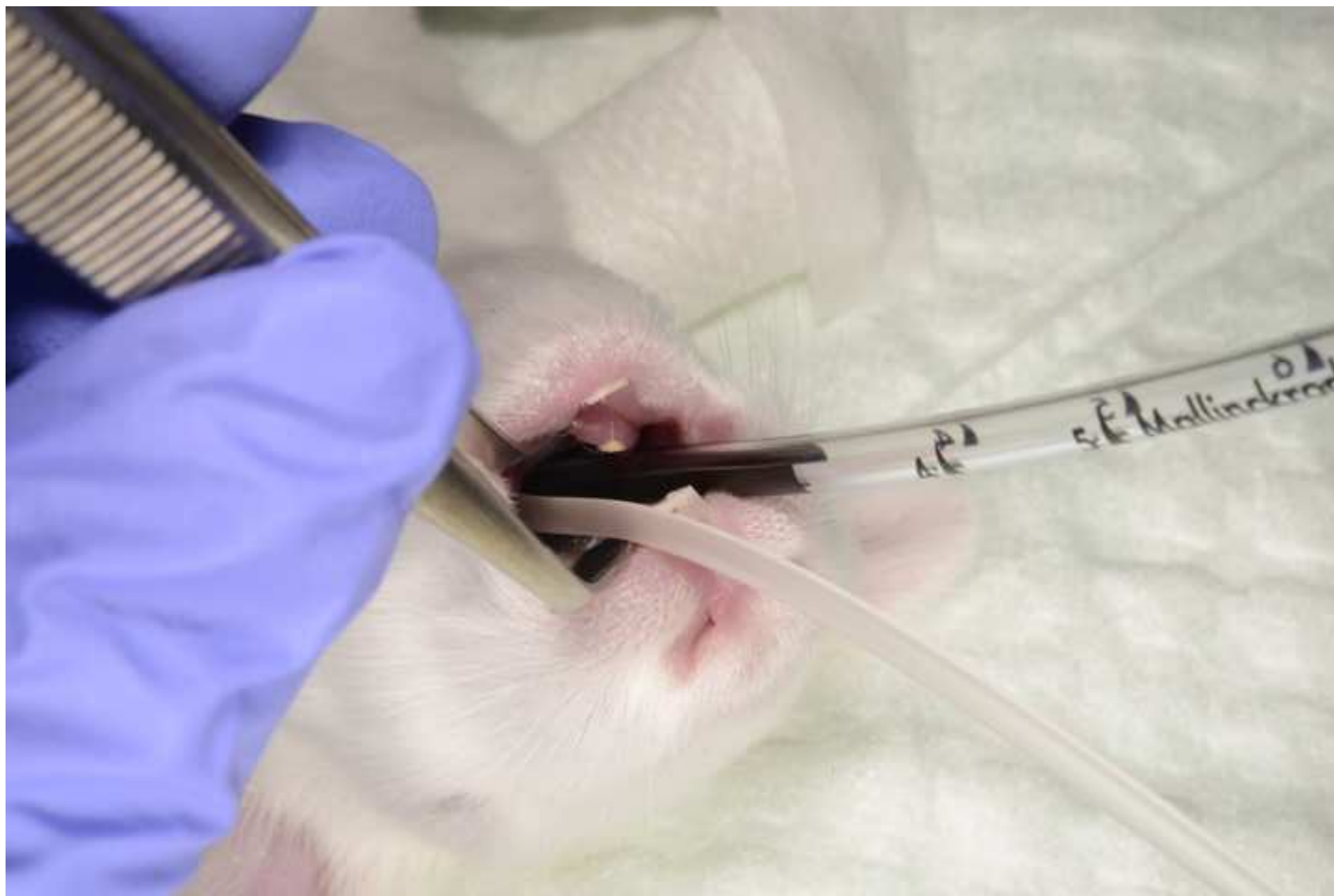
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ANIMAL NUMBER	WEIGHT (g)	SUCCESS	ATTEMPTS	DEATH
1	150	+	1	-
2	125	+	2	-
3	180	+	1	-
4	210	+	1	-
5	120	+	2	-
6	140	+	2	-
7	200	+	1	-
8	190	+	1	-
9	180	+	1	-
10	110	-	3	+
TOTAL	160.5	90%	1.5	10%

Name of Material/Equipment	Company	Catalog Number	Comments/Description
2 mm diameter Polyvinyl Chloride (PVC) endotracheal tube	Vygon	520.20	
6 French polyurethane feeding tube	Vygon	310.06	
Anesthesia (Ketamine and Xylazine)			
Multiparameter monitor Intellivue MP5	Phillips		
Plain and wide tip dissection forceps	LensforVision	AK005	
Sterile lubricant (Silkospray)	Rush		
Ventilator Servo 900c	Maquet		

REBUTTAL DOCUMENT

Editorial and production comments:

Changes to be made by the Author(s) regarding the written manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Thank you for the suggestion, grammar and spelling have been revised

2. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed?

3. 3.1: How large is the roll? *This information has been added to the protocol*

4. 3.3: How is the tube fixed? *This information has been added to the protocol*

5. What happens to the animal after the experiment?

The reviewer is right and this information was not included in the original text, this question has been answered in the new “point 6” of the protocol.

6. Please specify the euthanasia method if the animal is euthanized.

This information has been added to point 6 of the protocol.

7. Discuss maintenance of sterile conditions during survival surgery.

Following the reviewer’s suggestion, a description of the maintenance of sterility during the procedure has been explained in point 6 of the protocol.

8. Please specify that the animal is not left unattended until it has regained sufficient consciousness to maintain sternal recumbency.

All the animals were euthanized at the end of the procedure, so this point might not be necessary because of the endpoint of the experiment.

9. Please specify that the animal that has undergone surgery is not returned to the company of other animals until fully recovered.

In accordance with the previous answer, all the animals were euthanized at the end of the procedure, so this point might not be necessary because of the endpoint of the experiment.

10. Please include the table at 3:10 of the video in the manuscript text. Ideally, all figures in the video would appear in the written manuscript and vice versa. The video and the written manuscript should be reflections of each other.

“Table 2” has been included in the video, following reviewer suggestions.

Changes to be made by the Author(s) regarding the video:

1. "Blind Endotracheal Intubation in Newborn Rabbits", the intro text is running off the screen being cropped.

This part of the intro has been modified

2. 1:34 – Please revise the on screen text: Xylazine 5 mg/kg and ketamine 25 mg/kg
Text revised.

3. I noticed there’s warp stabilization on some of the shots around where the tube goes

into the mouth. It's a static shot and doesn't need to be stabilized. It's more distracting because everytime their hand moves in the frame, the image warps.

This has been modified in the video.

4. There are some jump cuts, where dissolves should go. Like after the title card, the video should fade up, not jump cut in.

This has been modified in the video.

5. Please put the ethics card put it between the end of the introduction and before the start of the protocol.

The ethics card has also been included

6. Please remove the watermark from the protocol sections of the video. It may only appear in the introduction and conclusion.

Watermark has been removed from the sections following editorial suggestions.

Please submit a high-resolution video here:

<https://www.dropbox.com/request/wM1PQeG8f7WOPJcSIDxc?oref=e>

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This manuscript provides what appears to be a relatively simple method for intubation of neonatal rabbits using minimal materials that are readily accessible.

Major Concerns:

No major concerns.

Minor Concerns:

Text manuscript:

2.3: If an additional dose is needed, would the authors suggest a specific fraction of the original dose?

We have specified that is the same dose.

2.3: "podal" should be "pedal" *Spelling error fixed*

3.2: Is the gastric tube lubricated? *We have specified that the tube is lubricated..*

3.3: How is the feeding tube fixed? *We have specified that the tube is fixed with adhesive tape to surgical field.*

3.5: Please expand this sentence to introduce that the table lists ventilator settings. Is there. *Following reviewer suggestion this sentence has been modified.*

Other questions:

Is thermal support provided during intubation? *Yes, we have specified that in 3.1*

Alignment of the head as straight and midline as possible is essential for most intubation techniques in rabbits. Is this true for this method as well (in which case, it would be worthwhile to add this point), or is it not as important?

In neonatal rabbits the alignment of the head is as important as in adult rabbits. We have specified that point in 3.1

In the figures and video, the rabbit's head looks mildly extended, but not hyperextended. Intubation of adult rabbits generally requires hyperextension of the head

to essentially a 90 degree angle relative to the horizontal surface of the table. Suggest revising accordingly.

We have added a sentence in 3.1 where we explain that in our sample we have good results with mild hyperextension.

Video comments:

A few comments on the slide pictured at 1:31

- "Pedal" is spelled incorrectly. *Sorry, this has been corrected.*

- Did the author obtain permission to use this anatomical image? *The image was drawn by the author.*

- Since revisions are needed anyway, please fix the spacing in the "mg/kg" for ketamine. *This has been done.*

- The image of the rabbit is not relevant to the information being discussed when this slide is presented. It may be more useful to show an image of the administration of the anesthetic to relate the anatomical illustration to what that actually looks like on the neonatal rabbit. *We don't have an image of this procedure therefore, as suggested by the reviewer, only the image of the injection site is shown in the final version*

Reviewer #2:

Title:

* change "newborn" to neonatal

This changed has been done

Keywords:

* combine "endotracheal intubation"

This has been included

Summary:

"We describe a novel technique of endotracheal intubation in newborn rabbits after esophageal catheterization with a gastric tube."

* This is not a novel technique. This is a modified technique and one that has been described in rabbits (Falcão, et al, 2011). Please reword this statement.

We have modified this statement.

Abstract:

"Within the different routes of advanced airway management, endotracheal intubation is the least aggressive, since it does not require surgery"

* While intubation may be less aggressive than surgery it is certainly not the least aggressive. It has been shown that intubation is traumatic in rabbits, especially blind intubation.

We have changed the sentence, introducing supraglottic devices.

See:

Comparison of a Supraglottic Airway Device (v-gel®) with Blind Orotracheal Intubation in Rabbits, Engbers, et al

Following reviewers suggestion, this reference has been added into the bibliography

"...this blind intubation model is presented as a good alternative, especially for

experiments requiring long sedation time."

* Avoid the use of "good" as this technique will likely induce trauma and you have not scientifically proven otherwise.

We have modified these expressions

* Additionally, animals that are intubated should be under general anesthesia, not under "sedation".

This expression has also been changed

* Authors should add objective results to abstract.

Some of the most important objective results have been added to the abstract.

INTRODUCTION:

"The oral cavity is deep and narrow, making direct visualization of the larynx difficult, even with the use of a laryngoscope"

* Include the anatomical description of the tongue in rabbits as this is a major issue with visualization.

Some anatomical features related to the tongue and mouth rabbit have been included to improve understanding .

* Should also include other physiological reasons— There are multiple publications in the literature that describe the anatomical differences in rabbits that makes intubation challenging as well as their increased predisposition to laryngospasm (*This has been included into the text*). Additionally, rabbits, even without clinical signs, have been reported to have significant acute histological post-intubation lesions, likely due to the increased mucosal perfusion (*This concept has also been mentioned in the discussion*).

"Several intubation techniques have been described in the literature, such as laryngoscope tracheal intubation, blind intubation, otoscope intubation, retrograde intubation, endoscope intubation tracheostomy and laryngeal mask ventilation"

* Please add supraglottic airway devices (ie vgel) in place of laryngeal mask as not all SGADs are laryngeal masks

Following your suggestions supraglottic airway devices has been added.

Ref: Comparison of endoscopic endotracheal intubation and the v-gel supraglottic airway device for spontaneously ventilating New Zealand white rabbits undergoing ovariectomy, Comolli, et al (*This reference has been included in the bibliography*)

"Our method for endotracheal intubation has proved to be an effective, secure and low-cost method of airway management achievable with a minimal learning curve."

* Authors are oversimplifying the issues involved with blind intubation in rabbits. They are stating this method is "proven" simply because intubation was successful but without evaluating the post intubation results. Due to the current literature indicating the trauma involved with intubating rabbits (*This concept has also been included in the discussion*), I would avoid comments like "minimal learning curve" unless you have proof that the procedure was performed correctly and a traumatically. *According to reviewer suggestion this kind of expressions have been avoided*

PROTOCOL:

* Under materials, please describe what is meant by "plain and wide" tip forceps.

A description of the forceps has been included and it appears in materials figure

"Sedate the animal with xylazine 5mg/kg and ketamine 25mg/kg..."

* Change sedate to anesthetize. *This has been changed.*
* Add / to 5 mg kg. *This has been included.*
* Please note- there are more modern alpha-2 drugs available than xylazine...dexmedetomidine would be preferable and readily reversible if needed. *Thanks for the suggestion, we have included the possibility of dexmedetomidine. We will probably use it in the future.*

"negative pedal reflex..."

-Do you mean "pedal" reflex? *(word changed)* It may be better to indicate the stage/plane of anesthesia (describe what this means) when an animal should be intubated. *Deep anesthesia specified.*

"A roll made of sterile gauze and placed in the dorsal region of the neck may be helpful"

* Please explain why this is helpful. *This has been explained in section 3.1*
* Remove the word "and" *This has been done.*

"Use plain and wide tip dissection forceps to open the oral cavity approximately one centimeter wide. Introduce a 6 Fr (French) polyurethane gastric tube until the cessation of its progress "

* Again, please explain what "plain and wide tip" means.

This part has been rewritten

* rather than advance until cessation, the tube should be pre-measured to the region of the stomach, to avoid potential injury (measure to the last rib).

This has been added to section 3.2

The feeding tube is moved to the left and fixed

* How is it fixed? *This has been better explained in section 3.3.*

"Impregnated with sterile lubricant until it reaches the 3cm mark."

* Impregnated indicates that the inside of the tube is lubricated, which could cause obstruction in such a small tube. What do authors mean? Covered with lubricant to the 3 cm mark?

Following the reviewer suggestion, the word "impregnated" was changed to "covered" for a better understanding

"If there are any difficulties in advancing the tube, it should be completely removed and reattempted until a smooth glide is achieved. The tube is also fixed. "

* Authors need to address the trauma to the larynx and epiglottis with multiple attempts of intubation. This has been confirmed in the literature for rabbits. *This concept has been added to section 3.4*

* How is the tube fixed? *This is now explained in section 3.4*

The correct position of the tube is confirmed by direct visualization of thoracic expansion and fogging of the tube. The detection of CO₂ in exhaled air and monitoring of the animal by pulse oximetry can also be helpful. * Would put the monitoring of ETCO₂ as one of the confirmatory measures as it will give you an idea of correct placement much faster than SPO₂

The role of ETCO₂ has been better explained in section 5.1.

* Bilateral auscultation of both lungs to make sure both lungs are expanding would also be a good idea.

This suggestion has been included, although in small rabbits is technically difficult to perform bilateral auscultation.

RESULTS:

"In our laboratory, this technique is carried out by medical personnel with experience in experimentation with New Zealand rabbits. Once blind intubation has been practiced, success is achieved on the first attempt in most cases, even if the procedure is performed by personnel not previously trained in airway management."

* Please explain as the statement is conflicting. Please better explain in your methodology. how many different individuals intubated the 10 rabbits with 90% success, and what was the experience level of these people?

Results section was expanded and better explained.

DISCUSSION

"In our center, we use 5-7-day old New Zealand rabbits as a neonatal model to study"

* Please provide genus and species. *We have included it in the manuscript.*

"As a result, we utilize procedures that require deep sedation and must use invasive airway management techniques for periods of time up to an hour."

* Do authors mean anesthesia or are you intubating sedated animals?

Sorry for the misunderstanding, "sedation" was changed to "anesthesia".

* What is considered "invasive" airway management techniques, please clarify

"When we tried to extrapolate these techniques to our pup rabbits, several problems arose."

* Neonatal rabbits are called "kits"

Thanks for the suggestion, we have included this word

"These characteristics makes direct visualization of the larynx a rather impossible task since no laryngoscopes of a small enough size."

* I disagree with this statement; a 1.9 or 1mm endoscope would permit direct visualization.

We have changed the statement, suggesting that is very difficult to find this material, but not impossible..

* Please correct the grammatical errors of this sentence.

We have corrected the grammatical errors.

"Due to the circumstances mentioned above, there are two fundamental mechanisms to secure the airway of the very small rabbit: tracheostomy and blind intubation.

Tracheostomy is an invasive approach that has been used in both adult and newborn rabbits"

* These are not the only options: Endoscopic/fiberoptic intubation (along the side of the ETtube if small) is a method that would allow for direct visualization without surgery, and is not only practical when done correctly, but is preferable.

Following the reviewer's suggestion, we have included the concept of endoscopic intubation with some bibliographic references.

* A tight fitting face mask is another method that has been described, that has its own drawbacks but would prevent laryngeal trauma.

This concept has been also included.

"This method has been described for the adult rabbit and has proved to be safe, cheap and easy to learn"

* There is not enough data/information in this article to support what the authors are claiming here. Successful does not mean "safe". There are many complications that can arise from blind intubation that the authors have not described, nor have they proven has not happened in this case. Were the rabbits recovered after the procedure? If not, was histopathology performed on the trachea? Please discuss so that the reader can understand all aspect of this technique.

We have changed this part of the discussion, emphasizing possible complications.

"The use of 2 mm diameter endotracheal tubes, introduced no more than 3 cm, has been easy to learn and effective in ventilating small animals."

* Please cite statement as it sounds like the authors are saying it has been proven in small animals.

The statement has been changed, we had only performed this technique in kits.

* There needs to be discussion regarding whether these sizes are internal or external tube diameters.

An explanation of this has been added.

* There also needs to be some brief discussion regarding airway resistance and Hagen-Poiseuille law as it pertains to airway resistance for these very small tubes.

Some explanation has been added. We didn't have problems with tube diameter and were able to measure blood gases after 1 hour of ventilation that showed effective ventilation and oxygenation.

"With the insertion of the probe, prior to intubation, however, successful intubation was achieved in almost 100% of cases."

* This statement is biased- please be objective and indicate the exact percentage.

A better explanation of this part has been included.

* In the article and video authors discuss that 90% of animals were intubated with blind intubation, but do not elaborate on this. Please explain: how many tries did it take? How long did it take to intubate? How many times did you intubate the esophagus instead? If authors do not have this data explain why.

An explanation of this has been written in the results section.

"As elements to be considered, we introduce the tube, previously lubricated, slowly and carefully because one of the main problems during the technique learning curve is perforation of the trachea due to the friability of tissues. To solve this, we recommend not to introduce the tube beyond 3 cm and discontinuing the insertion if we find structures that impede the advance; in this situation it is preferable to remove the tube and start over"

* There are several grammatical errors/ incorrect wording in this sentence. Please re write.

Sorry for that, grammar and spelling have been corrected.

* In this statement, the authors have discussed complications they have encountered. Please discuss- did these occur in any of the 10 rabbits? Typically, the ET tube is premeasured to reach the carina, prior to the tracheal bifurcation. How many rabbits, complications, attempts, etc did it take for authors to be comfortable with this technique before coming to this 3cm recommendation?

As we explain in the text, another investigator practiced the intubations to identify optimal techniques prior to performing intubations on the 10 rabbits described in this study

* the rabbit tracheal blood perfusion is high and described as 10 fold higher during intubation. acute histopathological injury post intubation injury has been reported in rabbits, and this risk is increased significantly with blind intubation; multiple attempts would further increase this risk and should be taken into consideration if the animals are to be recovered.

This concept has been included in the protocol and discussion.

"In our experience, the advancement of the device without apparent obstacles, the fogging of the tube in spontaneous breathing, the visualization of the thoracic expansion during ventilation, as well as the maintenance of vital signs have been sufficient to corroborate the suitability of the procedure in all cases."

* Please include the use ETCO₂ as this is also helpful in determining airway placement.

Done

"In conclusion, this model of intubation in newborn rabbits that has been described is a rapid, easy and secure technique that does not require advance airway management supplies and that has a very easy learning curve"

* What do the authors mean by "advance airway management supplies"

We have changed this sentence to make it more understandable.

* As mentioned previously, the authors are oversimplifying the issues involved with blind intubation in rabbits without data supporting the safety of the technique described. Due to the current literature indicating the trauma involved with intubating rabbits, I would avoid comments like "minimal learning curve" and "easy" unless you have proof that the procedure was performed safely and atraumatically (which according to the literature in larger rabbits is unlikely).

These kind of expressions have been eliminated from the manuscript.

"We believe this technique can be easily incorporated in the armamentarium of the airway management of neonatal rabbits, specifically for those that would be later extubated."

* Were any of these animals allowed to be recovered. Please describe. Perhaps analgesics should be recommended for those requiring multiple attempts (if animals are to be recovered)?

As explained in the protocol, animals were deeply anesthetized and then euthanatized.

General recommendations:

* Authors should add objective results to abstract (ie 90%, etc).

This has been included

* Figures should be able to stand on their own. Please describe figures better.

Figures description has been improved.

* Add drugs (ketamine and xylazine), monitor equipment, ventilator to listed materials/equipment

This also has been added to improve manuscript understanding

* Some unanswered questions: Did authors perform histopathology on the trachea to determine if tracheal damage occurred. Were any of the animals recovered?

This has been explained in protocol/discussion.

* Some important but valuable information is missing, including: how many attempts before success, how many different people intubating, how much time it took for intubation, any post-operative complications, etc.

The purpose of the paper was to describe the technique, so we didn't collect some of that information during the experiment..

* Need to address/discuss the trauma and other complications that can occur with blind intubation techniques, which has been described in the literature.

This has been included in discussion.

* Some references are listed in reviewer comments but additional important reference not listed are:

1. Morgan, Thamus J and Glowaski, Maria M, Teaching a New Method of Rabbit Intubation. Journal of the American Association for Laboratory Animal Science Volume 46, Number 3, May 2007, pp. 32-36(5)

2. Phaneuf, Lise R., et al. "Tracheal injury after endotracheal intubation and anesthesia in rabbits." Journal of the American Association for Laboratory Animal Science 45.6 (2006): 67-72.

All theses references have been added to bibliography

VIDEO

Many of the statements that need corrected in the article are also mentioned in the video and will therefore need adjustment.

* Please use a more clear image for the orotracheal anatomy of the rabbit in the video

* You indicate that you failed to intubate the smallest rabbit due to its narrow airway, when this rabbit was actually larger than the smallest rabbits you have described in your discussion. The article should indicate that you did not successfully intubate rabbits under 120 grams with this technique.



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Title: ENDOTRACHEAL BLIND INTUBATION IN NEWBORN RABBITS

URL: <https://www.jove.com/v/61874/title?status=a63880k>

Improvement requires.

1. Materials Preparation: 1. Materials needed: 2mm diameter Polyvinyl Chloride (PVC) endotracheal tube, 6 French polyurethane feeding tubes, plain and wide tip dissection forceps, and sterile lubricant (Figure 1).

Comment:

- Sterile lubricant was not included in Figure 1. Please revise the picture to include it.
Sterile lubricant has been ~~also~~ included in Figure 1.

2. Anesthesia:

3. Repeat dose until negative pedal reflex is achieved.

Questions/Comments:

- Do you only using negative pedal reflex to measure the depth of anesthesia? In general, you need to use palpebral reflex, corneal reflex in addition to using pedal reflex to measure the depth of anesthesia. Please clarify.
We included all this information.

#	Time in the video	comment	Change in video required Yes/No	Change in text is sufficient Yes/No	Suggested Changes
1	01:09-01:34	The author used only pedal reflex to measure the depth of anesthesia.	No	Yes	Under text 2.3 after pedal reflex, add palpebral reflex, corneal reflex to measure the depth of anesthesia. <i>Included</i>
2	02:00-02:07	The author used pointy sharp forceps to open oral cavity during intubation procedure.	No	N/A	For future procedure, please consider using blunt forceps to open oral cavity. Sharp Forceps may injure the oral mucosa of the animal and cause trauma. <i>We have explained that in the protocol</i>
3	02:53- 03:56	The author concluded that the success rate was 90% due to smallest rabbit (110 gram) due to the narrow airway. Please clarify if the animal 's body weight has to be in minimum 125 gram in order to have successful blind intubation procedure?	No	Yes	Please clarify or add sentence under Representative results that you need minimum of body weight 125 gram in order to have 100% success rate? <i>A sentence has been included in "results"</i>