

22 - ORIGINAL ARTICLE TECHNICAL SKILL

Perioperative care in an animal model for training in abdominal surgery. Is it necessary a preoperative fasting?¹

Cuidados perioperatórios em modelo animal de treinamento em cirurgia abdominal. É necessário jejum pré-operatório?

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ABSTRACT

PURPOSE: Demonstrate that the rabbit may be used in the training of surgery, in addition to present its perioperative care.

METHODS: Thirty two animals, with age and weight, respectively, from 3 to 5.5 months old and 3000 to 4200 grams, were undergone different periods of pre-operative fasting, exclusive intramuscular anesthesia (ketamine+xylazine), laparotomy with total gastrectomy and total splenectomy. It was dosed the pre-operative (initial) and post-surgical (end) serum blood glucose, in addition to quantify the gastric content after the resection of the part.

RESULTS: The anesthetic-surgical procedure presented a mortality rate of 3.125% (1:32) and a morbidity rate of 6.25% (2:32). It was evidenced an initial mean blood glucose = 199.4 mg/dl and the end = 326.1 mg/dl. In spite of extended fasting (minimum of 2 hours for the absolute fasting and maximum of 8.5 hours for liquids, and 20.5 hours for solids) all animals presented at the end of the surgical procedure any gastric content and a blood glucose increase. Those with fasting for liquids and solids when compared to the quantity of solid gastric content, presented a moderate negative degree of correlation.

CONCLUSION: The rabbit is a good model to be used in training of surgery, with a low morbi-mortality, able to be anesthetized intramuscularly, with no need of pre-operative fasting and does not present hypoglycemia even with the extended fasting period.

Keywords: Animal Experimentation. Surgery. Fasting. Blood Glucose. Rabbits.

RESUMO

OBJETIVO: Demonstrar que o coelho pode ser utilizado no treinamento em cirurgia, além de apresentar seus cuidados perioperatórios.

MÉTODOS: Trinta e dois animais, com idade e peso respectivamente, entre 3 a 5,5 meses e 3000 a 4200 gramas, foram submetidos a variados tempos de jejum pré-operatório, anestesia intramuscular exclusiva (quetamina+xilasina), laparotomia com gastrectomia e esplenectomia totais. Dosou-se a glicemia sérica pré-operatória (inicial) e pós-cirúrgica (final), além de quantificado o conteúdo gástrico pós-gastrectomia. **RESULTADOS:** O procedimento anestésico-cirúrgico apresentou taxa de mortalidade de 3,125 % (1:32) e morbidade de 6,25 % (2:32). Evidenciou-se glicemia média inicial = 199,4 mg/dl e final = 326,1 mg/dl. Apesar de jejuns prolongados (mínimo de 2 horas para jejum absoluto e máximo de 8,5 horas para líquidos, e 20,5 horas para sólidos) todos os animais apresentaram no final do procedimento cirúrgico algum conteúdo gástrico e aumento da glicemia. Aqueles com jejum para líquidos e sólidos quando comparados com a quantidade de conteúdo gástrico sólido e total apresentaram grau moderadamente negativo de correlação linear.

CONCLUSÃO: O coelho é um bom modelo para ser utilizado em treinamento de cirurgia, com baixa morbimortalidade, passível de ser anestesiado por via intramuscular, sem necessidade de jejum pré-operatório e ausência de hipoglicemia.

Descritores: Experimentação Animal. Cirurgia. Jejum. Glicemia. Coelhos.

Introduction

In the last 10 years, the medical science has been noticing even more the potential of the white rabbit (*Oryctolagus cuniculus*) of the New Zealand lineage¹ as an experimental animal model for the research in video-surgery, general surgery, orthopedics, gynecology, vascular, craniofacial and urologic²⁻¹⁶. However, there are few information with a good scientific evidence, regarding the perioperative care^{2-15,17}.

To discuss about the need of the pre-surgical fasting for the rabbits that will be submitted to any anesthetic-surgical procedure, we need to expose some particularities.

The rabbit is a monogastric herbal animal that has a hypocontractile stomach, with thin walls composed of a weak muscle (exception to the pyloric area)^{18,19}. As a consequence, the food itself pushes the gastric content up to the pylorus, where it is gradually injected by the small bowel by strong contractions, not being possible to occur vomit reflex^{2,17}.

In addition, it has the habit of cecotrophy¹⁷⁻¹⁹. It takes place when the animal intakes directly from the anus the cecotropes (pellets constituted by a high index of proteins and vitamins, formed after the bacterial fermentation occurred in the cecum)^{1,17}. Cecotrophy therefore occurs mainly in the periods between midnight and 02:00 am, and again at 08:00 am¹⁸. Once the cecotropes are consumed directly from the anus, the habitation on a fenestrated bottom does not hinder the cecotrophy^{1,18}. The time of permanence of the food bolus in the stomach varies from 3 to 6 hours, and, even with a previous fasting of 24 hours, the food still remains inside the stomach¹⁹.

In relation to the maintenance of its daily serum glycemic rates, we have the range of the normal settled between > 75.5 to < 150 mg/dl^{16,21}. Although the scarce literature commenting this theme^{17,21,22}, the rabbit is an animal that, under stress, frequently suffers an increase of the blood glucose levels by sympathetic discharge. Thus, in the occurrence of any cardiac stress, transportation, containment, presence of hypothermia, hemorrhage, type of euthanasia technique (for instance, beheading), fear and/or pain, will evolve with the hyperglycemia^{21,22}.

There are many advantages that recommend the rabbit as an experimental animal model². Among them, we have the easy handling. However, we must remember its particularity related to the bone fragility prone to fractures of limbs and high likelihood of cervical dislocation with consequent death, when handled incorrectly. Thus, in order to avoid injuries, we should take the animal by the skin of the dorsal-neck area with support for the weight of its body, avoiding that it is squirming or kicking with

their back legs, creating a tonic stillness^{2,17}. Another way to take it would be catch it with only one hand, putting it packed in our upper limb, and it must be remembered that it is a position that must be used for a short time, because it is stressful to the animal¹⁷.

For abdominal surgical procedures through laparotomy, it was proposed in several studies different anesthetic protocols for many isolated or combined routes: inhalation, endovenous, epidural and intramuscular, depending on the effects related to the anesthetic used and the physiological and behavioral parameters related to the rabbits^{2,4-15,17,22-24}.

Often rabbits are considered difficult to anesthetize^{3,23,24}, particularly for problems related to endotracheal intubation, due to the small size and shape of its oral cavity, making it difficult to view the larynx, especially for the long distance between incisors teeth and epiglottis, a trend to laryngospasm and respiratory arrest^{17,24}. Therefore, some authors suggest the execution of an intubation without viewing the glottis²⁴, or guided by fibroscopy³, or simply the exclusive execution of tracheostomy^{3,23,24}. Still, we must highlight that there is a high risk because of these characteristics, for the occurrence of glottis edema during the intubation process, due to the common incidence of local trauma, which could generate the need for early euthanasia²⁴.

The execution of euthanasia is a common practice, and it is important that, in addition to be fast, it is free from stress and pain. Methods of euthanasia may impact the study results, either directly with qualitative or quantitative changes of a parameter to be measured, or even generating histological changes, or indirectly, due to the circumstances associated to the technique, handling and exposure to other animals killed²². Most studies adopts as a technique of euthanasia the injection of overdose of barbiturates, for instance, pentobarbital sodium^{2,7,9,10,23}, or injection of potassium chloride^{6,8}, up to a recognition of the animal's death, through the absence of heartbeats, breathing movements and reflexes.

Through a prospective, consecutive study, it was conducted many periods of preoperative fasting, immediate preoperative blood glucose dosing and at the end of the abdominal surgery procedure, in addition to the execution of a anesthetic and euthanasia technique protocol proposal and euthanasia, with the purpose to demonstrate the perioperative handling of the rabbit and its advantages as an animal model for training in abdominal surgery.

Methods

Thirty-two white rabbits (*Oryctolagus cuniculus*) of the New Zealand lineage, male, weighing less than or above 3000

grams, healthy, provided by a single breeder (Granja Grota Azul - Campinas/SP), with a conventional pattern of sanitary breeding, light / dark cycle of 12 hours, kept in unit cages of galvanized mesh, with a fenestrated bottom and dimensions of 50x45x40 cm, with feed intake (140 grams/day/animal) and water *ad libitum*, were undergone to many periods of fasting preoperatively to water from 2 to 8.5 hours and for food (solids) from 2 to 22 hours. Then, there was the dosage of pre- and post-operative blood glucose levels, through serum blood glucose meter (OneTouch®-UltraMini).

Laparotomy with total gastrectomy and total splenectomy, in block, the opening of the stomach resected for evaluation of gastric content, anesthesia technique and standardized euthanasia. It was assessed the gastric content through the identification of solid constituents and weighing on a digital scale of high precision, in addition of the quantification in milliliters of liquid contents drained by the orogastric probe through a millimetered transparent bottle.

Transportation, handling and restraint - Animals in number from 3 to 5, were taken from their respective cages in the creator before 8 am and transported to the Surgical Technique and Experimental Surgery Laboratory, Medical Sciences School, UNICAMP-SP in plastic cages fenestrated on the sides and covered with a lid, with dimensions of 60x40x30 cm, for about 15 minutes. Upon arrival at the laboratory, they remained in galvanized and plastic cages (on the sides and at the bottom), with dimensions of 60x50x30 cm, in an environment with reduced light, silent, with a room temperature about 20 to 28°C for at least 40 minutes. After they were taken, one by one to initiate the anesthesia, handled and hold properly^{2,17}.

After applying the first dose of the anesthetic solution prepared (see anesthesia item), it was performed the weight of the animal, punctured access in the marginal ear vein with a needle type butterfly number (nº) 21 to 23 for blood collection in order to dose the initial blood glucose and intravenous hydration during the intra-operative procedure. Then, they were placed and contained in the supine position on the bench with the aid of ligature in its members with flexible plastic catheter of the serum.

Anesthesia

The animals were anesthetized after the fasting pre-established, through intramuscular injection of a solution prepared with ketamine hydrochloride (30 mg/kg) and xylazine (3 mg/kg). We performed the trichotomy of the abdominal area and local infiltration of the abdominal wall with 5 ml of lidocaine 2% diluted in 5 ml of distilled water. For the maintenance of anesthesia during the surgery, it was provided to the animal, approximately every

30-40 minutes or earlier, if signs of anesthetic superficial plan are noted (occurrence of responses to painful stimuli, presence of eye reflexes and cardiopulmonary changes and decreased of body temperature)²⁰, of 1/2 of the initial dose of the prepared solution through intramuscular path. Throughout the surgical anesthetic procedure, it was provided, via facial mask²⁴, supplemental oxygen and venous hydration with saline. The whole process was developed in the presence of a biologist with experience in experimental surgery and anesthesia in laboratory animals, to follow the procedures.

Blood glucose level

The blood was collected for measurement of glucose levels during ear vein puncture (initial blood glucose) and immediately after the removal of the stomach and spleen together (end blood glucose).

Surgical procedure (total gastrectomy with splenectomy)

After five minutes of abdominal wall infiltration with lidocaine, it was performed a median laparotomy. After opening the cavity, the stomach was pulled and applied to the great curvature, with two points separated with the 2.0 cotton thread to repair the stomach, where this organ was pulled by the weight of the clamps type Kelly (Figure 1).

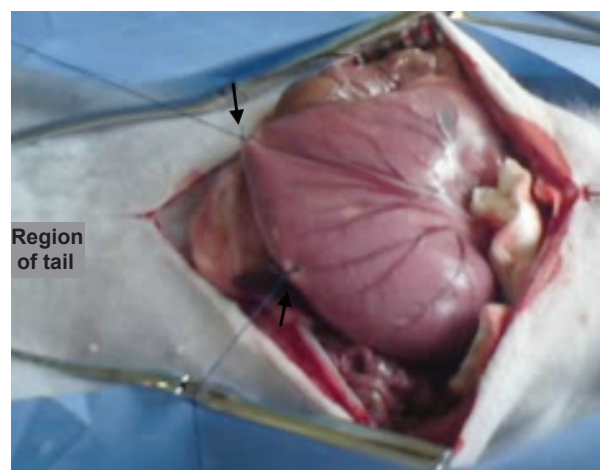


FIGURE 1 - Photograph demonstrating through the black arrows the reparation points with cotton performed in a great curvature to traction the stomach.

Twenty minutes after the laparotomy, we proceeded to the total gastrectomy and total splenectomy in block as follows: 1st Isolation and dieresis of abdominal esophagus (with a simultaneous withdrawal of the probe Levine); 2nd Clamping with hemostatic tweezers and vessels dieresis that irrigate the spleen and great curvature; 3rd Clamping with hemostatic tweezers and

vessels dieresis that irrigate the lesser curvature, 4th Dissection, isolation and link of the small intestine with pyloric dieresis of it and stomach removal in block with the spleen (Figure 2).

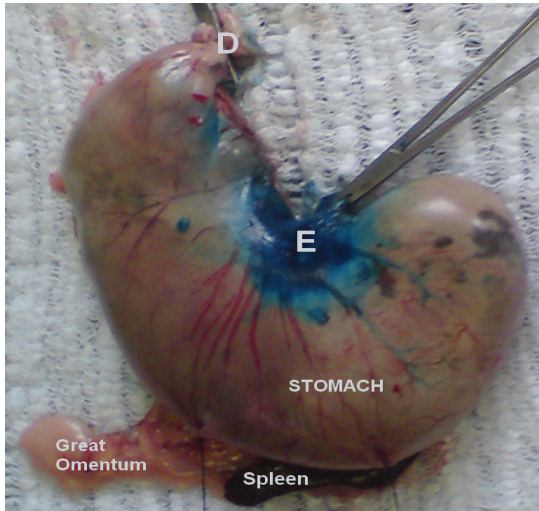


FIGURE 2 - Photograph demonstrating the surgical part resected in block. E=Esophagus; D=Duodenum.

After the surgery of the rabbit n^o. 16, associated to the surgical procedure for the next animals, the fixation of the xiphoid process with two simple cotton points 2.0, cephalad, in the chest wall, right after opening the abdominal wall, in order to avoid dissection of surrounding tissues and consequent pneumothorax, in addition to provide an improved presentation of the operative field (Figure 3).

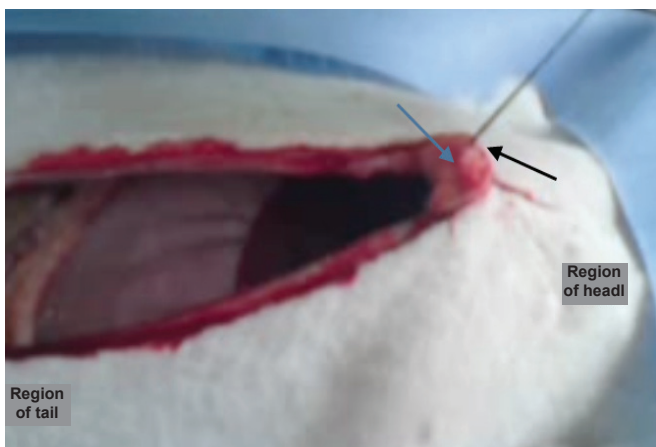


FIGURE 3 - Photograph demonstrating the fixation of the xiphoid process in the thorax wall. Black arrow identifies the point that the cotton thread penetrates the xiphoid process and the blue arrow identifies the xiphoid itself.

Euthanasia

It was performed right after the collection of blood for

final blood glucose level dosing, through the deepening of the anesthesia plan with an overdose (5 ml) of Sodium Thiopental 25 mg/ml intravenously. Later, it was infused approximately 10 ml of potassium chloride at 19.1% until the occurrence of the absence of apparent signs of life (presence of heartbeats, respiratory rates and reflexes).

This study is part of a project approved by the Ethics Committee on Animal Use-CEUA from State University of Campinas-SP, under protocol no. 2033-1.

Statistical analysis

For comparison of the measures assessed, it was used the Wilcoxon test and, in order to verify the degree of association between two variables, we used the Spearman correlation coefficient²⁵.

This ranges from -1 to 1, with values near to the extremes, indicating a higher degree of negative or positive correlation, respectively, in addition to the values near 0 represent the lack of correlation²⁵. According to Landis and Koch²⁸ when presenting values between 0.20 and 0.40, will have a regular correlation between the variables, when between 0.40 and 0.60 will have a moderate correlation, and when greater than 0.60, will have a substantial correlation.

Positive values of this coefficient will indicate a direct linear relationship, i.e., as one variable increases, the other one also increases, and negative coefficients will indicate an inverse relationship, i.e., when one variable increases, the other one decreases^{25,26}.

The statistical calculations were performed with the aid of the softwares Microsoft Excel 2002, SPSS 15.0 for Windows Evaluation Version and SAS System for Windows 9.2.

It was taken into account in this study the p value < 0.05 statistically significant.

Results

The sample of 32 rabbits presented in the age range from 3 to 5.5 months, mean = 4.578 (Standard deviation, SD = 0.7417), and body weight from 3000 to 4200 grams, mean = 3407.81 (SD 331.689).

The anesthetic surgical procedure performed in the study had a mortality rate of 3.125%, expressed by the death of an animal, which occurred during the laparotomy, 15 minutes after the completion of repair points in the stomach. In addition, it presented a morbidity rate of 6.25% (2:32). This expressed by the occurrence of shouting in an animal during the intramuscular

application of an anesthetic solution and a left pneumothorax case (rabbit nº. 16), in which a thorax drainage was performed in water seal system, and you can finish the whole procedure without other complications (Table 1).

TABLE 1 - Distribution of rabbits in accordance to the fasting time, initial and end glucose blood value, quantity of liquid, solid and total gastric content.

N	FASTING (hours)		GLUCOSE BLOOD (mg/dl)		CONTENT GASTRIC		GASTRIC CONTENT TOTAL (Liquid + solid)*
	Water	Solid	Initial	End	Liquid (ml)	Solid (grams)	
1	2	2	243	350	12	218.7	230.7
2	3	3	187	231	2	132.5	134.5
3	3.5	3.5	271	544	4	184.1	188.1
4	5	5	254	382	4	114.5	118.5
5	2	19	171	296	10	156.5	166.5
6	5	22	176	290	2	37	39
7	2.5	12.5	147	325	27	77.2	104.2
8	2.5	12.5	204	310	20	75.3	95.3
9	4.5	14.5	190	420	5	77.1	82.1
10	2	13	128	400	1	126.9	127.9
11	2	13	277	368	20	138.3	158.3
12	2.5	13.5	216	190	5	124.3	129.3
13	3	14	218	340	0	176.1	176.1
14	7	19	156	---- **	1.2	46.4	47.6
15	7	19	182	232	20	30.8	50.8
16	7.5	19.5	138	161	30	14.7	44.7
17	8	20	219	336	40	27	67
18	8.5	20.5	183	267	13	64	77
19	2	13	257	470	0	120.4	120.4
20	2	13	165	199	65	65.5	130.5
21	3	14	151	361	48	121	169
22	3.5	14.5	250	390	54	103.7	157.7
23	4	15	156	269	60	109	169
24	2	13	188	290	30	56.8	86.8
25	2.5	13.5	181	274	60	80.3	140.3
26	3	14	184	309	15	80.2	95.2
27	3.5	14.5	284	324	15	45.6	60.6
28	4	15	196	450	25	79.7	104.7
29	2	13	209	400	60	167.4	227.4
30	2.5	13.5	196	320	60	64.7	124.7
31	3	14	201	380	35	175.7	210.7
32	3.5	14.5	158	232	10	37	47

* = Trying to define the total gastric content as close to reality as possible, knowing that the density of water at 20°C, major constituent of the drained contents through the orogastric probe, is approximately 1 g/ml, it was taken into account that 1 ml of gastric fluid is approximately equal to 1 gram²⁷. Thus, we performed the conversion of the amount of fluid drained by the probe in milliliters to grams, following this principle, resulting in a total estimate of gastric contents.

** = It represents the occurrence of the death during laparotomy, making it impossible to measure this value.

In accordance to the data exposed in Table 1, trying to verify if there is a correlation among the variables (fasting, blood glucose, gastric content), we applied the Spearman Correlation (ρ)^{25,26,28} represented through the results disclosed in the Table 2.

TABLE 2 - Spearman correlation coefficients (ρ) between the fasting time and the gastric content.

	F Water	F Solid
CG Liquid (ρ)	-0.09262	0.08027
Correlation grade	Weak negative	Weak positive
P	0.6141	0.6623
CG Solid (ρ)	-0.54562	-0.57506
Correlation grade	Moderate negative	Moderate negative
P	0.0012	0.0006
CG Total (ρ)	0.37961	0.47664
Correlation grade	Weak positive	Moderate positive
P	0.0321	0.0058

Note: CG = gastric content; J = Fasting; N = 32.; p = statistical significance; Prob > |r| under H0: $\rho = 0$

Regarding the glucose blood, the animals presented the surgical procedure performed, an increase statistically significant of glucose blood ($p < 0.0001$), with an initial mean glucose blood = 199.4 mg/dl and end mean glucose blood = 326.1 mg/dl (Table 3).

TABLE 3 - Descriptive analysis and initial and end glucose blood comparison.

Variable	N*	Mean	SD	Minimum	Median	Maximum	p-value**
GLI Initial	31	199.4	41.6	128.0	190.0	284.0	
GLI Final	31	326.1	85.5	161.0	324.0	544.0	
DifGLI	31	126.8	74.2	-26.0	117.0	273.0	<0.0001

Note: N = number of sample considered; GLI = glucose blood; DifGLI = difference calculates by the subtraction GLI end - GLI initial; DP = standard deviation; *In N, it was not considered the animal n°. 14, as it has evolved to death during laparotomy, making it impossible to dose the final glucose blood; **Regarding the Wilcoxon test for the samples related.

Discussion

The sample consisted of young or early adulthood rabbits. The cost of these animals was, on average, R\$ 36.00

(approximately R\$ 12.00 per kilogram of body weight). It was presented as an affordable animal, easy transportation, handling, sweet and with a low morbid-mortality during the study.

It was found that the rabbit, in spite of the fast, always remained with some gastric content, as shown in Table 1, as reported in the sparse indexed literature^{18,19}.

It is believed that this fact should occur due to the continual habit of cecotrophy and ingestion of hair, that the rabbits have, regardless living in cages with fenestrated bottoms, because the cecotrophy occurs from an anal reflex after the arrival of the cecotropes in the anus, and the animal licking the area intakes it without them falling on the cage's floor. Another fact is also the habit of eating one's own body hair, although it is more common in pregnant female¹⁷, it was found traces of them constituting the gastric contents in all the study animals.

After analyzing the results, it was observed through the Spearman correlation coefficient that, with a statistical significance, fasting for liquid and solid presented a negative moderate linear correlation, it means, a moderate tendency for the larger times of fasting (for liquid = $\rho = 0.545$ and solid = $\rho = 0.575$) the lower the amount of solid gastric content measured. However, no statistically significant change in the amount of liquid content inside the stomach.

In addition, it is believed that in relation to total gastric content, in a justification for presenting a moderately positive correlation ($\rho = 0.476$), i.e., with the extended fasting presenting a trend to increase its content, it was believed that the animal undergoing long periods of fasting presented a trend of a higher intake of fluids while it has access to water, possibly in an attempt to decrease its hungry feelings.

Regarding the blood glucose, it was statistically significant ($p < 0.0001$) the increase in the initial blood glucose values measured in relation to the end blood glucose values. Its increase confirms what the current literature says, it means, under stress (fear, pain and surgical trauma), the animal becomes hyperglycemic with values at least greater than 147 mg/dl^{16,17,21,22}. In addition, it is worthwhile to highlight that all animals in the sample, in spite of being subjected to prolonged fasting times, showed no episodes of hypoglycemia (glucose blood < 75 mg/dl²¹).

Regarding the protocol of intramuscular anesthesia used in this study, it is believed that it is feasible, viable, cheap, easy to use, that has as the main complication the respiratory arrest of the animal. All animals, except the animal that died, received at most an extra dose (1/2 of the initial dose) for the maintenance of the anesthesia. In the case of the animal that died due to an

idiosyncratic response, it required 3 extra doses of anesthetic prepared solution, his death occurring due to the respiratory arrest right after the infusion of the last dose of anesthetic. However, before the entire sample, it was considered an acceptable mortality rate.

In relation to the surgical procedure (total gastrectomy and total splenectomy), it is important to highlight that the rabbit has an anatomy of easy identification, with humanoid similarities that can be used in other experiments, especially those related to the vascular surgery and digestive tract, in addition to the cephalic fixation of the xiphoid process in the chest wall to have assisted greatly in the presentation of the operative field and have decreased the incidence of iatrogenic pneumothorax.

Regarding the technique of euthanasia, it was fast, with a length time below 10 seconds, without the need for doses higher than 10 ml of potassium chloride 19.1% in order to finish the act.

Conclusion

The rabbit is an experimental animal model feasible to be used in training in surgery, with many advantages such as: accessible cost, easy to handle, sweet, low morbid-mortality, likely to be anesthetized via intramuscular, in addition not to have any trend to hypoglycemia and does not require preoperative fasting for the execution of abdominal surgical procedures.

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