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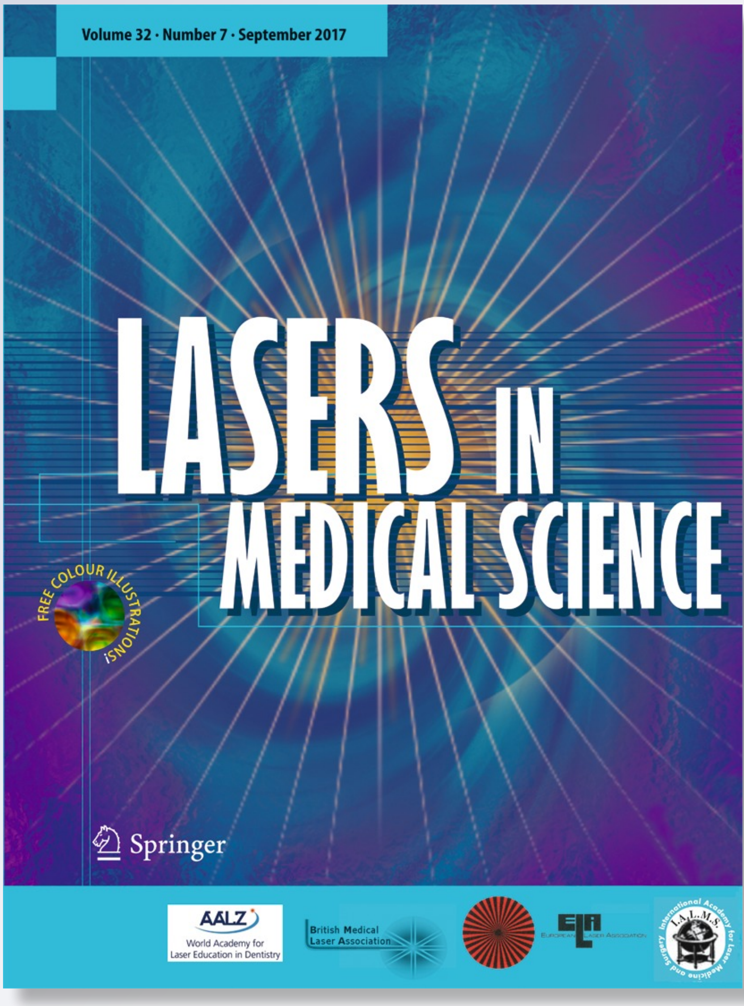


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ORIGINAL ARTICLE

# Application of phototherapy for the healing of the navels of neonatal dairy calves

Ana Lúcia Borges de Souza Faria [1] · Luis Augusto Lupato Conrado [1] · Luiz Sergio Vanzela [2] · Antonio Balbin Villaverde [3,4] · Egberto Munin [3,4]

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**Abstract** The present work evaluated the effects of LED light irradiation on the healing of the navels of neonatal dairy calves. Fifty-seven neonatal calves were divided into two groups. Animals had their umbilical stumps immersed in 10% iodine tincture for 60 s, and this process was repeated every 24 h for three consecutive days. The 29 animals in the first group did not receive LED therapy. The 28 animals in the second group received LED light irradiation at 640 nm with 300 mW power, 46.8 J/cm$^2$ energy density, 60 s irradiation time, and 0.385 cm$^2$ spot size. The animals were irradiated at four points (46.8 J/cm$^2$ per point) evenly distributed around the insertion site of the umbilical stump every 24 h for three consecutive days. Irradiation with LED light was applied before the umbilical stumps were immersed in the iodine solution. The time after birth at which the umbilical stump fell off of each calf was noted. The umbilical stumps of all animals fell off by the 25th day of age. After the umbilical stump fell off, the healing of the remnant wound was followed up to the 30th day after birth. The area of the wound was measured on the 15th, 20th, and 25th day after birth using digital photographs and computer-assisted area measurements. A two-tailed unpaired *t* test was applied to analyze the falling off the umbilical stump, whereas a Kruskal-Wallis one-way ANOVA test with a Dunn's multiple comparison test was used for the wound size evolution. GraphPad Prisma 5.0® and GraphPad StatMate 2.00® were used for the statistical analysis. The results revealed that phototherapy hastened the falling off the umbilical stump, accelerated navel healing, and reduced the mortality rate in newborn calves. Therefore, this study introduced a preventive and adjuvant after birth treatment that proved to be effective in reducing the incidences of omphalitis and newborn mortality.

**Keywords** Omphalitis · Wound healing · Umbilical diseases · Navel · Phototherapy

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## Introduction

The incidence of mortality during the first year of life in cattle is greatest during the neonatal period (i.e., the first 28 days after birth). Omphalitis is a significant risk factor for calf mortality in Brazil, which has a bovine population over of 190 million. The main causes of this umbilical disease are environmental, sanitary, traumatic, bacterial, and congenital factors that, alone or in combination, cause inflammatory and/or infectious disorders in the structures of the navel [1, 2].

Bacterial infections can ascend from the umbilical vessels and the urachus, and they may evolve into acute or chronic joint pathology with septicemia, meningitis, uveitis, hepatic abscesses, or endocarditis. In newborn farm animals, particularly calves, infections of the umbilicus and its associated structures are often correlated with the level of contamination of the environment in which the calf was born and lived during the first days of life as well as with the failure of passive-immunity transference and the delayed treatment of the navel [2, 3].

The efficacy of phototherapy using red or near-infrared light delivered by laser or LED light sources has long been known [4–6]. Tissue irradiation using either monochromatic laser light or narrow-band LED light has been proven to induce angiogenesis [7–9], increase local tissue perfusion [10, 11], and induce cellular proliferation [12–14], which ultimately leads to improved wound healing. Phototherapy with low-power light sources has also been demonstrated to exert anti-inflammatory effects [15–19] and, when applied to wound healing, induces the deposition of collagen fibers [20–22], which provide support for new tissue formation. Phototherapy with lasers or LEDs is being used in the management of muscle strength loss and fatigue in healthy active adults, young athletes, and elderly people [23–25]. Furthermore, light radiation alone also has an antimicrobial effect because it can excite intrinsic microbial photosensitizers that mediate the production of reactive oxygen species, kill microorganisms and enhance the immune response to bacterial infections [26–28].

The present study was aimed to investigate if the early preventive treatment of the navel using LED therapy would hasten the cicatricial process and the closure of the umbilical wound and thus reduce the time that newborn animals with critical umbilical wounds are exposed to infectious agents, which, in turn, would reduce the incidence of omphalitis and the risk of mortality. Controlling the incidence of umbilical infections depends on enacting preventive measures during the navel healing period. Such preventive practices are low-cost initiatives that avoid high costs in the future.

Therefore, the present study evaluated the efficacy of the application of red LED light phototherapy to the healing of the navels of newborn cattle. The hypotheses considered in this study are the LED light therapy hastens the falling off the umbilical cord stump and LED light therapy accelerates the closure of the umbilical wound of neonatal dairy calves.

## Materials and methods

The present investigation employed a sample population of 57 newborn bovines (28 males and 29 females). The study was conducted on a private farm in the city of Indiaporã, São Paulo State, Brazil (latitude 19° 58′ 48″ south and longitude 50° 17′ 23″ west with an elevation of 440 m). The genetic compositions of the animals varied; all animals were derived from lineages suitable for dairy production, but they had no specific race. The ongoing production system of the farm is semi-intensive in that the calves remained with their mothers for 6 h per day to allow them to ingest colostrum. Then, the calves were separated from their mothers and placed in paddocks in which they spent the rest of the day. The research project was approved by the University Camilo Castelo Branco (UNICASTELO, São Paulo, Brazil) board for ethics in animal research under protocol number 1-00032/2012.

A summary diagram is presented in Fig. 1 showing the different steps of the study.

## Group assignment

The calves were divided alternately into two experimental groups after birth, without any of them being excluded. The

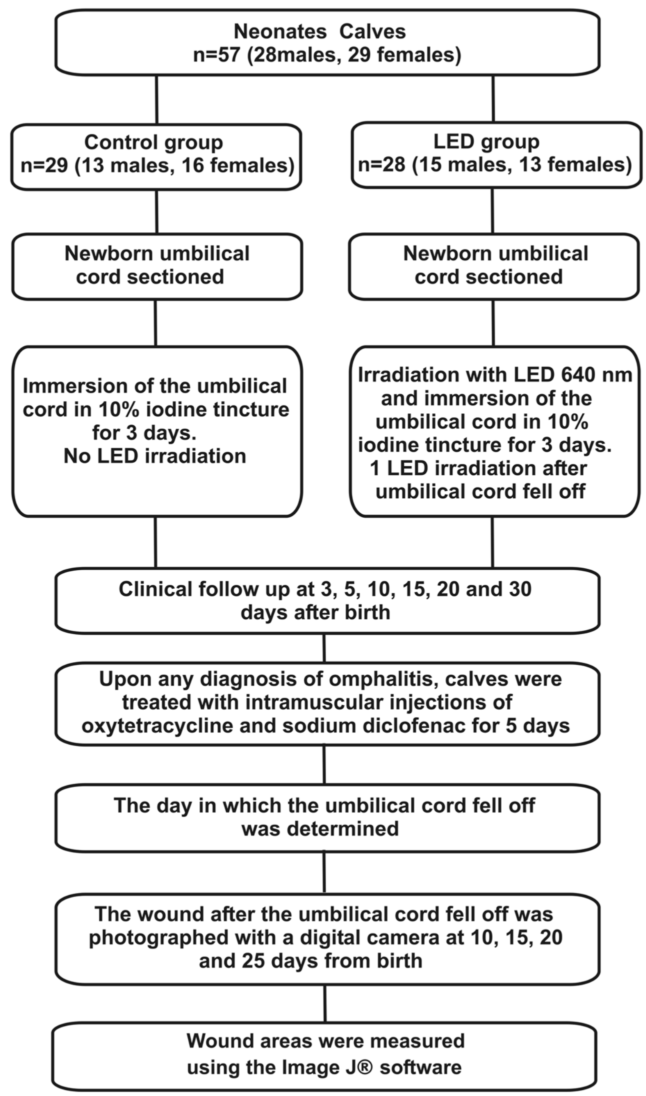


**Fig. 1** Flowchart of the study


**Fig. 2** Irradiation around the insertion point of the umbilical cord stump

control group consisted of 29 neonates (13 males and 16 females), and the LED-treated group consisted of 28 neonates (15 males and 13 females). Immediately after birth, the umbilical cord of each animal was sectioned at approximately 5 cm from the point of its insertion into the body using stainless-steel surgical scissors (Model R663-18D, Richter, São Paulo, SP, Brazil) that had been disinfected using 70% ethanol (Santa Cruz. Guarulhos, SP, Brazil). All animals from both study groups had the region around the insertion point of the umbilical cord shaved. This procedure improved the light penetration into the target tissue of the light-treated group while maintaining similar environmental exposure conditions for both groups. Sequentially numbered earrings were affixed to the calves for identification purposes.

## Experimental protocols

The animals in the control group received the conventional treatment that is employed on the farm, i.e., the immersion of the umbilical cord in a 10% tincture of iodine contained in a beaker for 60 s. In contrast, the animals in the LED-treated group were exposed to irradiation with red light from an LED source (Bios Therapy II, Bios Equipamentos Médicos, São José dos Campos, Brazil) on the region around the insertion point of the umbilical cord before the cord was immersed in the tincture of iodine. The procedures for both groups were repeated every 24 h for three consecutive days after birth. The LED light was applied at four symmetrically distributed points around the insertion point of the umbilical cord with the tip of the light guide in contact with the animal's skin at a 90° angle. Figure 2 visually illustrates the irradiation site. The LED spectrum emission was centered at a wavelength of 640 nm with a bandwidth of 40 nm (FWHM), and the amount of power delivered at the tip of the 7 mm diameter (0.385 $cm^2$), flat-ended light guide was 300 mW. The irradiation time per point was 60 s, which yielded a dosage of 18.0 J per point and a total dose of 72.0 J.

All animals in the LED-treated group received an additional dose of light radiation after the umbilical cord fell off. At that time, the radiation was applied in a single point at the center of the wound with the tip of the light guide in contact with the animal's skin at a 90° angle. The irradiation time was 60 s, which yielded a dosage of 18.0 J (46.8 J/$cm^2$) at that single point.

Upon any diagnosis of omphalitis, all animals, regardless of experimental group, were treated every 24 h for a period of 5 days with intramuscular injections of oxytetracycline at 20 mg/kg of body weight (oxitetraciclina 20% L.A. Biovet, veterinarian use, Jofadel Indústria Farmacêutica SA, Varginha, MG, Brazil) and sodium diclofenac at 1 mg/kg of body weight (Diclorofenaco J.A., 50 mL, veterinarian use, J.A. Saúde Animal, Patrocínio Paulista, SP, Brazil).

### Treatment evaluation

The involutions of the umbilical cord stumps or the remnant wounds were followed up clinically when the neonates were 3, 5, 10, 15, 20, 25, and 30 days old. A categorical scale was established for the evaluation of umbilical cicatrization; scores of A, B, and C were assigned based upon the following criteria: whether the umbilical wound was closed or semiclosed, whether the umbilical cord stump was present or absent, and whether the umbilicus was inflamed. Score A indicated umbilical cicatrization. To receive a score of A, the animal had to satisfy the following conditions: the absence

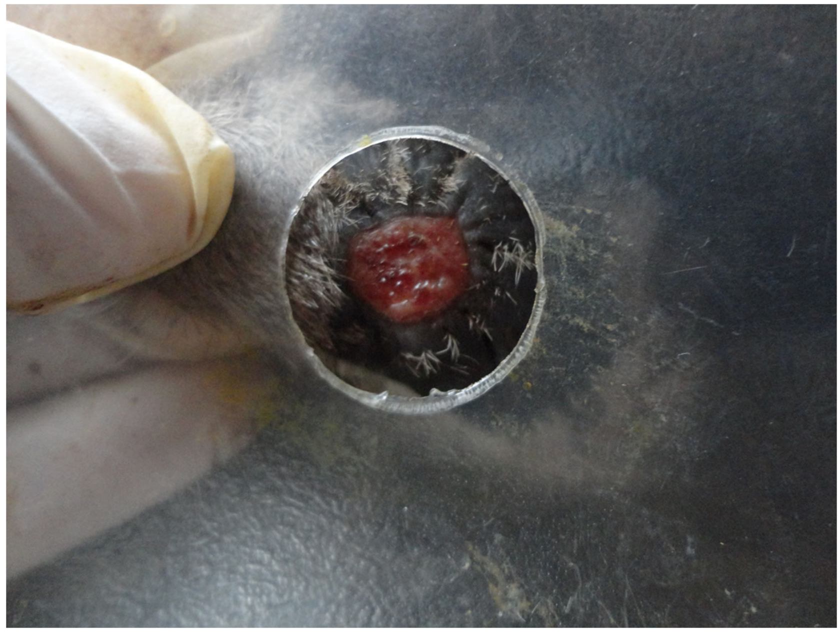

**Fig. 3** A healing wound taken through a polyethylene mask with a hole of a known dimension for computer-assisted comparative measurement of the wound area

of an umbilical cord stump and a non-inflamed umbilical site with a closed wound. Score B indicated an intermediate condition that could evolve into a categorical score of A. Score B was defined by the following criteria: the umbilical wound was semi-closed, the umbilical cord stump was present or absent, and the umbilical site was not inflamed. Categorical score C mainly indicated an inflamed umbilical site. The criteria that defined categorical score C included the presence or absence of the umbilical cord stump, a semi-closed umbilical wound when the cord was absent, and an inflamed umbilical site or umbilical cord that may or may not have contained pus.

After the umbilical cord fell off, the remnant wound was photographed with a Sony® digital camera, 16.1 Mp and 5× zoom (DSC-W570, Sony Co, Minato, Tokyo, Japan) to determine the evolution of the wound area over time (10, 15, 20, and 25 days after birth). For this task, a sheet of polyethylene with a punched hole of known area in its center was placed over the wound as a mask (Fig. 3). The hole in the sheet provided a reference for the comparative determination of the wound area through image analysis. The area of the hole was 3.93 $cm^2$. The ImageJ® software (National Institutes of Health, Bethesda, MD, USA) was used for the image-based area measurements.

**Table 1** Absolute numbers and percentages of animals from which the umbilical cord stumps had fallen off in the LED-treated and control groups at different times

| Group | Days after birth | | |
|---|---|---|---|
| | 0–15 *n* (%) | 16–25 *n* (%) | 0–25 *n* (%) |
| LED treated | 19 (67.9) | 11 (32.1) | 28 (100) |
| Control | 10 (34.5) | 18 (65.5) | 29 (100) |

## Statistical analysis

A parametric two-tailed unpaired *t* test was applied for the comparison of the umbilical cord fall off times between the LED-treated and control groups. The umbilical wound healing dynamics for both experimental groups were studied using a three-variable statistical analysis, i.e., time, the wound size of the control group, and the wound size of LED group were examined with a Kruskal-Wallis test (non-parametric ANOVA) followed by a Dunn's multiple comparisons post

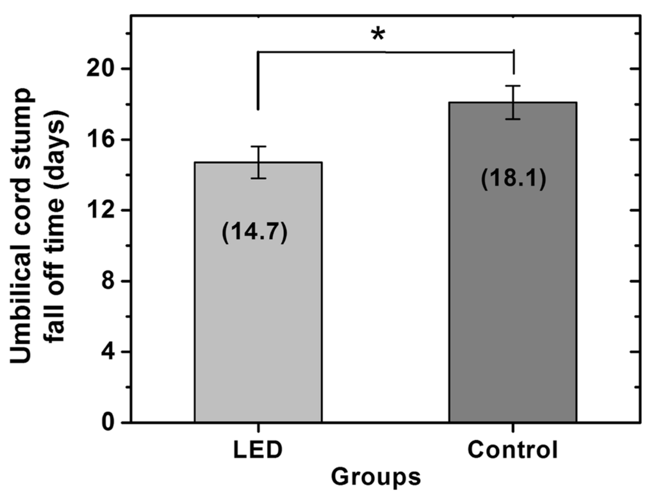


**Fig. 4** Mean numbers of days required for the umbilical cord stumps to fall off in the irradiated and non-irradiated groups. The results are expressed as the means ± the SEMs. *$p < 0.05$ between groups

**Table 2** Absolute numbers and percentages of animals with categorical scores of A (closed umbilical wound), B (non-inflamed, semi-closed wound or the presence of the cord stump), and C (inflamed semi-closed wound or the presence of the cord stump)

| Group | 15th day after birth | | | 25th day after birth | | | 30th day after birth | | |
|---|---|---|---|---|---|---|---|---|---|
| | A<br>*n* (%) | B<br>*n* (%) | C<br>*n* (%) | A<br>*n* (%) | B<br>*n* (%) | C<br>*n* (%) | A<br>*n* (%) | B<br>*n* (%) | C<br>*n* (%) |
| LED treated<br>($n = 28$) | – | 25<br>(89.3) | 3<br>(10.7) | 12<br>(42.8) | 15<br>(53.6) | 1<br>(3.6) | 27<br>(96.4) | 0<br>(0) | 1<br>(3.57) |
| Control<br>($n = 29$) | – | 26<br>(89.6) | 3<br>(10.4) | 7<br>(24.1) | 16<br>(55.2) | 6<br>(20.7) | 20<br>(69.0) | 6<br>(20.7) | 3<br>(10.4) |

hoc test to identify the pairs of variables that exhibited statistically significant differences. The GraphPad software (GraphPad Software Inc., La Jolla, CA, USA) Prisma 5.0® and StatMate 2.00® were used for the statistical analysis and the test power determination. By choosing $\alpha = 0.05$, test powers of 80% or higher was obtained, depending on the comparison test that was studied.

## Results

To study the fall off dynamics of the umbilical cord in both the control and LED-treated groups, the animals were observed daily until the 25th day after birth at which point the umbilical cord stumps had fallen off all of the animals. Table 1 provides the percentages of animals from which the umbilical cord stumps had fallen for two time periods, i.e., 0–15 and 16–25 days of age. From this table, it can be observed that the umbilical cord stumps fell off earlier in the LED-treated group than in the control group. For example, during the first 15 days after birth, the umbilical cord stumps had fallen off 67.9% of the animals in the LED-treated group and only 34.5% in the control group; thus, the difference was twofold. From day 16 to day 25, the umbilical cord stumps had fallen off the remaining 32.1% of the animals in the LED-treated group and the remaining 65.5% of the animals in the control group. Figure 4 displays the mean numbers of days at which the umbilical cord stumps fell off in the irradiated and non-irradiated groups. Parametric statistical analysis using a two-tailed Student's *t* test ($\alpha = 0.05$, test power = $1 - \beta = 80\%$) revealed a statistically significant difference between the two groups ($p < 0.01$); the umbilical cord stump fell off earlier when the animals received LED light irradiation.

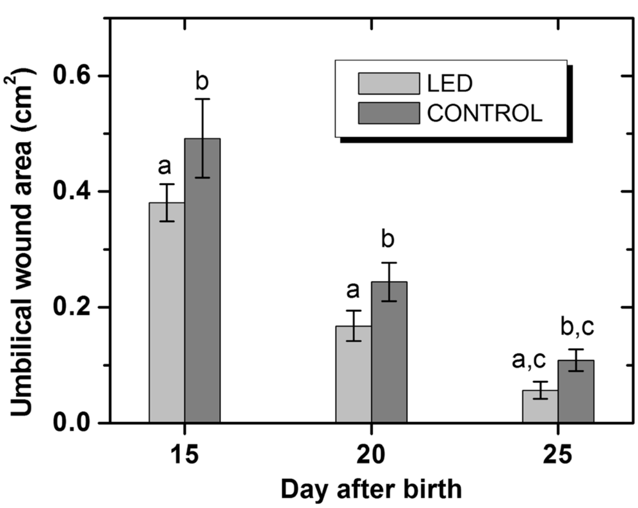


**Fig. 5** Umbilical wound healing dynamics after the umbilical cord stump had fallen off. The umbilical wound areas are plotted for the LED-treated and control groups at 15, 20, and 25 days of age. The results are expressed as the means ± the SEMs. Equal *lower case letters* indicate statistically significant differences ($p < 0.05$)

Table 2 presents the numbers of animals that were assigned categorical scores of A (closed wound), B (non-inflamed, semi-closed wound or the presence of the cord stump), and C (inflamed semi-closed wound or the presence of the cord stump) at 15, 25, and 30 days after birth. At 25 days of age, 42.8% of the animals in the LED-treated group exhibited a cicatrized umbilical site (score A), whereas only 24.1% of the animals in the control group presented with this condition. The numbers of animals with scores of B were very similar in the two experimental groups at the 25th day after birth; 53.6% of the LED-treated group and 55.2% of the control group received scores of B. A significant difference between the study groups was found in the calves that were assigned a score of C at the 25th day after birth; 20.7% of the animals in the control group presented with omphalitis compared with only 3.6% in the LED-treated group. At the 30th day after birth, nearly all of the LED-treated animals presented cicatrized navel wounds (with the exception of one animal that exhibited omphalitis) compared with only 69% of those in the control group. The numbers of animals with scores of B or C was greater in the control group compared with the group that was treated with phototherapy. The three animals with omphalitis at day 30 in the non-treated group died at later times, whereas the one animal with omphalitis in the LED-treated group survived.

Figure 5 displays the umbilical wound healing dynamics of both experimental groups. The mean umbilical wound areas as revealed by measurements after the umbilical cord stump had fallen off at 15, 20, and 25 days of age are illustrated for both groups. Notably, the graph indicates that the wound areas decreased over time in both groups, but the wound areas were smaller in the LED-treated group than in the control group at all experimental times. The Kruskal-Wallis non-parametric test that was applied to the three variables—time, LED group wound size, and control group wound size ($\alpha = 0.05$, test power = $1 - \beta = 80\%$) revealed a statistically significant difference among the variables ($p < 0.007$). Next, a Dunn's multiple comparisons test was performed. The results revealed that the mean umbilical wound areas present a statistically significant decrease ($\alpha = 0.05$, test power = 0.97) over time in both the LED and control groups ($p < 0.01$ for LED and $p < 0.05$ for control). Moreover, a statistically significant difference between the LED-treated and control groups ($\alpha = 0.05$, test power = 80%) was observed at 25 days of age ($p < 0.05$).

## Discussion

This study evaluated the effects of light treatment with a red LED light on the healing of the navels of newborn cattle. The results revealed that the umbilical cord stump fell off earlier in newborn cattle that received LED light treatment as illustrated in Table 1 and Fig. 4. Additionally, the remnant wounds after the cord stumps had fallen off cicatrized earlier in the LED-treated animals. At the 30th day after birth, nearly 100% of the LED-treated animals presented a cicatrized navel wound, whereas in the control group, 31% of the animals presented an umbilical wound that was still open. Other studies have reported on the beneficial effects of phototherapy on the healing of wounds with diverse etiologies [29, 30]. To the authors' knowledge, there are no previously published reports describing the application of phototherapy for navel healing in farm animals. The mechanisms by which laser or LED radiation acts on wound healing are described in many important classic articles, such those by T Karu, E Mester, W Posten, MR Hamblin, and other authors [4, 6, 7, 9, 14, 20, 22, 31–37].

The findings presented here might have great influence on cattle reproductive facilities due to the application of this technique as a preventive and adjuvant after birth treatment for newborn calves.

Good sanitization of animal quarters in farm environments is difficult to achieve, and newborns are therefore exposed to diverse conditions. Because the navel cicatrization process was accelerated by treatment with luminous radiation, the period during which the wound was exposed to infectious agents was reduced. Indeed, in this experimental study, at the 30th day after birth, three (10.3%) animals in the control group were diagnosed with omphalitis, and all of these animals died due to complications arising from this disorder. In contrast, in the LED-treated group, only one (3.57%) animal presented with omphalitis, and this animal survived. Therefore, phototherapy was effective in reducing the incidence of omphalitis and the risk of mortality in newborn calves.

The occurrence of umbilical myiasis was observed in an animal in the control group. A single dose of LED light irradiation (640 nm, 18.0 J) was locally applied to this animal to assess whether the light elicited any effect on the larvae. After 24 h, the sizes of the larvae increased, and the infestation worsened. These effects were probably elicited by an increase in the local blood flow induced by the LED radiation, which delivered oxygen and nutrients and led to the greater development of the larvae. This animal was removed from the experiment.

As a routine protocol for after birth care, the farm at which the present study was conducted employs an immersion time of 60 s of the umbilical cord stump in a 10% iodine solution every 24 h for three consecutive days after birth. This practice implies that animal handling costs are routine for the farm. Because the animals are already being handled for preventive treatment with the iodine solution, the addition of a light treatment procedure to the existing protocol should not significantly increase the costs while effectively accelerating cicatrization and reducing the mortality rate of the neonates; thus, this is a cost-effective preventive treatment that is worthy of application.

The two hypotheses on which the present study is based are confirmed by the obtained data, which show faster falling off of the umbilical cord stump when calves are treated with LED light and later, faster closure of the wound left by the umbilical cord stump fall.

## Conclusion

The preventive treatment of the navel using LED light therapy hastened the falling off the umbilical cord stump and accelerated the closure of the umbilical wound and therefore reduced the time that the newborn animals with critical umbilical wound conditions were exposed to infectious agents. Consequently, the incidence of omphalitis was reduced as was the newborn mortality rate.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interests.

**Ethical approval** The research project was approved by the University Camilo Castelo Branco (UNICASTELO, São Paulo, Brazil) board for ethics in animal research, under protocol # 1-00032/2012.

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