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Original Article

Ultrasonographic monitoring of abdominal wound healing in ewes

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ABSTRACT

Objective: The present study was done to monitor the progress of abdominal wound healing after experimental laparotomy in ewes using Doppler ultrasonography.
Materials and methods: Laparotomy was performed in seven apparently healthy indigenous ewes for collection and transfer of embryos. Wound morphology was studied in terms of width of sutured area of internal wound (ventral raphae) and swelling of suture areas, width and contraction rate of external wound. Internal wound healing process was monitor at two days interval by real time B-mode ultrasonography using transabdominal (13 MHz frequency) transducer. The echoes focus or zone of best resolution was adjusted to the point of interest on the screen.
Results: The average widths of raphae wounds declined from 7.7±0.18 mm on day 0 to 0.9±0.34 mm on day 20 and were completely healed on day 22 in all ewes. The average widths of skin wound decreased to 0.36±0.14 mm on day 26 from 8.6±0.33 mm on day 0.
Conclusion: It was observed that internal wound healed rapidly than external wound.

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INTRODUCTION

A wound may be defined as damage or discontinuation to the normal anatomical structure and function (Robson et al., 2001). Wound healing is a process in which the body tissue repairs itself through the coordination of extracellular and intracellular events (Ennis et al., 2003). New tissues are formed by the wound healing process that is structurally and functionally identical to its previous state (Janis et al., 2010).

At present, several methods are available to detect wound healing progress which include dimensional, visual and physiological assessments. Accurate wound measurement techniques will help clinicians to monitor the wounds which will indirectly improve care of patients with wounds (Flanagan, 2013). A common method like the ruler method has inconsistent measurements limited by subjective interpretation and inter-observer variability (Bryant et al., 2001; Keast et al., 2008).

Ultrasound is an essential diagnostic imaging tool for the investigation of diseases of the abdominal cavity (Kofler and Hittmair, 2006; Pastore et al., 2007). This technique is safe, non-invasive and useful in many cases. It is a sensitive method for the detection of certain lesions (Kofler and Hittmair, 2006; Pastore et al., 2007).

Laparotomy is essential during multiple ovulation and embryo transfer (MOET) technique in field condition. Ultrasonography was used to monitor healing of ventral abdominal midline incisions after exploratory laparotomy in small animals (Wilson et al., 1989). More accurate diagnosis has been facilitated through the evolution of imaging techniques (Daneman and Navaro, 2009). For these reasons, ultrasonographic monitoring of wound healing is very important and relevant to the current state of applied research in ewes. To the best of our knowledge, no researches on progress of abdominal wound healing by ultrasonography been performed in Bangladesh. Hence, this research was designed for gross and ultrasonographic monitoring of the progress of abdominal wound healing after experimental laparotomy in ewes.

MATERIALS AND METHODS

Ethical statement: Considering animal welfare, aseptic wounds were made following AVMA (American Veterinary Medical Association) Animal Welfare Principles to provide minimal discomfort to the experimental ewes. Local analgesic was done before creation of the wounds to minimize pain sensation.

Experimental location: The experiment was done at operation theatre, Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh, from July to December 2016.

Experimental design: Seven apparently healthy ewes were randomly selected for creation of wound for embryo flushing and transfer. Surgical wounds (External wound and Internal wound) were then closed. The external wounds were grossly observed using slide calipers. The internal wounds were observed using ultrasonography and measured using ultrasound measuring system

Experimental animals: Seven apparently healthy ewes weighing 10-12 Kg and 2-3 years old were used in this experiment. The ewes were kept in a well-ventilated, concrete floor, tin-roofed shed located at the Animal Research Farm, Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh. The animals were kept under good veterinary supervision *ad libitum*.

The animals were dewormed with anthelmintics (A-Mectin Plus® Acme Pharmaceuticals Ltd, Bangladesh) and vaccinated against PPR (PPR vaccine® Livestock Research Institute, Bangladesh), FMD (Rakhsha, Indian Immunology Ltd., India). The ewes were maintained with roadside grass from 9:00 am to 5:00 pm and supplemented with 300 gm of concentrate once daily. The concentrate was a mixture of crushed maize (25%), wheat bran (50%), soybean meal (20%), fish meal (1%), Di-calcium phosphate (DCP) (1.5%), vitamins-mineral premix (0.5%) and iodized salt (3%).

Surgical technique: Before creation of wound all the instruments were sterilized by autoclaving. Clinical examination of ewes were performed to ensure that the animal was good enough physically and apparently free from infections or infestation. The ewes were kept in fasting condition for 24 h prior to operation. The ewes were restrained on right dorso-ventral recumbency on the operation table. All wounds were produced following paramedian laparotomy approach. The operation site of the ewes were shaved, cleaned with soap water and disinfected with 10% povidone iodine solution (Povisep®). The animals were premadicated with Xylazine hydrochloride (Xylazill-100®) dosed at 0.1 mg/Kg bwt. General anesthesia was performed with combination of Ketamine hydrochloride (G-Ketamine®) dosed at 8 mg/Kg bwt and Xylazine hydrochloride (Xylazill-100®) dosed at 0.1 mg/Kg bwt. A draping towel was used to wrap the animal body other than the operation site. An incision was given on the skin measuring 5 cm length.

Skin was separated from underlying tissues by blunt dissection. A careful incision was given to cut the fascia beneath the skin. The muscles were incised and the creation of wound was completed. After flushing and embryo transfer the muscle and fascia were closed with simple continuous pattern using chromic catgut (2-0). Skin wound was closed with simple interrupted mattress suture and reinforced surgeon's knot using silk. Sutures were placed 8 mm apart. Distance between needle placement and cutting edge was 5 mm.

Postoperative care: After suturing, antibiotics (Procaine penicillin 30 lac IU and Benzyl penicillin 10 lac IU; Pronapen®, Renata Ltd., Bangladesh), anti-inflammatory drugs (Ketoprofen dosed at 3.0 mg/Kg bwt; Ketovet®, Techno Drugs Ltd., Bangladesh), anti-histaminic (Pheniramine maleate dosed at 1.0 mg/Kg bwt; Asta-vet®, The Acme Laboratories Ltd., Bangladesh) and insect repellent spray were used. A low residue, highly digestible diet was offered within 24 h of surgery. Sutures were removed at day 9 of post-operation. These animals were kept carefully to avoid interference with granulation tissue formation.

Gross observation of wound by slide calipers: Wound contraction rate was measured from decreased width of suture area. Data were collected (mm) at two days interval until healing. Healing of wound was indicated as-
Excellent: absence of inflammation, exudation, infection, dehiscence and gradual decrease of cutting edge width;
Good: minimum inflammation and exudation, no dehiscence, gradual decreasing of cutting edge width;
Fair: presence of marked inflammation, infection and exudation.

Observation and measurement of wound by ultrasonography: The Doppler ultrasonography machine (MyLab™ Five, Esaote, Italy) was used for this study. The skin structure and wound healing process were observed at two days intervals by real time B-mode ultrasonography using micro convex transabdominal transducer (13 MHz Frequency). Ultrasound transmission gel (Aquasonic®, Parker laboratories, Inc., USA), was applied over the surface of the transducer and skin. The ultrasound transmission gel was applied to the skin. This prevents friction so they can rub the ultrasound transducer on the skin and the probe was applied beside the wound area of skin. The transducer was placed and observed the echoes on the screen. The echoes focused or zone of best resolution (gain) also adjusted to the point of interest. The zoom should be used mainly for the final investigation of detail and for preparing the documentation. The transducer was moved in a slow constant pattern, while maintaining the defined scanning plane. Transducer was held fixed when the patient moves, *e.g.*, during respiration. After fixing the image on screen width of suture was measured by ultrasound machine measurement system and data were recorded. After the procedure, the gel was cleaned from the ewes' skin. The whole procedure typically lasts less than 30 minutes.

Statistical analysis: All data were shown as mean±SEM. One way ANOVA (Analysis of Variance) factor analysis was performed to compare data among groups. The obtained data were analyzed with SPSS statistics 20.0 software. *P* value, $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The advancement of abdominal wound healing was monitored with physical examination and also Doppler ultrasonography technique. Morphological and ultrasonographic measurements of the wounds were evaluated for the experiment.

Swelling of suture areas and width of external wounds were evaluated. We recorded width of sutured area from day of operation to 29 days postoperatively with two days interval to understand the contraction of healing process. Results are presented in **Table 1**.

Table 1. Width of wound (skin and raphae) in ewes

Day	Width of skin wound (mm)	Width of raphae wound (mm)
D_0	8.6±0.33**	7.7±0.18**
D_1	10.4±0.10**	8.36±0.36**
D_4	7.8±0.41**	6.7±0.22**
D_7	6.8±0.51**	5.5±0.28**
D_{10}	5.3±0.42**	4.2±0.23**
D_{13}	4.3±0.45**	3.1±0.29**
D_{17}	3.2±0.35**	2.0±0.32**
D_{20}	2.1±0.35**	0.9±0.34**
D_{23}	1.1±0.28**	0.38±0.24*
D_{26}	0.36±0.14^{NS}	-
D_{29}	0.06±0.03^{NS}	-

***=P<1% of Significance, *=P<5% of Significance, NS= Not Significant*

It was found that width of sutured area increased at day 1 in skin wounds and then decreased gradually. Width of skin wound healing reduced significantly ($P<0.01$) in relation to healing time and the gradual decreasing of width of wounds indicates the ongoing healing process. **Figure 1** showed that average contraction rate was 0.36 mm per day in case of skin wound. Swelling was seen up to three days post-operation and later it was absent. Swelling was largest on day 1 and then significantly reduced on day 2 and day 3. Moreover, complete healing of external wound was observed on day 26. In this study, healing process was observed in each ewes to monitor the individual effect. Results are shown in **Table 2**.

There were no significant differences in swelling of suture areas (mm), average contraction rate (mm/day) and complete healing days among ewes. More swelled area (13.72 mm) was observed in ewe H-31. Greater contraction rate (0.40 mm/day) was found in ewe H-27. Shortest healing time (23 days) was recorded in ewe H-20 and H-31 and longest healing time (29 days) was observed in ewe H-02. For external wound, a dry and thin scar was considered as completely healed wound (**Figure 1**). Healing of skin wound was excellent in all ewes.

An echoic structure (almost white color) was observed on screen (**Figure 2a**) when transducer was placed on site of raphae wounds. Width of echogenic image was measured with Doppler ultrasound machine software system and results are shown in **Table 1**. As seen in skin wounds, width of raphae wound was greater on day 1 and then decreased gradually and significantly (*P<0.01*) according to time of healing. Average contraction rate was 0.34 mm/day in raphae wound which is lower in comparison to that of skin wounds. Raphae wound healed earlier (22 days) in ewes. Image of raphae wound was seen as homogenous hypo echoic (grey color) when raphae wound healed completely (**Figure 2b**). Healing of raphae wound was observed in individual ewes and results are presented in **Table 2**. Greater contraction rate (0.45 mm/day) and healing time (29 days) were observed in ewes H-28. Shortest healing time (20 days) was recorded in ewes H-20, H-24, H-27 and H-33 respectively.

The purposes of this investigation were to monitor the abdominal wound healing after experimental laparotomy in indigenous ewes. Laparotomy was performed following paramedic approach on right side of ventral abdomen. Wound morphology in term of swelling of suture areas, width and contraction rate of wound was studied. Complete healing time of both internal and external abdominal wounds was also recorded. Moreover, this is the first attempt to monitor the healing progress of internal abdominal wound with abdominal ultrasonography in ewes used for embryo collection and transfer in Bangladesh.

In this study, the increased swelling and sutured area width were noted up to day 3 postoperatively. This might result from the inflammatory response caused by the greater tissue handling needed for suture placement and foreign body reaction after suture (Runk et al., 1999). The healing process could be slowed or even exacerbate the original wound by the excessive or prolonged inflammation. Because collateral damage is caused high levels of reactive oxygen species (ROS) production by neutrophils (Novo and Parola, 2008).

Width of sutured area was recorded from day of operation (Day 0) up to complete scar formation in case of skin wounds and up to presence of hypoechoic image of monitor in case of raphae wounds postoperatively to understand wound contraction process morphologically. Results showed that width of sutured line markedly

Figure 1. Completely healed skin wound

Table 2. Swelling of skin wound area (mm), average contraction rate (mm/day) and complete healing (day) in ewes

Animal ID.	Swelling of suturing area in skin wound (mm)			Average contraction rate (mm/day)		Complete healing (days)	
	Day 1	Day 2	Day 3	External wound	Internal wound	Skin wound	Internal wound
H-02	13.52	6.32	0.48	0.36	0.29	29	26
H-20	12.85	5.76	0.68	0.29	0.28	23	20
H-24	12.64	5.48	0.91	0.37	0.45	25	20
H-27	13.23	6.58	0.43	0.40	0.38	28	20
H-28	12.48	4.33	0.24	0.39	0.31	28	29
H-31	13.72	5.43	0.63	0.32	0.44	23	20
H-33	12.77	4.61	0.41	0.37	0.24	25	23
Mean	13.03	5.50	0.54	0.36	0.34	26.00	22.57

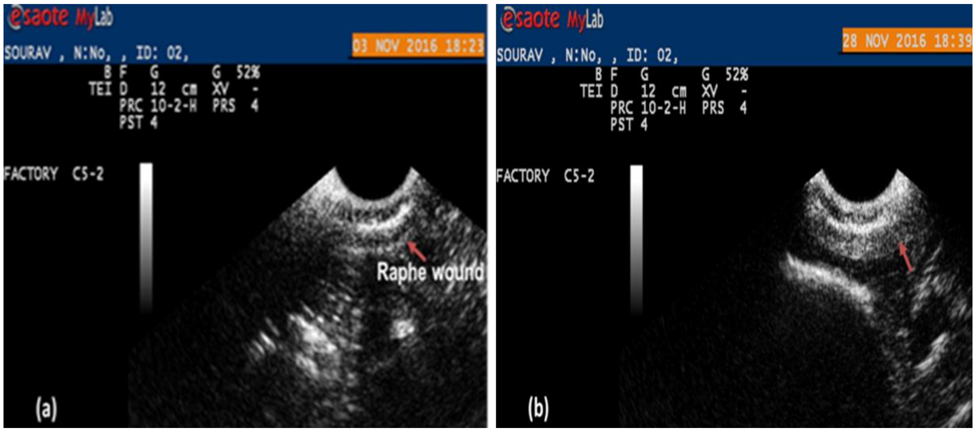

Figure 2. Ultrasonography observation of post-operative raphae wound healing; (a) Day 0 (b) Complete healing of raphea wound.

decreased from day 7 (**Table 1**) in all wounds irrespective of site. Contraction may be defined as the centripetal movement of wound edges facilitating wound closure that is maximal at 5-15 days after injury.

Micro convex probe (13 MHz) was used in this study. Areas of tissue that had high acoustic impedance (*e.g.*, collagen) were presented as white and tissue with lower acoustic impedance (*e.g.*, cellular or fluid filled) were represented as black areas in ultrasound scans. Internal muscle was highly echodense at the wound base, with a regular alignment of fibres within the bands themselves. This might be due to the marked ultrasound attenuation by the muscle connective tissue enabling the base of the wound to be identified as echo-rich bands. No edema or swelling was present in internal wound through ultrasonography. There is a limited literature on ultrasonographic monitoring of wound healing in animals. Zuki and Boyd (2003) have reported the ultrasonographic appearance of wound. They have also stated that with time the wound appeared hypo echoic with some degree of an echogenic center and casting acoustic shadowing artifact. They used linear probe with 7.5 MHz frequency. The size of the ultrasound pulse at different depths within the imaged area determines the amount of blurring and image detail.

The muscles reveal medium-level echoes with a lamellar pattern of the muscle fibers (Gokhale, 2007). We observed homogenous hypoechoic areas when raphae wound was healed completely. Similar result has been reported by Olerud et al. (1990). They had demonstrated the disordered collagen fibres of a 3-month-old scar as echo-poor image. It has been reported that the most common finding of fibrosis in muscle was disorganized hyper echoic structure (Laine et al., 1985). The disorganized hyper echoic structure is suggestive of fibrous tissue formation which is essential for the healing process in soft tissue injury. During the inflammatory phase, ultrasound has a stimulating effect on the mast cells, platelets, white cells with phagocytic roles (Li et al., 2003; Watson, 2008). Ultrasound acts as an inflammatory optimiser (Watson, 2008).

Ultrasound enhances the functional capacity of the scar tissues when applied to tissues (Tsai et al., 2011; Yeung et al., 2006). Rippon et al. (1998) have reported ultrasonography as a useful, reliable, quantifiable technique for wound healing assessment. They have also stated that the technique has allowed visualization and quantification of wound healing components such as accumulation of collagen, reepithelialisation and volume of wound.

CONCLUSION

Hence, both external and internal wound healing progress after laparotomy can be successfully monitored using ultrasonography. Abdominal wall ultrasonography can help clinicians to make the decision easier in further management of abdominal wound. Future study should be directed to identify differences in the ultrasonic attenuation coefficient of wound tissue at different time points.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTION

SM designed the research protocol and carried out surgical procedures and ultrasonographic monitoring. MH participated in ultrasonographic monitoring and drafted the manuscript. NSJ performed ultrasonographic monitoring, interpreted the ultrasonograph and contributed in critical checking and preparation of this manuscript. DSB and MS helped in ultrasonographic monitoring. MRA supervised throughout the experiment and provided valuable suggestions.

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