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Subcutaneous Implantation of Unidirectional Self-Inflating Anisotropic Tissue Expander Has No Effect on the Physiological Parameters and Behaviours of Horses

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ABSTRACT

The tissue expansion technique is one of the most important innovations in skin reconstructive surgery in human and veterinary medicine. This study investigated horses' physiological and behavioural responses to subcutaneous implantation of a unidirectional self-inflating anisotropic tissue expander. The tissue expanders were subcutaneously implanted on six horses at three different locations: the frontal region of the head, the lateral side of the right shoulder, and the dorsomedial part of the cannon region of the right forelimb. Prior to the tissue expander implantation, each horse was clinically examined, and the observed vital parameters and behaviour were recorded as baseline data. For six days post implantations, the horses were clinically examined, implantation sites monitored, and the horse's behaviour was recorded both during the day by visual observation

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and at night with a video camera. The results showed that all horses tolerated the subcutaneous gradual skin expansion by implantation of the tissue expanders, and all surgical sites healed without any complications. The implantation of the tissue expanders does not affect the vital parameters or overall horse behaviour either during the day or at night. In conclusion, the subcutaneous implantation of unidirectional anisotropic tissue expanders in horses resulted in successful skin growth with no physiological and psychological discomfort; hence skin expansion is a good option to be considered when pursuing equine skin reconstructive surgery.

Keywords: Behaviour, horse, self-inflating tissue expander, subcutaneous expansion

INTRODUCTION

In equine practice, a large wound is commonly managed as an open wound with scar formation resulting in deformation of the injured area (Theoret & Schumacher, 2017; Zöllner et al., 2012). To ensure anatomical restoration of the injured or defective site, reconstructive surgery is usually required (De Lorenzi et al., 2018; Keller et al., 1994; Whittaker et al., 2020).

The tissue expansion technique is a surgical technique to generate excess skin adjacent to a defect mechanically (Fang et al., 2013). The significance of this technique is that it allows the replacement of lost tissue with analogous tissue with matching texture, colour, and other important local features (Agrawal & Agrawal, 2012; Tepole et al., 2012). In humans, the skin expansion technique is used to manage soft tissue defects because of neoplastic resection, burns, congenital conditions, and adjacent or distant skin expansion for use as a local or free flap, respectively. In the equine clinical setting, surgical coverage of large wounds, particularly at the distal part of the horse's legs and face, cannot be easily performed due to inadequate skin for closure (Zöllner et al., 2012). Thus, healing is characterised by excessive scar formation resulting in deformation of the injured area. Although skin grafting has been used to fill the skin defect, however, tissue expansion technique has been proposed as an alternative technique to skin grafting because it is more favourable in terms of higher success rates and cosmetic results since the defect is replaced with analogous tissue with matching texture, colour and other important local features (Agrawal & Agrawal, 2012; Al-Majhali et al., 2021; Whittaker et al., 2020).

Considering the recent awareness and the potential use of the tissue expansion technique in equine reconstructive surgery, the knowledge of possible behavioural responses to gradual subcutaneous expansion to unidirectional self-inflating tissue expander implantation became necessary. Currently, no research has evaluated the behaviour of horses to tissue expander implantation. Behaviour in animal species, including horses, is one of the most readily observed measures of welfare (Budras et al., 2012; Chung et al., 2018; Mason & Mench, 1997),

and it is mostly studied by assessing the animal response to its environment or procedures. To substantiate the significance of the behavioural evidence, including physiological assessment in the behavioural study is always considered an important step (Yarnell et al., 2013).

Therefore, in this study, we investigate the effect of subcutaneous implantation of unidirectional anisotropic tissue expanders on the physiological parameters and behaviour of horses. It was hypothesised that there would be no physiological parameters and behavioural changes to horses' subcutaneous implantation of tissue expanders.

MATERIALS AND METHODS

Ethical Consideration

The study was approved by the Institutional Animal Care and Use Committee of Universiti Putra Malaysia (UPM/IACUC/AUP-R066/2018).

Animals

A total of six horses comprising four geldings and two mares were used in this study. The horses were aged 17–22 and weighed 450 to 500 kg. They were housed separately in a clean stall box (3.5 m × 4.5 m × 4 m) with good bedding and fed on hay, pelleted feed, and clean water provided ad libitum. The horses had up-to-date vaccination against equine influenza, Japanese Encephalitis, and tetanus. All the horses were from Ceremonial Mounted Squadron, Malaysian Armed Forces.

Subcutaneous Implantation of Unidirectional Self-Inflating Anisotropic Tissue Expanders

The horses were implanted with the tissue expanders (Expaniderm, Oxtex®, United Kingdom) subcutaneously at three locations: the rostral part of the frontal region of the head, the lateral side of the right shoulder, and the dorsomedial part of the cannon region of the right forelimb. All procedures were performed under the aseptic condition with the horses on standing sedation with xylazine (Chanazine® 10%, Chanelle Pharma, Ireland) with normal saline infusion (100 µg/ml, at a constant infusion rate of 14 drops/min). The 2% lignocaine hydrochloride (DSN Pharma Sdn. Bhd, Malaysia) was infiltrated at the sites of the surgical implantation. After which, a horizontal incision was made at the implantation site, and the skin was then undermined with blunt dissection to create a subcutaneous pocket. A piece of anisotropic tissue expander was then gently inserted into the subcutaneous pocket and secured in position by placing a few tacking sutures to prevent migration of the tissue expander subcutaneously. The incision site was then sutured with 3-0 Ethilon® (USA) using a cross-mattress suture pattern. During the post-implantation, the antibiotic of procaine penicillin and dihydrostreptomycin sulphate (Norbrook, United Kingdom) (22,000 IU/kg, intramuscularly), and anti-inflammatory of flunixin meglumine (Norbrook, United Kingdom) (1.1 mg/kg, intravenously) was given once a day for three days. A period

of 14 days was allowed for the cutaneous expansion process as recommended by the manufacturer. The experiment lasted for 18 days.

Behavioural and Physiological Vitals Assessment

Three days prior to the tissue expander implantation, each horse was observed for normal behaviour in the stall box (Table 1). Physiological parameters were determined, including rectal temperature, heart rate, and respiratory rate. The physiological parameters were assessed 30 min before the behavioural observational section. The horse behaviour was observed and recorded at an interval of 10 min for four hours during the daytime and another four hours at night. All observation sessions were performed around 10:00 a.m. to 2:00 p.m. and between 9:00 p.m. to 1:00 a.m. The frequency and duration based on the ethogram before the implantation of tissue expanders were recorded and considered as baseline data (D_o). Two camera traps (Ltl Acorn, Ltl-5210A, United Kingdom) were installed in the stable where the horses are kept and adjusted to record the horses' behaviour and responses according to a schedule throughout the day/night.

Table 1
Description of horse behaviours examined before and after implantation of tissue expanders

Behaviour	Description
	Measured in Frequency (number of occurrences/hour)
Head shaking	Shaking the head from side to side
Head-turning	Turning the head together with the neck to a particular side
Nose scratching	Rubbing a nose area on the wall or any hard object
Circling	Radial locomotion in the stable
Eating	Mastication and swallowing
Drinking	Ingest water by dipping lips at or slightly below the surface of the water and drawing the water with a sucking action, and swallowing
	Measured in Duration (hour)
Lying	Lying down on the sternum with legs folded under the body
	Lying down on the side with legs stretched out
Scratching of the implant site	Rubbing of the forehead
	Rubbing of the shoulder area
	Nibbling, biting, licking, or rubbing the lower forelimb
Standing rest	Weight-bearing on all four legs
	Weight-bearing on three legs with right hindleg slightly flexed
	Weight-bearing on three legs with left hindleg slightly flexed
Walking	Forward and backward movement of more than 1 limb resulting in a new position within the stable
Exploring	Foraging without eating or smelling the local environment while standing or walking

Note. The ethogram description of horse behaviours was based on previous studies (Ashley et al., 2005; Price et al., 2003; Torcivia & Mcdonnell, 2020)

Post-surgical implantation of tissue expanders, the horses were observed for any change in behavioural patterns and comfort level for up to 6 days (D_1, D_2, D_3, D_4, D_5, and D_6). On each day prior to behavioural observation, the rectal temperature, heart rate and respiratory rate were taken 30 min before recording the horse's behaviour. The frequency and time duration of the specific behaviour were analysed based on the visual observation and recorded video of the horse in the stable.

Statistical Analysis

The data were expressed as mean and standard deviation. The mean frequency or percentage difference of the behavioural parameters between pre- and post-implantation was analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc for multiple comparisons. The p-value < 0.05 was considered significant. All statistical analysis was performed using GraphPad Prism version 8.0.2 (GraphPad Software, USA).

RESULTS

Physiological Assessments

All horses tolerated the surgical implantation procedure, and the incision sites healed without complications. There was no evidence of suture breakage, implant rupture, hematoma, or discharge. The physiological parameters of the horses pre- and post-implantation of tissue expander subcutaneously in horses are shown in Figure 1. There was no significant effect ($p > 0.05$) of tissue expander implantation on the rectal temperature (Figure 1A) and respiratory rate over the six days observational period (Figure 1C). However, a significant difference ($p = 0.0245$) in heart rate within the observational days was observed among the horses (Figure 1B).

Behavioural Assessments

The mean proportion of time duration for each behaviour during the day and night of the pre-and post-implantation observational period are presented in Tables 2 and 3,

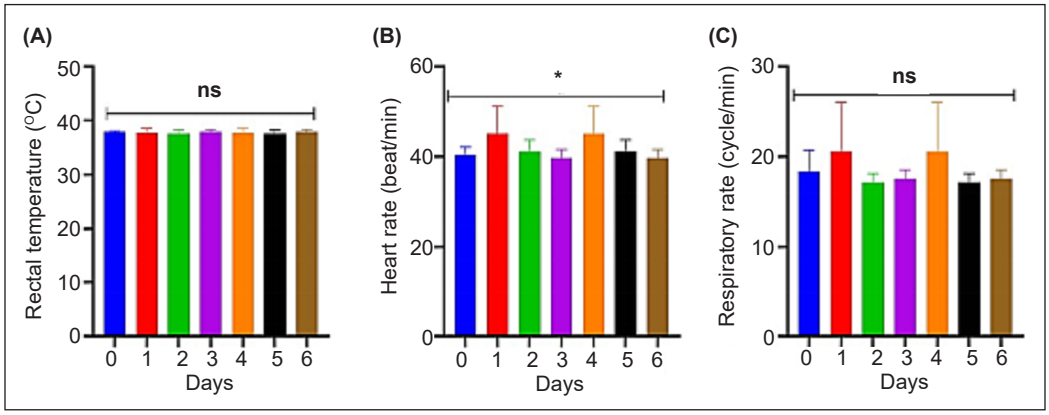

Figure 1. Rectal temperature (A), heart rate (B), and respiratory rate (C) determined pre and six days post-implantation of tissue expander subcutaneously in horses
Note. * $p < 0.05$ significance different over the time duration; ns = No significant difference between the time duration

Table 2
The proportion of time spent on each behavioural parameter observed during the daytime pre- and post-implantation period

Behaviour	Percentage of observation time/hour						
	Pre-implantation	Post-implantation					
	D_o	D_1	D_2	D_3	D_4	D_5	D_6
Standing rest	40.49±4.88	42.53±7.76	41.74±5.35	37.78±4.13	44.79±3.61	39.68±3.75	41.71±4.78
Walking	7.46±1.41	7.11±0.50	7.29±0.83	6.51±2.11	7.66±0.31	7.57±1.36	7.52±1.41
Exploring	11.25±2.86	10.65±4.03	12.35±3.09	9.03±5.20	12.22±1.86	12.36±2.67	11.81±2.07
Eating	37.23±5.70	38.61±4.47	36.32±6.47	39.54±7.34	33.24±4.81	37.87±1.15	37.34±4.46
Lying down	3.18±5.79	1.11±2.72	2.315±5.67	6.898±7.78	2.083±3.49	2.362±5.79	1.297±3.18
Forehead scratching	0.01±0.03[b]	0	0	3.36±5.15[a]	0	0	0.05±0.07[b]
Shoulder scratching	0.14±0.16	0	0.12±0.14	0.23±0.33	0.05±0.11	0.09±0.11	0.26±0.31
Lower limb scratching	0.21±0.25	0	0.17±0.19	0.26±0.39	0.05±0.11	0.17±0.14	0.33±0.39

Note. All values expressed in mean and standard deviation; [a,b] Means across the row with different superscripts are significantly different at $p < 0.05$

Table 3
The proportion of time spent on each behavioural parameter observed during the night pre- and post-implantation period

Behaviour	Percentage of observation time/hour						
	Pre-implantation	Post-implantation					
	D_o	D_1	D_2	D_3	D_4	D_5	D_6
Standing rest	62.08±10.39	58.59±5.68	62.18±7.82	61.71±16.65	56.94±9.67	67.32±7.53	68.84±6.36
Walking	4.08±0.85	4.19±0.86	4.44±1.39	4.77±1.21	4.35±1.68	4.49±1.60	5.16±0.87
Exploring	6.57±1.46	6.80±3.60	5.47±1.63	5.63±2.64	5.78±4.08	6.32±3.11	8.45±1.09
Eating	23.87±8.72	27.18±8.73	21.16±11.02	23.10±16.17	22.52±11.56	20.00±2.04	14.86±4.05
Lying down	3.12±3.01	3.24±3.56	6.67±8.17	4.58±7.34	10.28±19.39	5.28±12.93	2.45±3.81
Forehead scratching	0	0	0	0	0.05±0.11	0.05±0.11	0.02±0.06
Shoulder scratching	0.14±0.22	0	0.05±0.07	0.19±0.27	0.05±0.07	0.07±0.08	0.19±0.25
Lower limb scratching	0.04±0.06	0	0.05±0.07	0.02±0.06	0.02±0.06	0.05±0.07	0.02±0.06

Note. All values expressed in mean and standard deviation

respectively. During the day observation, the horses spent most of their time either standing rest (40.5%), exploring (11.3%), or eating (37.2%). There was no significant ($p > 0.05$) change in the time spent in all the behavioural parameters observed both during the pre-and post-implantation, except for forehead scratching, which was significantly ($p = 0.04$) higher in the daytime. Similarly, the time spent at night on each behaviour did not change significantly ($p > 0.05$) between the pre- and post-implantation periods. Compared to the daytime observations, the horses spent more time standing at rest, eating and recumbent rest (lying down), and less time exploring the stall box. Overall, the horses spent most of the time standing square on their 4 legs or with slightly flexed right or left hindlimb feet. There was no difference in the time spent in these different standing postures between the pre-and post-implantation day and night periods of observations (Figures 2A and 2B).

The frequency of other miscellaneous behaviour, such as headshaking, head-turning, circling, and scratching of the nose and/or abdomen, did not significantly change when compared between the pre- and post-implantation for day and night observation periods (Tables 4 and 5).

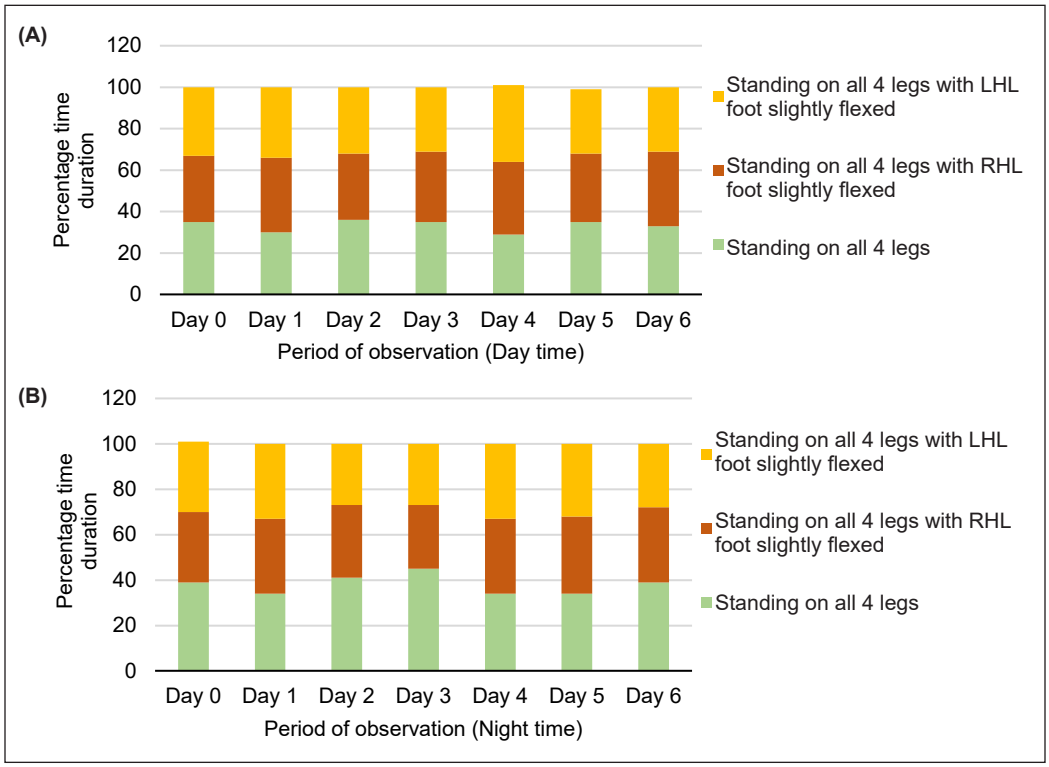

Figure 2. Average percentages of time spent standing without other activity during the day (A) and night (B) period of the observations
Note. LHL = Left hind limb; RHL = Right hind limb

Table 4
Mean frequency of other miscellaneous behaviours observed during the day

Behaviour	Mean frequency of observation time						
	Pre-implantation	Post-implantation					
	D_o	D_1	D_2	D_3	D_4	D_5	D_6
Head shaking	13.33±9.46	13.33±6.09	8.00±5.55	8.67±5.85	8.67±5.39	10.00±7.82	14.17±13.41
Head-turning	4.00±1.67	3.33±2.07	2.50±1.76	2.83±1.47	3.50±1.38	1.83±0.98	3.00±2.10
Circling	1.33±0.82	1.17±0.98	0.67±0.82	1.00±0.89	1.00±1.55	1.17±1.33	0.5±0.84
Drinking	2.83±4.62	1.50±3.67	3.17±6.31	5.00±6.87	1.83±2.14	0.83±0.75	2.167±3.92
Nose scratching	0.67±1.03	0.33±0.82	0.17±0.41	0.33±0.82	1.17±2.86	0.67±1.63	0.17±0.41
Abdomen scratching	0.50±0.84	0.17±0.41	0.33±0.82	0.50±0.84	0	0.17±0.41	0

Note. All values expressed in mean and standard deviation

Table 5
Mean frequency of other miscellaneous behaviours observed during the night

Behaviour	Mean frequency of observation time						
	Pre-implantation	Post-implantation					
	D_o	D_1	D_2	D_3	D_4	D_5	D_6
Head shaking	8.0±8.67	8.5±10.01	10.0±10.49	7.50±7.18	6.17±4.96	7.67±7.99	9.67±10.75
Head-turning	2.17±1.72	1.5±1.38	1.83±1.17	2.67±1.63	2.167±2.32	1.167±1.17	1.67±0.82
Circling	0.83±0.75	0.33±0.52	3.33±6.25	1.83±2.14	0.67±1.21	1.5±2.35	0.67±0.82
Nose scratching	0.67±0.82	0.33±0.82	0.17±.41	0	1.0±2.45	0.5±1.23	0.17±0.41
Abdomen scratching	0.5±0.84	0.17±0.41	0.5±1.23	0.83±0.98	0.5±0.84	0.33±0.52	0

Note. All values expressed in mean and standard deviation

DISCUSSION

It is the first study to describe that horse does not show any significant change in behaviour following subcutaneous tissue expander implantations with the hydrogel unidirectional self-inflating anisotropic tissue expanders. When assessed day and night, all horses showed no physiological and behavioural changes associated with the tissue expander implantation. All horses tolerated the implantation procedure, and the surgical sites healed without complication. There was no evidence of suture breakage, implant rupture, hematoma, or discharge. These findings are consistent with earlier observations (Al-Majhali et al., 2018; Chandawarkar et al., 2003; Whittaker et al., 2020). The elimination of pain after surgical implantation of the tissue expander by parenteral analgesia was to ensure the observed physiological and behaviour were not due to pain. The rectal temperature, respiratory, and heart rate of each horse over the six-days observation period, when compared with baseline data (pre-implantation), revealed normal vital parameters. Similarly, the rectal temperature and respiratory rate showed no significant change when considering all horses together. Although the heart rate revealed significant change over the observed period, the mean heart rate for each observed day is within the normal reference value. The slight changes in heart rate could be attributed possibly to the individual horse variation effect. This finding is similar to a previous behavioural study on pain where no correlation between the vital parameters and pain surgery, particularly following post-operative analgesia, was observed (Price et al., 2003).

In this study, the behavioural indices observed during the day and night pre-implantation of the tissue expanders considered normal for horses include standing, walking, exploring, lying down (recumbent rest), eating, and drinking. Based on the proportion of time, the horse spent most of the day standing, eating, and exploring the stall box. While at night, they spent the highest of the observed periods standing, resting and eating. Post-implantation, there was no significant change in the proportion of time spent on each of the behavioural parameters observed about the baseline (pre-implantation) during day and night over the six-day observational period. Scratching at the implantation areas was considered insignificant as it may reflect itching due to the inflammatory response to healing processes. Moreover, this behaviour was occasionally observed during the pre-implantation period suggesting a response to an external irritant (Velnar et al., 2009).

The findings of standing rest to be the predominating behaviour are not surprising, as it is typical for horses to spend most of the hours of the day and night in standing rest and lesser time in recumbent rest (Chung et al., 2018; Evans, 2000; Ransom & Cade, 2009). Horses can stand for long periods and even drowse and sleep while standing due to their unique passive stay apparatus of the equine forelimbs and hindlimbs. Thus, the horse could stand on their feet with minimum muscular effort (Dyce et al., 2009). Furthermore, horses may shift

weight between the hindlimb with slight flexing of the foot (Budras et al., 2012; Fuchs et al., 2016). This study's less time spent on recumbent rest seems lower than the average recumbent resting duration reported previously. In those reports, the type of bedding has been identified as an important factor that affects the duration of recumbent rest: sternal and/or lateral recumbency (Pedersen et al., 2004; Werhahn et al., 2010). Besides the bedding type, the depth and nature of bedding materials, such as cleanliness, texture, softness, and odour, could also influence the recumbent rest duration. However, the present observation of shorter recumbent rest duration is similar to what was documented for horses housed in shaving bedding, the same bedding material used in this study (Greening et al., 2013).

The duration of hay and/or concentrated eating during day and night, either pre- or post-implantation, were consistently normal for horses. Similar to the earlier report on the normal digestive behaviour of horses, the horses spent about 1/3 of the daytime and 1/4 of the night observation period engaged in eating (Greening et al., 2013).

When compared between the pre-and post-implantation period, the non-significant change in spent time on the behaviour of horses, such as eating, drinking, circling, head shaking or turning, and exploring during day and night horses tolerated the skin expansion technique without any discomfort or unwanted responses. Among the unwanted responses is reflected restlessness behaviour, which may be seen as either decreased eating, drinking, and exploring time or increased circling, head shaking, and/or turning (Ashley et al., 2005). More importantly, with a strong correlation between the longer time spent on standing rest and eating, standing rest behaviour has been described as an appropriate indicator of good welfare in horses (Ninomiya et al., 2007).

CONCLUSION

The subcutaneous implantation of unidirectional anisotropic tissue expanders in horses does not affect the physiological and behaviour of the horses. Hence tissue expansion technique is a good option to be considered when pursuing equine skin reconstructive surgery for equine skin reconstructive surgery.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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