Optimal implantation site of transponders for identification of experimental swine

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Abstract: Use of transponders, small electronic identification devices, in experimental swine is expected to be more reliable than the current common use of ear tags. However, it is necessary to determine the optimal implantation site for transponders with high readability, retentionability (i.e., long-term retention in tissues without detachment or loss), and biocompatibility, as this has not yet been investigated. Thus, we aimed to determine the optimal implantation site. Two types of transponders were subcutaneously implanted into four different sites (ear base, ear auricle, ventral neck, and back) in 3 domestic swine each. The transponders were scanned at 1, 2, 3, and 84 days after implantation. The location of the transponders was examined by X-ray and echography at 84 days. The transponders in the back were successfully scanned in a shorter time than those in other implantation sites, without any re-scanning procedures. X-ray examination revealed one transponder in the ventral neck was lost, whereas those in the other sites were retained in their original location for 84 days. Echography indicated that the transponders in the back were retained more deeply than those in other implantation sites, suggesting better retentionability. Acceptable biocompatibility was confirmed in all implantation sites, as evidenced by the finding that all transponders were covered by a connective tissue capsule without severe inflammation. In conclusion, the present results demonstrated that the back is the optimal implantation site for transponders in experimental swine.

Key words: experimental animal, implantation site, swine, transponder

Introduction

The ability to identify experimental animals from initiation through to termination of preclinical studies is important to prevent wrong treatments or data collection caused by mix up of animals. Transponders, which are small electronic devices that are implanted into subcutaneous tissues or body cavities of animals, have gained attention as a reliable identification method in recent years.

Swine have become frequently used in preclinical studies of medical devices because of the anatomical and physiological similarities between swine and humans [3, 11, 12, 17]. Currently, ear tags and ear notches are common swine identification methods. However, ear tags have the problems of tag detachment and application-site infection [4, 14]. For ear notches, low readability is a critical disadvantage, because errors may occur in the translation from ear notch location to alphanumeric identification numbers (ID).
Transponders are a promising alternative to ear tags and ear notches in swine. Transponders implanted in the ear base were shown to be lost less frequently and to produce fewer infections than ear tags [14]. In addition, ID is displayed directly on the reading device without the need for translation.

High readability is required for the use of transponders because animal identification procedures are frequent in preclinical studies, but this has not been investigated in swine. High retentionability (i.e., long-term retention in tissues without detachment or loss) and biocompatibility of transponders are also needed to confidently and safely identify experimental swine throughout the study period. In agricultural swine, it was reported that retentionability of transponders varied depending on implantation site [2], while excellent biocompatibility of transponders was widely confirmed [1, 2, 4–10, 13, 14]. However, it is necessary to determine the optimal implantation site for transponders with high readability, retentionability, and biocompatibility by comparing four implantation sites: ear base, ear auricle, ventral neck, and back. In addition to the ear base and ear auricle, which were frequently used in previous porcine studies [1, 2, 6–10, 14], we also chose the ventral neck and back, in which high retentionability of transponders is expected because the thick subcutaneous fat tissues at these sites would allow transponders to be implanted more deeply. The swine species (domestic swine, cross of Landrace and Large White) and the age at implantation (3 months) were determined based on their frequent use in preclinical studies of medical devices. The duration of implantation (3 months) was chosen because it is the longest observation period recommended in the use of domestic swine for preclinical studies [15].

**Materials and Methods**

Transponders

Two types of transponders were used in the current study: IMI-1000 Implantable Micro Identification (hereafter abbreviated as “IMI”; BioMedic Data Systems, DE, USA) and JMC Microchip (hereafter abbreviated as “JMC”; Japan Microchip Technical Development, Tokyo, Japan) (Fig. 1, Table 1). These transponders were
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chosen in consideration of their usefulness in experimental animals. In these transponders, enough numerical information (10 or more digits) can be saved to identify animals. In addition, the information can be changed in test facilities, which suits the actual situation where experimental animals are often labeled with facility-specific ID. Each transponder was pre-loaded in a sterilized single-use injection needle. The sizes of the injection needle and the transponder were similar between IMI and JMC. A unique ID was encoded on each transponder. The ID of the transponders were read with a portable reading device supplied from the suppliers of each transponder.

Animals

All animal procedures were performed at Terumo Corporation (Kanagawa, Japan). This study was approved by the Institutional Animal Care and Use Committee of Terumo Corporation.

Six healthy domestic swine, Sus scrofa domesticus (cross of Landrace and Large White, SPF, castrated males at 3 months of age, weighing 45–46 kg), provided by San-esu Breeding (Chiba, Japan) were used. All swine were housed in pens individually. They were habituated over a 1-week period. Forage for miniature swine (M-16, CLEA Japan, Inc.) was provided once daily. Drinking water complying with the water-quality standards defined in Japanese regulations was available ad libitum. The temperature of the animal housing room was maintained between 18.0 and 28.0°C; lights were on from 8:00 a.m. to 8:00 p.m. (12-h light/dark cycle). IMI and JMC were implanted in three swine respectively for 84 days. Four transponders were inserted at four different implantation sites in each swine: ear base, ear auricle, ventral neck, and back (Fig. 2A). Only the left ear bases and ear auricles were used for implantation. In the backs, the transponders were implanted in the subcutaneous tissue approximately 5 cm to the right or left of the spinous process.

All animals orally received aspirin (330 mg) and clopidogrel (75 mg) daily from 3 days prior to the implantation of transponders until euthanasia, because antiplatelet therapy was indicated in some preclinical studies of intravascular implanted devices [16]. All transponders were implanted under general anesthesia to ensure full insertion of injection needles into subcutaneous tissues, achieving deep implantation of transponders. Swine were fasted for 12 h before the general anesthesia.

They were tranquilized with intramuscular administration of medetomidine hydrochloride (0.04 mg/kg) and midazolam (0.2 mg/kg) in the back. The administration site was arranged far from implantation sites of the transponders. After onset of sedation, anesthesia was induced with intravenous administration of thiamylal sodium to
effect (2.8–6.1 mg/kg). Hair at the planned implantation sites was clipped and the sites were disinfected with 70% ethanol. The injection needle of the transponder was fully inserted into subcutaneous tissues at the implantation site. Angles of the injection needle to the body surface were adjusted so there was no damage to the muscles or auricular cartilage under the subcutaneous tissues (Figs. 2B and C). The plunger of the injection needle was pushed fully and the transponder was delivered into the subcutaneous tissues. The implantation sites were observed daily for 7 days from implantation and weekly from 7 days after the implantation to the day of euthanasia.

Readability

Scanning was performed to evaluate readability of transponders (Table 2). The transponders were scanned with portable reading devices at 1, 2, 3, and 84 days after implantation, without anesthesia or sedation. To guide the swine to take a position suitable for scanning, the experimenter stood in front of the pen, held a hand above or below the swine’s head, and moved the hand to guide the swine’s nose to the desired location. For example, the swine was guided to lift his chin during scanning of transponders implanted in the ventral neck. The portable reading device was moved close to each implantation site from outside of the pen to read the transponder IDs.

Table 2. Methods and time points for evaluation of each parameter

<table>
<thead>
<tr>
<th>Evaluation parameter</th>
<th>Method</th>
<th>Time point</th>
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<tbody>
<tr>
<td>Readability</td>
<td>Required number of scanning attempts until</td>
<td>1, 2, 3, and 84 days after implantation</td>
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<tr>
<td></td>
<td>successful reading of the ID</td>
<td></td>
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<tr>
<td></td>
<td>Time to complete reading the ID at the last scanning attempt</td>
<td>1, 2, 3, and 84 days after implantation</td>
</tr>
<tr>
<td>Retentionability</td>
<td>Presence of the implanted transponder</td>
<td>84 days after implantation</td>
</tr>
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<td></td>
<td>Depth of the transponder from the body surface</td>
<td>84 days after implantation</td>
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<tr>
<td>Biocompatibility</td>
<td>Presence of macroscopic abnormalities in the harvested tissue</td>
<td>84 days after implantation</td>
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<td></td>
<td>Inflammatory response and tissue reaction</td>
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<td></td>
<td>Echography</td>
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<td></td>
<td>X-ray examination</td>
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<td></td>
<td>Macroscopic examination</td>
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<td></td>
<td>Histopathological examination</td>
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</tbody>
</table>

Fig. 3. Scanning of the transponders with portable reading devices. (A) Scanning a JMC implanted in the ear base. (B) Scanning an IMI implanted in the back. The portable reading device was moved close to the implantation sites from outside of the pen to read the transponder IDs.
Retentionability

X-ray examination and echography were performed to evaluate retentionability of the transponders (Table 2). At 84 days after implantation, all swine underwent general anesthesia for X-ray examination and echography. Swine were tranquilized and anesthesia was induced with the same protocol as for transponder implantation. After anesthetic induction, swine were intubated and connected to mechanical ventilation. Anesthesia was maintained with inhalation of 2–4% sevoflurane throughout the procedure. The presence of the implanted transponders was examined by observing not only the implantation sites but also the whole body with the X-ray diagnostic apparatus, Infinix Celeve-i INFX-8000V (Toshiba Medical Systems, Tochigi, Japan). The depth of the transponders from the body surface was measured with the ultrasonograph, Vivid-i (GE Healthcare Japan, Tokyo, Japan).

Biocompatibility

Histopathological examinations were performed to evaluate biocompatibility of the transponders (Table 2). After X-ray examination and echography, all swine were euthanized by exsanguination while still under general anesthesia. The tissue including the transponders of each implantation site was harvested and immersed in 10% neutralized buffered formalin. After adequate fixation, the transponder was removed from the tissue. The harvested tissues were grossly observed to examine if there were any abnormalities. The tissues were delipidated, dehydrated, and embedded in paraffin. The ear auricles were decalcified after delipidation. Paraffin blocks were cut into sections of approximately 3 µm thickness. The sections were stained with hematoxylin and eosin and Masson’s trichrome. Inflammatory responses and tissue reactions were evaluated on each section by light microscopy according to the criteria described in Table 3, which was made in reference to an international standard for the assessment of the local effects after implantation of biomaterials intended for use in medical devices (ISO 10993-6 Annex E) [5].

Statistical analysis

Data are presented as means ± SD. The number of scanning attempts required until successful reading and the time to complete the reading of an ID at the last scanning attempt were compared between the four implantation sites by the Steel-Dwass test; *P*<0.05 was considered statistically significant. The statistical analyses were performed with EXSUS software (version 7.7.1; Arm Systex Co., Ltd., Osaka, Japan) in combination with SAS software (version 9.2 TS2M3; SAS Institute Inc., Cary, NC, USA).

Results

All transponders were successfully implanted. No inflammation, swelling or other abnormalities were no-

<table>
<thead>
<tr>
<th>Cell type/response</th>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymorphonuclear cells</td>
<td>Rare, 1–5/phf</td>
<td>5–10/phf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
<td></td>
<td></td>
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<tr>
<td>Lymphocytes</td>
<td>Rare, 1–5/phf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
<td></td>
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<td></td>
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<tr>
<td>Plasma cells</td>
<td>Rare, 1–5/phf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>Rare, 1–5/phf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giant cells</td>
<td>Rare, 1–2/phf</td>
<td>Heavy infiltrate</td>
<td>Sheets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td>Minimal</td>
<td>Mild</td>
<td>Moderate</td>
<td>Sheets</td>
<td></td>
<td></td>
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<tr>
<td>Neovascularization</td>
<td>Minimal capillary proliferation, focal, 1–3 buds</td>
<td>Groups of 4–7 capillaries with supporting fibroblastic structures</td>
<td>Broad band of capillaries with supporting structures</td>
<td>Extensive band of capillaries with supporting fibroblastic structures</td>
<td></td>
<td></td>
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<tr>
<td>Fibrosis</td>
<td>Minimal amount of fat associated with fibrosis</td>
<td>Several layers of fat and fibrosis</td>
<td>Thick band</td>
<td>Elongated and broad accumulation of fat cells about the implant site</td>
<td>Extensive fat completely surrounding the implant</td>
<td></td>
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<tr>
<td>Fatty infiltrate</td>
<td>Minimal</td>
<td>Mild</td>
<td>Moderate</td>
<td>Sheets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>Minimal</td>
<td>Mild</td>
<td>Moderate</td>
<td>Sheets</td>
<td></td>
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</table>

phf: per high power (×400) field.
noticed at any of the implantation sites by daily gross observation. The body weights of the swine were 45.0–46.0 kg at implantation and 82.0–87.2 kg at euthanasia (84 days after implantation) with a gain of 37.0–42.2 kg.

**Readability**

The IDs of all the transponders were successfully read at 1, 2 and 3 days after implantation. At 84 days, the ID of one of three JmC transponders implanted in the ventral neck could not be read and was found to be lost upon X-ray examination. The number in ear bases was similar to that in ventral necks ($P=0.1964$) with JmC. The number in ear auricles was similar to that in ventral necks ($P=0.4312$) with JmC. All transponders in ear auricles and backs were read with only one scanning attempt at all time points.

Figure 4 shows the frequency of the number of scanning attempts required until successful reading of an ID. Transponders in ear bases required more than one scanning attempt in 7 of 12 attempts with IMI and in 7 of 11 attempts with JmC. The number of scanning attempts required for ear bases was significantly larger than at ear auricles ($P=0.0127$), ventral necks ($P=0.0494$), and backs ($P=0.0127$) with IMI, and was significantly larger than that at ear auricles ($P=0.0076$) and backs ($P=0.0076$) with JmC. The number in ear bases was similar to that in ventral necks ($P=0.1964$) with JmC. The number in ear auricles was similar to that in ventral necks ($P=0.7494$) with IMI and was similar to that in ventral necks ($P=0.4312$) with JmC. The number in ear auricles was equal to that in backs with IMI and JmC. All transponders in ear auricles and backs were read with only one scanning attempt at all time points.

Figure 5 shows the time to complete reading the ID at the last scanning attempt. The time to complete reading the ID at the last scanning attempt in ear bases was similar to that for ear auricles ($P=0.8763$), ventral necks ($P=0.3854$), and backs ($P=0.9301$) with IMI, and was similar to that for ear auricles ($P=0.7924$), ventral necks ($P=0.9444$), and backs ($P=0.7218$) with JmC. The time for ear auricles was similar to that for ventral necks ($P=0.8224$) and backs ($P=1.0000$) with IMI, and was similar to that for ventral necks ($P=0.9828$) and backs ($P=0.9989$) with JmC. The time for ventral necks was similar to that for backs ($P=0.7430$) with IMI, and was similar to that for backs ($P=0.9608$) with JmC. The time for backs tended to be shorter than for other implantation sites.

Figure 6 shows a dot plot of the depth of the transponders from the body surface. The transponders in backs tended to be located deeper than those in other implantation sites.
The time for backs tended to be shorter than for other implantation sites but was not statistically different between the four implantation sites.

**Retentionability**

One of three JMC transponders in the ventral neck was lost, as indicated by X-ray examination at 84 days after implantation. All JMC transponders in the other three implantation sites and all IMI transponders were retained in the original implantation sites. Figure 6 shows the depth of the transponders from the body surface by echography. The transponders in the backs tended to be located deeper than those in other implantation sites.

**Biocompatibility**

By gross observation of the implantation sites at necropsy, red brown areas were found in one of three ear bases (3.0 mm × 3.0 mm) and one of three ventral necks (1.0 mm × 1.0 mm) with IMI (Fig. 7), whereas no gross abnormalities were found in the implantation sites with JMC.

In the histopathological examination, infiltration of inflammatory cells, mainly composed of macrophages and lymphocytes, was occasionally observed, but the average score of each inflammatory cell type was less than 2 (Table 4). All transponders were covered by a connective tissue capsule (Figs. 8A1 and 2). Minimal or mild hemorrhage was found in one of three ear bases with IMI, one of three ventral necks with IMI (Figs. 8B1 and 2) and one of three ear bases with JMC.

**Discussion**

In the current study, we compared readability, retentionability, and biocompatibility of transponders at four different implantation sites (ear base, ear auricle, ventral...
neck, and back) in experimental swine kept in an experimental animal facility. For scanning, the number of scanning attempts required until successful reading of the ID and the time to complete reading the ID at the last scanning attempt tended to be smaller and shorter, respectively, in backs, indicating that readability in backs was higher than at the other implantation sites. Conversely, the number of scanning attempts required at ear bases was significantly larger than at other implantation sites, indicating that readability at ear bases was lower than at the other implantation sites. X-ray examination and echography showed transponders in backs were retained at the original implantation sites in all animals and they tended to be located more deeply than those at other implantation sites, indicating that retentionability in the backs was higher than at the other implantation sites. The histopathological examination showed acceptable healing, as evidenced by the finding that all transponders were covered by a connective tissue capsule and without severe inflammatory responses, indicating excellent biocompatibility at all implantation sites.

In the current study, two types of commercial transponders, IMI and JMC, were chosen as convenient transponders for animal experimental facilities; both types of transponder can save enough information to identify animals and the information can be changed within the facilities. The size of the transponders and the length of the dedicated injection needles were similar.

Fig. 8. Histopathological images of the implantation site. (A1) A typical histopathological image of an implantation site (a back implanted with JMC; Masson’s trichrome staining). (A2) Higher magnification of the box in A1. The space previously occupied by the transponder (T) was covered by a connective tissue capsule. The inflammatory reaction was minimal. (B1) A representative image of an implantation site with mild hemorrhage (a ventral neck implanted with IMI; Masson’s trichrome staining). (B2) Higher magnification of the box in B1 (hematoxylin and eosin staining). The space previously occupied by the transponder (T) was covered by a connective tissue capsule. Moderate inflammation and mild hemorrhage were found.
between the two types of the transponders.

Although both domestic swine and miniature swine are used in preclinical studies of medical devices, domestic swine are more frequently used because of greater similarity of organ sizes compared with humans. For this reason, we used domestic swine in the current study. Since domestic swine are not recommended to be used in studies in which the duration is more than 28 days due to rapid growth [15], implantation of the transponders for three months in the current study was considered a sufficient period of time.

For scanning, readability was high in backs and low in ear bases. This result was reasonable considering the habits of swine. Swine are curious and move their heads constantly in the experimenters’ presence. Therefore, ear bases, ear auricles, and ventral necks often moved away from the scanner during transponder scanning, while backs did not move as frequently.

By X-ray examination, one of three JMC transponders in the ventral neck was lost. Because we successfully read this transponder until 3 days after implantation, this transponder was considered to have dropped out of the body at 4 days or later. In porcine studies comparing different sizes of transponders, smaller transponders dropped out less frequently than larger one [1, 2, 14]. Although the length of JMC transponders (12 mm) was smaller than that of transponders used in previous porcine studies (23 mm) [2, 14], loss of JMC transponders implanted in the ventral neck (one of three transponders, 33%) was higher than that in ear bases reported in the previous porcine studies (18.1–29.8%) [2, 14]. Considering the above, ventral necks are unfit as implantation sites for transponders in experimental swine due to low retentionability. Katharina et al. reported that 11.5-mm-long transponders implanted in the ear base of swine migrated by 7 mm during 2 weeks [14]. It is possible that the transponders implanted in the ventral neck migrated more than the transponders in ear bases because of external forces derived from continuous head motion, as described above, leading to dropout from the body. This estimation is in line with the echography results; the locations of the remaining transponders in the ventral neck tended to be shallower than those in backs, although transponders in ventral necks were expected to be implanted as deeply as those in backs because both the ventral neck and back have thick fat tissues.

The body weight of swine used in the current study was 45.0–46.0 kg at implantation and 82.0–87.2 kg at euthanasia, with a gain of 37.0–42.2 kg. Despite the rapid growth during the 3-month study period, the transponders in the backs tended to be located more deeply than those in other implantation sites. These results indicate that retentionability in backs was higher than in ear bases, the implantation site recommended by the World Small Animal Veterinary Association (WSAVA) [18]. Possible reasons for the superiority of backs are as follows. First, transponders are thought to have been inserted deep in backs at implantation without damaging muscular tissues because of the presence of thick subcutaneous fat tissues. Second, less motion of the backs may have contributed to better retentionability compared with other implantation sites.

Excellent biocompatibility was confirmed in all four implantation sites in the current study. By daily observation of the animals, no abnormalities were found at any implantation site. By histopathological examination, all transponders were covered by a connective tissue capsule with an acceptable range of inflammatory responses at all implantation sites. These results indicate that tissue reactions at all implantation sites were acceptable. The current 3-month histopathological responses to the transponders are similar to those seen in ear bases of swine in a previous study; tissue reactions such as exudation and cellular proliferation that were observed at 7 and 21 days were almost terminated and the transponders were covered by connective tissues at 5 months [10]. In the current study, mild hemorrhages were found in ear bases and ventral necks but they were not considered serious for animal health. This mild injury was probably due to frequent head motion, resulting in repetitive physical stress of the transponders to the surrounding tissues.

The current study has the following limitations. First, the sample size for each implantation site was small, potentially leading to failure to detect low-frequency loss of the transponders and adverse events. However, the current study has provided a novel and important suggestion that the back, which has not been investigated previously, is a better implantation site for transponders in swine than ear bases, the recommended implantation site of an international guideline [18]. Second, we focused on only domestic swine instead of miniature swine. Nevertheless, domestic swine are more widely used in preclinical studies of medical devices than miniature swine. Investigation using miniature swine is needed in future. Third, we used only two types
of commercial transponders (IMI and JMC) and thus the optimal implantation site of other types of transponders may be different. Nevertheless, it is expected that the results would be generally similar if other types of transponder are used because the current study found similar results for IMI and JMC. Fourth, readability was assessed only for singly housed swine, leading to a concern that identification errors may occur in group-housed swine if the ID of a different animal in the same pen is mistakenly read. However, this error seems unlikely because the readable distance between scanner and transponder is short (5 cm for IMI and 10 cm for JMC). Fifth, all animals were 3 months of age at implantation; optimal implantation sites may be different for younger or older animals. Sixth, assessment time points after implantation were limited to 1, 2, 3, and 84 days for scanning and 84 days for retentionability (X-ray and echography). Because of the lack of data at intermediate time points, we cannot say when or how the transponder was lost from the neck. Seventh, the durability of implanted transponders against physical shock was not investigated; durability should be considered in animal models of disease with impaired locomotion, in which transponders may undergo physical shocks when animals stagger or fall.

In conclusion, the current study demonstrated that back is the optimal implantation site for transponders in experimental swine because back showed high readability, high retentionability, and excellent biocompatibility. Conversely, ear bases, which are site recommended for implantation by WSAVA, do not appear optimal because the readability was lower than at ear auricles, ventral necks and backs, and the retentionability in ear bases tended to be lower than in backs.

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