

# The Subcutaneous Capsules for Foreign Body in Fetal Rabbits: Preliminary Report

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In terms of wound healing, there are fundamental intrinsic and extrinsic differences between fetuses (scar-free healing) and adults. The fetus exhibits less typical inflammatory response (significantly neutropenic) with an underdeveloped self-nonsel self immunologic identity and a lack of cellular immunity. The recruitment of inflammatory cells to a wound may play an important role in the resulting cellular processes and ultimately affect the quality of the healing response. Foreign bodies can act as a source of infection and immunologic reactions. In contrast, there have been few studies of the wound healing of fetus with foreign bodies, where in adults, wounds are healed by tissue regeneration rather than capsule formation and a foreign body reaction.

In this study, the wound healing process in an adult rabbit and fetus group, in which either silicone or a sponge was inserted in the uterus, were compared. All specimens showed capsule formation with fibroblast, collagen deposition, neovascularization, and infiltration of inflammatory cells. However, the fetal specimen exhibited mainly acute inflammatory responses and the capsule contained less fibroblasts and collagen deposition. In addition, myofibroblast expression, which mediates wound contracture, was lower in the fetal specimen. These findings were common with cotton implants, which were expected to induce a severe inflammatory response.

The inflammatory response induced by foreign materials in fetal tissue showed similar response with that of incisional wound healing. This study may provide a basis for the use of implants such as silicone in future fetal surgery.

**Key Words:** Foreign body reaction, fetus

## INTRODUCTION

Since Rowlatt first reported the ability of the

fetal wounds to heal without scar formation in 1979,<sup>1</sup> there has been a large effort to establish the mechanism of fetal wound healing.<sup>2-7</sup>

There are fundamental intrinsic and extrinsic (environmental) differences between fetuses and adults, which would certainly impact on wound healing.<sup>2-7</sup> Firstly, fetal skin wounds are bathed continuously in warm, sterile amniotic fluid known to be rich in growth factors<sup>2,3</sup> crucial to fetal development and extracellular matrix components such as hyaluronic acid (HA)<sup>2-7</sup> and fibronectin.<sup>8</sup> Secondly, the fetus is significantly neutropenic and has not yet developed a self-nonsel self immunologic identity.<sup>9</sup> The fetal wounds heal by tissue regeneration by macrophages rather than by a typical inflammation response in adults. Macrophage have many growth factors, are involved in cellular matrix turnover, and participates in wound healing and remodeling of the cellular matrix.<sup>3,7,9</sup> Moreover, in the wound healing of a fetus, growth factors, such as TGF- $\beta$  and EGF, are also involved in cellular proliferation, interaction and differentiation. The most significant difference in the wound healing of fetuses and adults is that in fetuses, there is a minimal inflammation reaction with limited fibroblast involvement, minimal collagen collection but a regular collagen distribution. A midgestation human fetus heals by mesenchymal proliferation and without the scar formation normally observed in the adult.

Wound healing is a very complex and dynamic process. It is also a process where normal tissue is replaced by scar tissue. Foreign bodies can act as a source of infection and in immunogenic reactions. Wound healing with foreign bodies can induce specific immunologic reactions. However,

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wound healing with foreign bodies is slightly different from the normal wound healing process. Thus far, there have been limited studies of wound healing of fetuses with foreign bodies, where wounds are healed by tissue regeneration rather than by capsule formation and foreign body reactions in adults.

In this study, an adult rabbit group and a fetus group where either silicone or sponge was inserted were compared. The aim of this study was to determine the foreign body reaction in the fetus, the distribution of fibroblasts, collagen deposition, and myofibroblasts induction on the capsules by a histological and immunohistochemical examination.

## MATERIALS AND METHODS

Time related New Zealand white rabbits were obtained from the Hanlim Laboratory Animals Center (Kyonggi, Korea). Fetal foreign body reactions studies were performed on the 23-24th day of gestation (term, 31 days) in 10 fetuses, with harvesting after a normal delivery. For comparison, old pregnant rabbits were used to provide the mature adult response.

### Wound Implants

The wound tissue was sampled using a subcutaneous cotton implant and a Silastic implant. A  $5 \times 5 \times 2$  mm cotton and Silastic implant were used for the fetal rabbits and  $10 \times 10 \times 4$  mm implants were used for the adult rabbits.

### Fetal Surgery

The rabbits were initially housed for 18 days of pregnancy and kept for 4 to 5 days prior to surgery in order for the rabbits to adapt their new environment. On day 23-24 of gestation (term, 31 days), each doe underwent a laparotomy and a hysterotomy after receiving atropine (0.08 mg/kg) and ketamine (10 mg/kg) intramuscularly and mask enflurane anesthesia. Using a sterile technique, a lower midline laparotomy incision was made. The size, number and position of the fetuses were determined by palpation.

One uterine horn was positioned in the wound and kept warm and moist with saline-soaked gauzes. A 4-0 vicryl purse-string suture was placed overlying the dorsal side of a fetus on the antimesometrial side of the uterus. The hysterotomy incision was made within the purse-string suture through all layers including the amniotic sac. A 0.5 cm incision in the fetal back was made to introduce the cotton or Silastic implant. The fetal incision was closed with 6-0 nylon. The purse-string suture was tightened after reconstituting the amniotic fluid volume with physiological warm saline mixed with antibiotics. A running 3-0 Vicryl suture was used to close the maternal peritoneum and abdominal fascia in one layer. The skin was closed with a running 4-0 nylon suture. Old pregnant adult female rabbits received identical implants.

### Wound Harvest

The implants were removed after a normal fetal delivery, and those with tissue around the wound were harvested and the fetuses were sacrificed with a pentobarbital overdose. The adult implants were harvested at same number of days postoperatively.

### Histology

The grade of the inflammatory response, wound healing state and the grade of capsular formation with the pattern of collagen bundles were examined both grossly and microscopically. A histologic examination of the harvested implants and the surrounding capsular tissues was performed using light microscopy. The specimens were fixed in formalin, sliced in half longitudinally, embedded in paraffin by a routine method, and an interrupted series of  $7\text{-}\mu\text{m}$  sections were produced. Thin sections of the specimens from fetal and adult wounds were stained with hematoxylin & eosin and Masson's trichrome stain. The numbers of fibroblast cells from capsular tissues were measured for the cell density. The positively stained fibroblast cell counts per high power fields ( $\times 400$ ) were counted using light microscopy. Two fields of view were analyzed in each section of capsule

tissue.

### Immunohistochemistry for $\alpha$ -Smooth Muscle Actin

Tissue sections from each group were deparaffinized with xylene and then dehydrated using graded alcohols. After rehydration, the sections were then exposed to normal goat serum for 10 minutes. Incubation of primary antibody was performed using a monoclonal antibodies specific to  $\alpha$ -smooth muscle actin (Dako, Carpinteria, CA, USA) for 60 minutes. The signal was detected by an EnVisionTM+ kit (Dako) and diaminobenzidine as chromogen. The smooth muscle cells in the blood vessels located throughout the tissues served as positive controls. The number of stained fibroblast cells in the capsular tissue was measured. Positively stained fibroblast cell counts per high power fields ( $\times 400$ ) were counted using light microscopy. Two fields of view were analyzed in each section of capsular tissue.

## RESULTS

A total of 6 specimens were obtained from the fetal surgery performed on 10 rabbits. Four fetuses were lost during pregnancy, probably due to a postoperative infection or excessive manipulation during the operation. The specimen showed capsule formation with fibroblast, extracellular matrix such as collagen, neovascularization, and an infiltration of inflammatory cells.

### Cellularity

All the implant induced capsule formation. The capsule was comprised of fibroblasts, extracellular matrix such as collagen, neovascularization and an infiltration of inflammatory cells. The numbers of fibroblasts in the capsule was higher in the adult cotton implant group ( $97.5 \pm 32.4$  fibroblasts/fields of view) than in the fetus ( $75.6 \pm 18.2$  fibroblasts/fields of view). In the silicone implant group the same results were obtained (adult:  $96 \pm 20.9$  fibroblasts/fields of view, fetus:  $65.9 \pm 10.8$  fibroblasts/fields of view) (Fig. 1).

### Collagen Matrix

The fibroblasts in the capsule were arranged parallel to the implant and the collagen productivity was found to be greater in the adult group. Masson's trichrome staining revealed less mature and less compactly arranged collagen bundles in the fetal specimen (Fig. 2).

### Inflammatory response

The vessels in the vascular layer of the capsule were usually dilated and infiltrated by inflammatory cells. However, the fetal and adult specimens had different types of inflammatory cells; neutrophils, eosinophils, and plasma cells, which are characteristic of an acute inflammatory response in fetal specimens and lymphocytes, which are characteristic of chronic inflammation in adult specimens.

### Immunohistochemistry for $\alpha$ -Smooth Muscle Actin

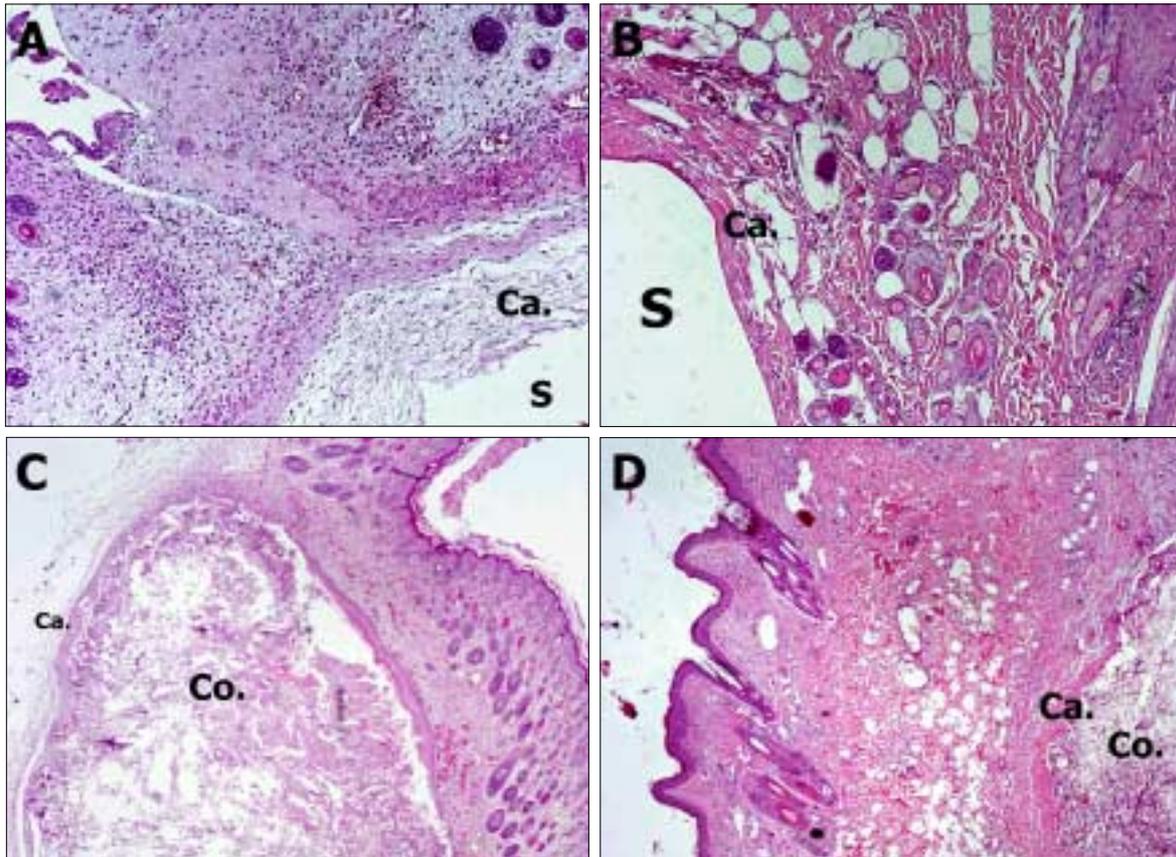
Fibroblasts identified by  $\alpha$ -Smooth Muscle Actin antibody staining were present in all specimens. The number of positive  $\alpha$ -Smooth Muscle Actin antibody stained fibroblasts was greater in the adult cotton implant capsular tissue group ( $25.5 \pm 4.98$  fibroblasts/fields of view) than in the fetus group ( $3.3 \pm 2.1$  fibroblasts/fields of view) (Fig. 3). However, in the silicone implant group, the numbers were reversed with the fetus group ( $26 \pm 3.9$  fibroblast/fields of view) having a higher number than the adult group ( $10.8 \pm 4.3$  fibroblasts/fields of view).

### Silastic implant (Fig. 1A, 1B, 2A, and 2B)

All specimen formed capsules. However, the capsule in the fetal specimen differed from the adult specimen in several ways. The inner layer was incompletely formed and the collagen layer was formed by fibroblasts, neovascularization and acute inflammatory responses were observed in the fetal specimen.

### Cotton implant (Fig. 1C, 1D, 2C, and 2D)

The capsule around the cotton implant was



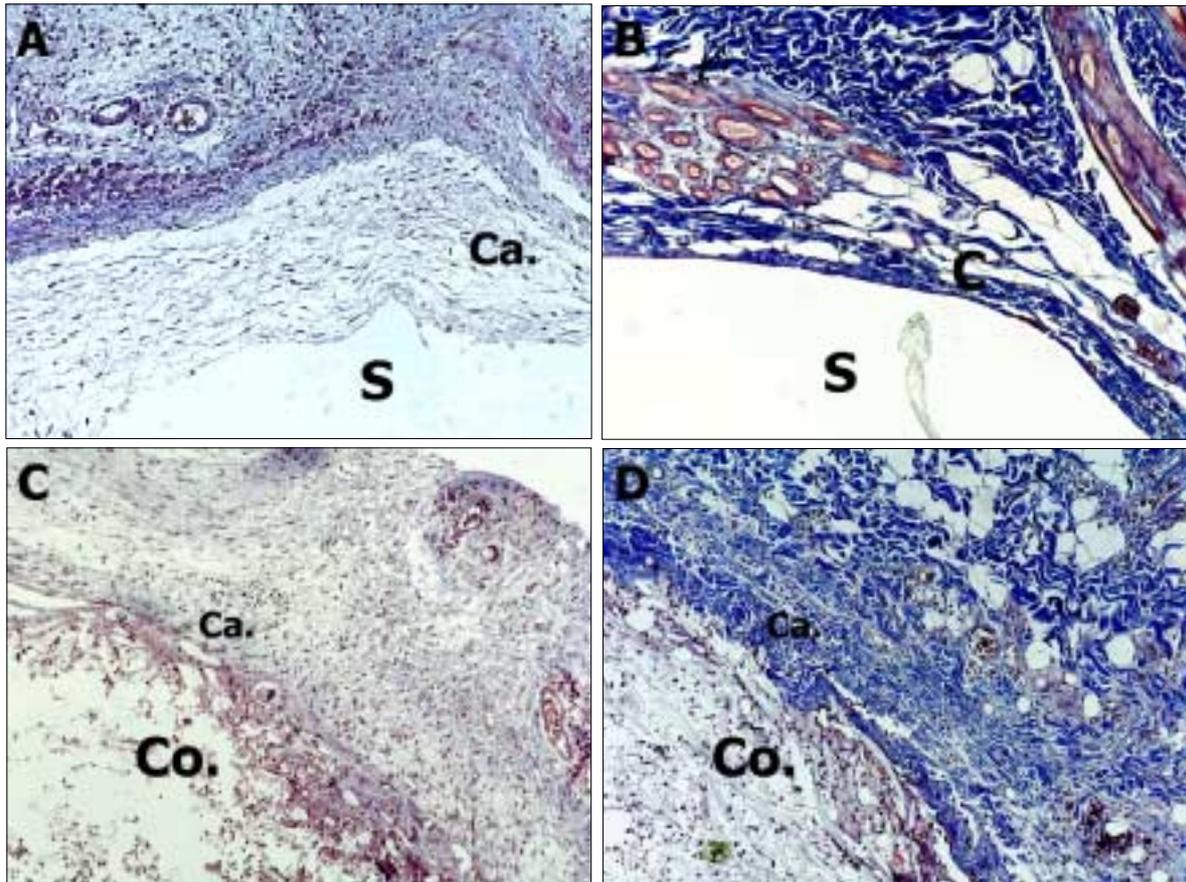
**Fig. 1.** Capsule formation (Ca.) with fibroblast, extracellular matrix such as collagen, neovascularization, and the infiltration of inflammatory cells (H&E stain  $\times 100$ ). The fetal tissue response to foreign materials (S; silicone, Co.; cotton) is characterized by a dominance of an acute inflammatory response and less fibrosis and collagen deposition than that of adults. (A; fetal silicone implant, B; adult silicone implant, C; fetal cotton implant, D; adult cotton implant)

more mature compared to the granulation tissue formed by the silicone implant. The cotton implant surface showed tissue ingrowths and adherence without inner layer formation. A relatively compact collagen layer with fibroblasts was observed. Neovascularization was greater in the fetal specimen and collagen deposition was greater in the capsule formed by cotton implants, which is similar to dermal collagen. An immunohistological examination with  $\alpha$ -SM Actin antibodies showed that a larger proportion of cells in the adult specimen that were identified as fibroblasts were actually myofibroblasts when compared to the fetal specimens.

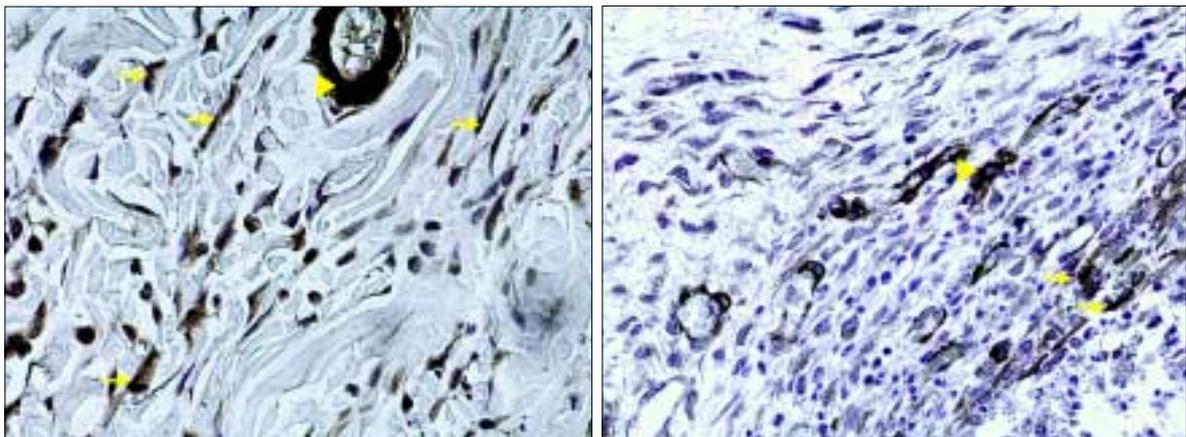
## DISCUSSION

There are fundamental intrinsic and extrinsic

differences between fetal and adult skin that certainly impact on wound healing. One of the most consistent features of fetal wound healing is the lack of acute inflammation compared to the adults.<sup>3-7,9</sup> The fetus exhibits smaller amount of typical inflammatory responses (significantly neutropenic) with an underdeveloped self-nonself immunologic identity and a lack of cellular immunity than adults. Because of the prominent role that inflammation plays in adult wound repair, the minimal fetal inflammatory response to injury may play a pivotal role in the unique fetal wound healing. The induction of an acute inflammatory response in fetal wounds results in increased fibroplasias and collagen deposition, simulating a more adult-like healing process.<sup>10</sup> In addition, several reports have demonstrated that penetrating sutures in fetal experimental surgery can arouse a mononuclear inflammatory response and



**Fig. 2.** Relatively less cellular and less compact organized fibrosis is observed in the fetal capsule tissue (Masson's trichrome;  $\times 100$ )(A; fetal silicone implant, B; adult silicone implant, C; fetal cotton implant, D; adult cotton implant, S;silicone, Co.; cotton, Ca.; capsule).



**Fig. 3.** The sections were stained with an antibody specific to  $\alpha$ -Smooth Muscle actin ( $\times 400$ ). In contrast to the fetus (Right,  $3.3 \pm 2.1$  fibroblasts/fields of view), the numbers of positive  $\alpha$ -Smooth Muscle actin antibody stained fibroblasts (arrow) is greater in the adult cotton implant capsular tissue group (Left,  $25.5 \pm 4.98$  fibroblasts/fields of view), as well as in the smooth muscle cells of the blood vessels (arrow heads).

stimulate growth factor production and fibroblast activation, all of which contribute to scar forma-

tion.<sup>11</sup> This study revealed that the histological and immunologic response of fetal tissue to for-

ign materials is characterized by a dominance of acute inflammatory responses, less fibrosis and collagen deposition than in adults. Furthermore, myofibroblast induction was greater in the cotton implant group where the inflammatory response was greater. As the wound healing process of the fetus and infant shows significant differences, the immunologic system also differs. However, the induction of an inflammatory response resulted in fibrosis, collagen deposition and myofibroblast formation, which showed some similarity to that of adults. Additionally, the fibroblast expressed according to the degree of inflammatory response also correlated with collagen deposition.

Capsule formation by the implant is the immunologic mechanism that isolates the foreign material by encapsulation when it cannot be destroyed or eliminated by an immunologic response between the contact surface between the implant and the body. According to Pasyk et al.,<sup>12</sup> the capsule was found to have four distinctive zones. The inner zone was composed of a fibrillar aggregation, long fibrin like fibers and cellular layer with macrophages. A fibrous layer contained elongated fibroblasts forming a central zone and myofibroblasts, which were pressed between very thick bundles of collagen fibers oriented parallel to the implant surface. The transitional zone contained looser bundles of collagen fibers and a few blood vessels. The outer zone was an established vascular layer with dilated blood vessels and newly formed small vessels. Similar findings were found in the capsule of the fetal implants on both sides of the outer layer with an extension of newly formed and dilated vessels. The immunologic response to be foreign body implants in the fetus was examined where wound healing is characterized by regeneration without scar formation. Linear and circumferential fibrosis was observed in all capsules. Capsules from the fetus implant revealed early connective tissue formation, which were relatively thin, less cellular, and less compactive. However, capsules from the adult group (cotton & silicone) were thick, irregular, and compactive. The thicker capsules, as a result of collagen deposition and fibrosis, were observed in the cotton implant than with the silicone implant. The fibrosis and collagen deposition of the fetal implant was some regular and

organized. It is believed that the capsule formation with enhanced fibrosis and collagen deposition in fetus, is a likely consequence of recruited neutrophils, eosinophils, and plasma cells at the sites of the perivascular areas.

The mechanisms that bring about the lack of scarring in fetal wounds relate to the control of collagen synthesis and fibrillogenesis.<sup>3-7</sup> The role of collagen in the fetal wound matrix is controversial.<sup>13-15</sup> Recent reports supports that the fetus lays down a collagen-rich repair matrix without scarring and fetal fibroblasts produce more type III and V collagen than their adult counterparts. The high concentration of hyaluronic acid (HA) present in fetal skin may suppress scar formation. Krummel et al.<sup>13</sup> implanted a PVA sponge within a perforated Silastic cylinder into fetal rabbits and reported no detectable hydroxyproline. In contrast, Adzick et al.<sup>14</sup> reported elevated hydroxyproline levels as a measure of collagen deposition in a rabbit model of fetal wound healing using implanted Gore-Tex. However, the histological events in fetal wound-healing animal models were reported to be similar to those found in the healing incisional model. Collagen deposition was greater in the cotton implant group where the foreign body reaction was greater than in the silicone implant group and the deposited collagen showed a more regular and organized pattern than that of adults. This finding suggests that collagen deposition plays an important role in capsule formation by a foreign body reaction. Moreover, it is more organized and less distorted in fetal tissue as shown by the scarless wound healing with the organization of the fetal wound matrix.

Wound contraction is a vital component of wound repair. However, in the extreme it may lead to excessive scar formation and pathologic wound contracture.<sup>16-18</sup> Myofibroblast have been implicated as the effector cell for open wound contraction and subsequent contracture formation.<sup>17</sup> This specialized fibroblast contains the contractile microfilaments of  $\alpha$ -smooth muscle (SM) actin, which may generate contractile forces. In the early and midgestational fetus, open dermal wounds expand and are characterized by a lack of myofibroblasts. The relative absence of inflammatory responses during the early and middle

prenatal periods may contribute to these findings. The acute inflammatory response was able to be induced by introducing of foreign body into the fetal tissue, and the capsule expressed myofibroblasts containing  $\alpha$ -smooth muscle actin; the contractile microfilaments. However, the fetal tissue specimen with the silicone implant showed a higher myofibroblast expression level, which is in contrast to predictions considering the lower inflammatory response. This finding is believed to be due to the position of the implant, which was placed nearer to the muscle layer.

In conclusion, the inflammatory response induced by a foreign material in fetal tissue showed similar response to that of incisional wound healing. This study might provide a basis for using implants such as silicone in future fetal surgery.

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