Scar Formation in the Fetal Alimentary Tract

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● **The aim of this study was to determine whether the fetal alimentary tract shares the unique scarless healing properties of fetal skin. Full-thickness incisional gastric wounds were created and sutured closed in fetal lambs at 60, 75, and 120 days' gestation (full term, 145 days), and in adult control sheep. At the time of harvest, 14 days postwounding, dense fibrous adhesions were found intraperitoneally in all fetal and adult animals. Histologically, all fetal and adult gastric wounds healed with pronounced scar formation. In contrast to the adult wound, there was no significant inflammatory response in the fetal wounds. Because scar formed in the absence of inflammation in fetal gastric wounds, there is no obvious relation between scarring and the inflammatory response at this location. This study shows that not all fetal tissues exhibit scarless repair properties.**

INDEX WORDS: Fetal wound healing, scar, alimentary tract.

SCARRING is a general and distinct feature of adult tissue repair. Excessive scar formation within the abdomen occurs after disease, trauma, and surgery, and leads to organ fibrosis, intraperitoneal adhesions, and anastomotic strictures. Whereas fetal skin heals without inflammation and scarring in humans[1,2] and in a variety of fetal animal models,[3,4] the healing properties of the fetal alimentary tract have not been formally investigated. The aim of this study was to determine whether fetal abdominal surgery with creation of a gastric wound is also followed by scar formation and intraperitoneal fibrous adhesions, as observed postnatally.

MATERIALS AND METHODS

Fetal gastric wounds were created in seven sheep fetuses at 60 days' gestation, in five fetuses at 75 days' gestation, and in two fetuses at 120 days' gestation (full term, 145 days). For the fetal sheep surgery, we used standard techniques previously described.[5] Briefly, the ewe underwent general halothane/oxygen anesthesia, the uterus was exteriorized through an infraumbilical midline laparotomy, and the fetus was exposed via a hysterotomy. Direct access to the fetal stomach was gained through a left subcostal fetal laparotomy. A 1.5-cm full-thickness wound into the stomach was made between two holding stitches using microscissors under ×3.5 loupe magnification. After marking the wound edges with India ink, the gastrotomy was closed with 6-0 Prolene interrupted sutures (Ethicon, Inc, Somerville, NJ), and the fetal laparotomy was closed. The fetus was returned to the uterus, and amniotic fluid volume was restored with warm sterile saline. The hysterotomy was closed with a TA-90 stapler (US Surgical Corporation, Norwalk, CT), the maternal laparotomy was closed in layers, and the ewe was returned to her stall. Gastric wounds were harvested 14 days after wounding. The fetus was delivered as described above and euthanized by intracardiac Beuthanasia-D (Schering, Kenilworth, NJ). Access to the stomach was gained through a separate laparotomy. The gastric wounds were excised, and the specimens were fixed in 4% paraformaldehyde and then processed for histology with H&E and Gomori's trichrome stain. Creation and harvesting of the gastric control wound in the adult ewe were performed analogously.

RESULTS

At the time of harvest, three fetal gastric wounds created at 60 days, four fetal wounds created at 75 days, two fetal wounds created at 120 days, and one adult gastric wound could be analyzed. The other fetuses (four operated on at 60 days and one at 75 days) were not used because of postoperative intrauterine demise or fetal laparotomy dehiscence with gastroschisis formation. All gastric wounds healed. In all fetuses, we found dense fibrous adhesions between the intestine and the abdominal wall, as well as between the bowel loops. Fibrosis was especially prominent in the area of the gastric wounds.

Histologically, all fetal gastric wounds showed scar formation to a similar extent (Fig 1), regardless of the gestational age at the time of wounding. The scarring was most extensive in the subserosal area, where abundant amounts of collagen bundles were deposited in a random, disorganized manner (Fig 2). The muscle layers showed a loss of tissue architecture, evidenced by disorientation and paucity of muscle fibers and by scant collagen deposition. The mucosal layers in all instances showed regeneration and were indistinguishable from those of the controls (Fig 3). There was no obvious inflammatory cell infiltrate in any fetal wounds.

The adult gastric wound had similar histological features, although the gastric wall was thicker and the tissues were more differentiated. Additionally, there was a large inflammatory cell infiltrate, with mononuclear and polymorphonuclear cells present.

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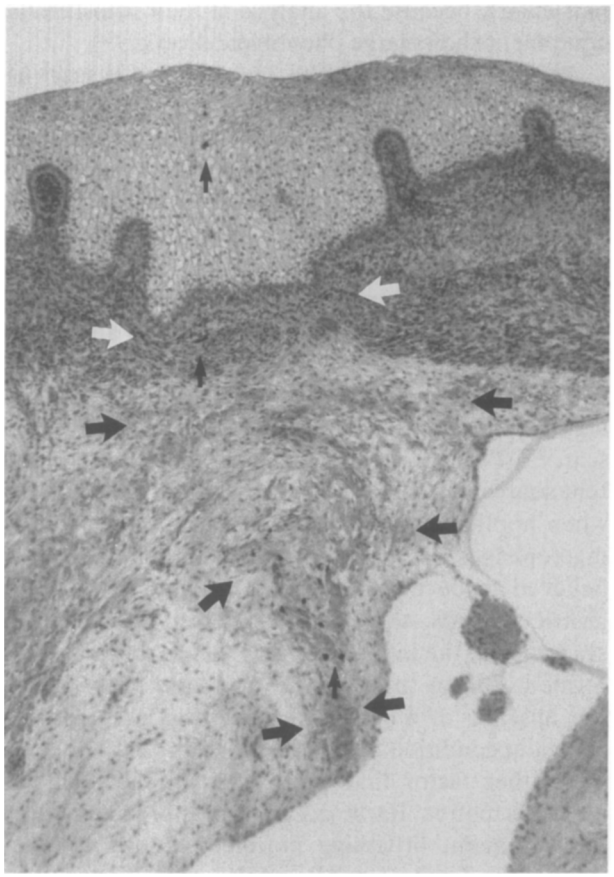

Fig 1. Fetal gastric wound created at 60 days' gestation and harvested 14 days later. The former wound site is identified by India ink particles (small black arrows). The mucosal layer has regenerated, the muscle layers show few and disoriented muscle fibers (white arrows), and there is significant subserosal scarring (large black arrows). (Gomori's trichrome stain, original magnification ×200.)

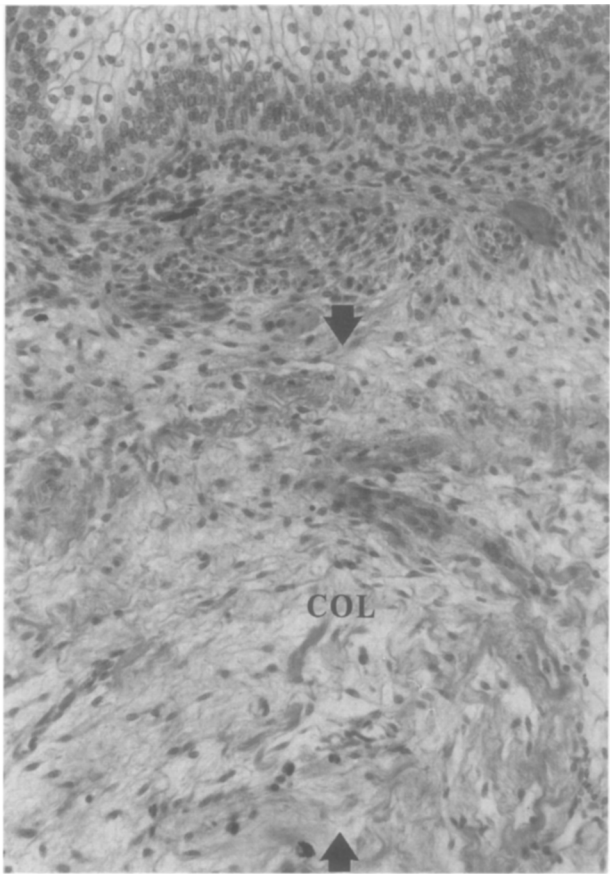

Fig 2. Detailed view of subserosal scarring (same wound as in Fig 1). Large amounts of collagen (COL) are deposited in a disorganized fashion (between arrows). (Gomori's trichrome stain, original magnification ×400.)

DISCUSSION

This study shows that midgestation fetal gastric wounds do not heal scarlessly, unlike the regenerative skin healing documented at the same gestational age.[6] Even when the wounds were created early in gestation (60 days), substantial subserosal scarring was found invariably. Furthermore, intraperitoneal adhesions occurred as observed postnatally. Three clinical correlates support these observations. First, intraperitoneal adhesions form after diaphragmatic hernia repair in human fetuses.[7] Second, in extremely premature (ie, "fetal-like") human infants, necrotizing enterocolitis can induce fibrotic intestinal strictures,[8] and abundant intraperitoneal adhesions form after laparotomy in these babies. Finally, there is increasing evidence that biliary atresia, another fibrotic intraabdominal condition, begins weeks before birth.[9]

Scar formation in the gastric wall was most intense in the subserosal connective tissue; it was minimal near and within the muscle layers. Analogous findings have been noted in rat skin wounds, where scarring

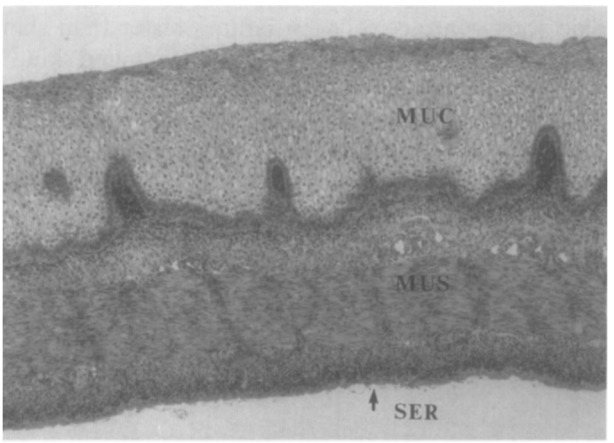

Fig 3. Normal anatomy of the fetal stomach at 90 days' gestation. MUC, mucosa; MUS, muscle layers; SER, serosa. (Gomori's trichrome stain, original magnification ×200.)

was minimal near the dermo-epidermal junction and more intense in the deep dermis.[10] Interestingly, in the upper dermis of adult skin, the collagen composition and configuration are more fetal-like, with a greater preponderance of collagen type III.[11]

Fetal striated muscle repair studies have been performed previously. Cheek and lip wounds in the fetal rat,[12,13] rabbit,[14] sheep,[15] and rhesus monkey[16] healed without scarring and with regeneration of the striated muscle elements. In the monkey, muscle regeneration occurred only in midgestation and was lost before collagen scar formation occurred.[16] In contrast, fetal sheep diaphragmatic muscle wounds created at 100 days' gestation healed with scar formation, regardless of whether the diaphragm was exposed to amniotic fluid.[17]

Previous studies in our laboratory have shown that scarless fetal skin repair can occur in the absence of amniotic fluid or perfusion by fetal blood.[2] Conversely, adult skin wounds scar even when placed in the sterile, intraamniotic milieu.[18] These findings suggest that the type of healing response is intrinsic to different fetal tissues. Why do fetal skin and gastric tissue heal differently? Because fibroblasts are responsible for the production and deposition of various collagen types, the different healing patterns may be caused by tissue-specific fetal fibroblast phenotypes. One permits healing without scarring ("nonscarring fibroblast"), and the other permits or promotes scar formation ("scarring fibroblast"). For example, cultured adult human dermal fibroblasts and colon fibroblasts are affected differently by a variety of cytokines and other regulatory factors, suggesting that adult gastrointestinal fibroblasts and dermal fibroblasts are phenotypically different.[19] Even dermal fibroblasts can have disparate properties, because cultured adult human fibroblasts from hypertrophic scars contract collagen lattices faster than skin fibroblasts from the same person's unwounded skin,[20] and human fetal and adult dermal fibroblasts respond differently to transforming growth factor beta (TGFβ).[21] Alternatively, different fetal wound environments might induce changes in fetal fibroblast behavior. Because adult fibroblasts can be altered in phenotype by the wound environment,[22] it is conceivable that fetal wounds in different tissues exhibit unique matrix and cytokine profiles that regulate fibroblasts differently. It is becoming evident that fibroblasts represent a heterogenous cell population, particularly because the analysis of their cytoskeletal equipment shows large phenotypic diversity.[23]

Another cell type possibly involved in the scarring process of the fetal alimentary tract is the fetal intestinal smooth muscle cell. Cell culture studies have shown that human fetal intestinal smooth muscle cells produce twice as much collagen as do human fetal dermal fibroblasts.[24] Thus, fetal intestinal smooth muscle cells also may produce large amounts of disorganized collagen during intestinal repair.

A striking difference between fetal and adult skin healing is the absence of inflammatory cells in early and midgestational skin wounds.[25] The poor inflammatory response has been related to the absence of scar.[18,26] For instance, adult macrophages are a potent source of TGFβ, a cytokine that induces fibrosis when applied to wounds. The collective paucity of macrophages, TGFβ, and scar in fetal skin wounds is believed to be interdependent.[27] However, in fetal gastric wounds, there is no obvious link between scarring and the inflammatory response, because scar formed even in the absence of inflammation. Thus, the absence of an inflammatory response is not a sufficient condition for scarless repair.

Another factor that could affect the healing response is motion. Early second-trimester lamb fetuses have frequent breathing movements with contractions of the diaphragm.[28] Peristalsis of the gastrointestinal tract is present at this stage of development; amniotic fluid is swallowed and transported to the small bowel. Motion is present in the fetal stomach and diaphragm, which scar, and movement may be less or absent in the skin, which does not scar.

In summary, this study shows that fetal abdominal surgery causes fibrous intraperitoneal adhesions and scarring, as occur postnatally. Thus, not all fetal tissues exhibit scarless repair properties. Since scar formed in the absence of inflammation in the fetal gastric wounds, there is no direct cause-and-effect relation between scarring and the inflammatory response. Because human fetal surgery is now a clinical reality in the treatment of highly selected life-threatening diaphragmatic, thoracic, and urologic malformations, these findings have clinical implications.

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REFERENCES

1. Harrison MR, Adzick NS: The fetus as a patient: Surgical considerations. Ann Surg 213:279-290, 1991

2. Lorenz HP, Longaker MT, Perkocha LA, et al: Scarless wound repair: A human fetal skin model. Development 114:253-259, 1992

3. Adzick NS, Longaker MT: Scarless fetal healing: Therapeutic implications. Ann Surg 215:3-7, 1992

4. Longaker MT, Adzick NS: The biology of fetal wound healing: A review. Plast Reconstr Surg 87:788-798, 1991

5. Harrison MR, Jester JA, Ross NA: Correction of congenital diaphragmatic hernia in utero. I. The model: Intrathoracic balloon produces fatal pulmonary hypoplasia. Surgery 88:174-182, 1980

6. Longaker MT, Whitby DJ, Adzick NS, et al: Studies in fetal wound healing VI. Second and early third trimester fetal wounds demonstrate rapid collagen deposition without scar formation. J Pediatr Surg 25:63-69, 1990

7. Harrison MR, Langer JC, Adzick NS, et al: Correction of congenital diaphragmatic hernia in utero V. Initial clinical experience. J Pediatr Surg 25:47-57, 1990

8. Schwartz MZ, Richardson CJ, Hayden CK, et al: Intestinal stenosis following successful medical management of necrotizing enterocolitis. J Pediatr Surg 15:890-899, 1980

9. MacGillivray TE, Adzick NS: Biliary atresia begins before birth. Pediatr Surg Int 9:116-117, 1994

10. Levenson SM, Geever EF, Crowley LV, et al: The healing of rat skin wounds. Ann Surg 161:293-307, 1965

11. Fleischmajer R, Perlish JS, Burgeson RE, et al: Type I and type III collagen interactions during fibrillogenesis. Ann N Y Acad Sci 580:161-175, 1990

12. Robinson BW, Goss AN: Intra-uterine healing of fetal rat cheek wounds. Cleft Palate J 18:251-259, 1981

13. Rowsell R: The intra-uterine healing of foetal muscle wounds: Experimental study in the rat. Br J Plast Surg 37:635-642, 1984

14. Kaban LB, Longaker MT, Stern M, et al: Wound healing and facial growth after cleft lip repair. Oral Maxillofac Surg Clin North Am 3:735-745, 1991

15. Longaker MT, Stern M, Lorenz HP, et al: A model for fetal cleft lip repair in lambs. Plast Reconstr Surg 90:750-756, 1992

16. Lorenz HP, Whitby DJ, Longaker MT, et al: Fetal wound healing: The ontogeny of scar formation in the non-human primate. Ann Surg 217:391-396, 1993

17. Longaker MT, Whitby DJ, Jennings RW, et al: Fetal diaphragmatic wounds heal with scar formation. J Surg Res 50:375-385, 1991

18. Longaker MT, Whitby DJ, Ferguson MWJ, et al: Adult skin wounds in the fetal environment heal with scar formation. Ann Surg 219:65-72, 1994

19. Martens MFWC, Huyben CMLC, Hendriks T: Collagen synthesis in fibroblasts from human colon: Regulatory aspects and differences with skin fibroblasts. Gut 33:1664-1670, 1992

20. Garner W, Rittenberg T, Ehrlich P: Hypertrophic scar fibroblasts accelerate wound contraction. Surg Forum 44:714-717, 1993

21. Lorenz HP, Chang J, Banda MJ: Transforming growth factors beta 1 and beta 2 synergistically increase collagen gene expression in fetal fibroblasts but not in adult fibroblasts. J Cell Biochem 17E:137-144, 1993

22. Regan MC, Kirk SJ, Wasserkrug HL, et al: The wound environment as a regulator of fibroblast phenotype. J Surg Res 50:442-448, 1991

23. Sappino AP, Schuerch W, Gabbiani G: Differentiation repertoire of fibroblastic cells: Expression of cytoskeletal proteins as marker of phenotypic modulations. Lab Invest 63:144-161, 1990

24. Perr HA, Grider JR, Mills AS, et al: Collagen production by human smooth muscle cells isolated during intestinal organogenesis. Anat Embryol 185:517-527, 1992

25. Ferguson MWJ, Howarth GF: Marsupial models of scarless fetal wound healing, in Adzick NS, Longaker MT (eds): Fetal Wound Healing. New York, NY, Elsevier, 1992, pp 95-124

26. Adzick NS, Longaker MT: Characteristics of fetal tissue repair, in Adzick NS, Longaker MT (eds): Fetal Wound Healing. New York, NY, Elsevier, 1992, pp 53-70

27. Whitby DJ, Ferguson MWJ: Immunohistochemical studies of the extracellular matrix and soluble growth factors in fetal and adult wound healing, in Adzick NS, Longaker MT (eds): Fetal Wound Healing. New York, NY, Elsevier, 1992, pp 161-176

28. Pooro ER, Walker DW: Chest wall movements during fetal breathing in sheep. J Physiol 301:307-315, 1980