
Experimental

The Fetal Cleft Palate: III. Ultrastructural and Functional Analysis of Palatal Development following In Utero Repair of the Congenital Model

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The role of fetal surgery in the management of congenital anomalies and intrauterine abnormalities is appropriately restricted on the basis of feasibility and risk-to-benefit analyses of intrauterine intervention. Recently, the authors demonstrated that in utero cleft palate repair of the congenital caprine model is technically feasible and results in scarless healing of the mucoperiosteum and velum, with subsequent development of a potentially functional bilaminar palate with distinct oral and nasal mucosal layers, following single-layer repair of the fetal mucoperiosteal flaps. A slight indentation at the site of repair was the only remaining evidence of a cleft. At 6 months of age, normal palatal architecture, including that of mucosal, muscular, and glandular elements, was seen grossly and histologically. The present work investigated the ultrastructural and functional aspects of the palate following in utero cleft repair to determine what benefits might be derived from fetal intervention. Six goats pregnant with twins were gavaged twice daily for 10 days (gestational days 32 to 41; term, 145 days) with dry, ground *Nicotiana glauca* plant delivering between 2.4 and 14 mg/kg per day of anabasine, doses that were adjusted in response to maternal toxicity. At 85 days' gestation, six fetuses underwent in utero palatoplasty using a modified von Langenbeck technique with elevation of bilateral mucoperiosteal flaps and lateral relaxing incisions. A single-layer repair of the mucoperiosteal flaps was performed using interrupted 6-0 Vicryl sutures. Six fetuses remained as unrepairs clefted controls. Six months after in utero palatoplasty, each group of goats underwent nasoendoscopy to evaluate palatal function; two unclefted 6-month-old goats served as controls. Subsequently, soft palate muscle was harvested from each of the goats and was evaluated by light and electron microscopy. Velar muscle was also harvested from the unclefted control goats and was similarly studied. Nasoendoscopy demonstrated functional palates capable of

dynamic velopharyngeal closure following in utero cleft repair; this motion was similar to that observed in unclefted animals. Unrepaired clefted goats did not demonstrate any evidence of velar motion or velopharyngeal closure. Soft palate muscle from this group demonstrated evidence of myofibril degeneration, atrophy, and loss compared with unclefted control velar muscle. Ultrastructural changes included sarcomere "scalloping," partial Z-line degeneration and loss, and progressive I-band degeneration and loss. Repaired clefted soft palate muscle was remarkably similar to unclefted control muscle. Significantly less myofibril, Z-line, and I-band degeneration and loss were observed with minimal evidence of sarcomere scalloping. In utero cleft palate repair results in a functional soft palate with restoration of ultrastructural architecture of the velum. These findings were attributed to reconstitution of the velar muscular sling, which is disrupted during the clefting process and remains abnormally inserted into the posterior edge of the palatal bone and along the bony cleft. Although repaired velar muscle does demonstrate some evidence of ultrastructural change compared with control muscle, these findings are significantly less pronounced than those observed in the unrepaired clefted muscle. (*Plast. Reconstr. Surg.* 109: 2355, 2002.)

Fetal surgery was pioneered almost two decades ago specifically for the management of congenital anomalies and intrauterine abnormalities that are incompatible with life if not treated prenatally.¹⁻⁵ The benefit of a procedure that potentially prevents fetal loss is obvious. The benefit of a procedure that poten-

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tially jeopardizes fetal viability is certainly less clear. With recent advances in fetal surgery techniques and technology and efforts to reduce maternal morbidity, the indications for intrauterine intervention have broadened slightly to include myelomeningocele reconstruction in selected cases.⁶⁻⁹ Although this condition is not life-threatening, the potential benefit of fetal surgery in preventing significant complications, including paralysis and loss of bladder and bowel function, is substantial. As techniques become further refined over the next several decades and fetal intervention poses less serious risks to fetal viability and maternal safety, it is likely that the indications for prenatal surgery will further expand. However, no procedure should be performed before proven and predictable benefits have been demonstrated, regardless of the feasibility of the operation.

Recently, we demonstrated that in utero cleft palate repair of the congenital caprine model is technically feasible.^{10,11} Moreover, intrauterine palatoplasty results in scarless healing of the mucoperiosteum and velum, with subsequent development of a potentially functional bilaminar palate with distinct oral and nasal mucosal layers, following single-layer repair of the fetal mucoperiosteal flaps. A slight indentation at the site of repair was the only remaining evidence of a cleft. At 6 months of age, normal palatal architecture, including that of mucosal, muscular, and glandular elements, was seen grossly and histologically. The present work investigated the ultrastructural and functional aspects of the palate following in utero cleft repair to determine what benefits might be derived from fetal intervention.

METHODS

Cleft Palate Induction

The method used for the induction of palatal clefting in the congenital caprine model has been previously described by us,¹⁰ and the description herein is therefore abbreviated. Six goats pregnant with twins were gavaged twice daily for 10 days (gestational days 32 to 41; term, 145 days) with dry, ground *Nicotiana glauca* plant, delivering approximately 2.4 to 14 mg/kg per day of the teratogenic alkaloid anabasine. We have previously described the process by which *N. glauca* and the dosage administered is titrated to the maternal response.¹⁰ Pregnancy had been confirmed in each case on day 30 of gestation by ultrasound

(Aloka 500V ultrasound with the 5-MHz medium animal abdominal probe, Corometrics Medical Systems, Inc., Wallingford, Conn.); the day of breeding is referred to as day 0. After completion of the 10-day treatment period and on day 70 of gestation, the goats were again examined by ultrasound to determine fetal viability and confirm the presence of twin fetuses. Using this protocol, we have produced complete clefting of the secondary palate in more than 98 percent of treated fetuses.^{10,11}

In Utero Cleft Palate Repair

At 85 days' gestation, six clefted fetuses from three pregnant goats underwent in utero palatoplasty using a modified von Langenbeck technique, as we have previously described.¹¹ After administration of general endotracheal anesthesia, infusion of prophylactic antibiotics (ceftriaxone, 1 g intravenously), and fetal head exposure through a conservative hysterotomy incision, lateral relaxing incisions were performed through the full thickness of the mucoperiosteal palatal flaps extending from the alveolar ridge, approximately 2 to 4 mm lateral to the anterior aspect of the cleft, posteriorly 20 to 22 mm. An incision along the medial edge of the cleft on either side was extended from the anterior point of the cleft posteriorly 20 to 25 mm, depending on the curvature of the posterior velum and the feasibility of repair at that level. The soft palate musculature was dissected free from its abnormal insertion along the posterior edge of the maxilla. Bilateral mucoperiosteal flaps were elevated from the underlying palatal bone from medial to lateral, extending to the lateral relaxing incisions. A single-layer tension-free midline closure of these flaps was performed using interrupted 6-0 Vicryl sutures. The lateral bare areas were left open. Before repair of the hysterotomy incision, intrauterine administration of another dose of ceftriaxone (500 mg for each uterine horn that was operated on) and amniotic fluid replacement with warm normal saline were performed. Six clefted fetuses from three pregnant treated goats remained as unrepairs controls.

Analyses

Nasoendoscopy. When the goats were 6 months of age, each group underwent nasoendoscopy to evaluate soft palate motion and velopharyngeal closure; two unclefted 6-month-old goats served as untreated controls. This was performed using topical 2% lidocaine spray to min-

imize discomfort of the nasal and pharyngeal passages. Velar motion and velopharyngeal closure were observed over a 10-minute period before and after phonation. Gentle stimulation of the goats by stroking the neck or abdomen resulted in phonation in all animals.

Electron microscopy. After the animals were killed with an overdosage of Beuthanasia-D (Schering-Plough, Kenilworth, N.J.) administered intravenously, soft palate muscle was harvested from each of the clefted goats and evaluated by light and electron microscopy. The velar muscle was harvested from three specific midline sites: (1) immediately posterior to the posterior edge of the hard palate, (2) the posterior margin of the uvula, and (3) an arbitrary point equidistant between these two sites. Soft palate muscle was similarly harvested from the unclefted control goats and studied. Tissue obtained for histologic evaluation was fixed in 10% formalin; tissue obtained for electron microscopy was fixed in Karnovsky fixative (2.5% glutaraldehyde/2% paraformaldehyde).

Statistical analysis. Fisher's exact test was used to assess differences observed in soft palate motion and velopharyngeal closure between the unrepaired and repaired clefted goats.

RESULTS

All 12 fetuses from six treated pregnant goats demonstrated complete clefts of the secondary palate. In addition, all fetuses survived the *in utero* palatoplasty and the duration of the experimental period until they were killed at 6 months of age. There was no incidence of preterm labor or other complications, including palatal repair dehiscence, fistula formation, or infection, throughout the perioperative and experimental periods.

Nasoendoscopy

Each of the goats tolerated the nasoendoscopy procedure. Unclefted control goats dem-

onstrated strong, consistent motion of the velum and lateral pharyngeal walls (Table I). With stimulation and phonation, velopharyngeal closure occurred in each animal (Figs. 1 and 2). Unrepaired clefted goats did not demonstrate any evidence of palatal motion or elevation or velopharyngeal closure with phonation. With transnasal passage of the endoscope, the wide clefts of the palatal shelves were easily appreciated from above and the tongue was clearly visualized between the palatal defect in each case; with transoral passage of the endoscope, the vomer was easily observed through the palatal defect in each case. Repaired clefted goats demonstrated strong motion of the velum and lateral pharyngeal walls, similar to that produced by the unclefted control goats. With phonation, velopharyngeal closure occurred in each case, demonstrating functional improvement when compared with the unrepaired clefted group. The difference in palatal motion observed between the unrepaired and repaired clefted goats was statistically significant ($p = 0.0022$).

Histologic Evaluation

At the light microscopy level, only minor changes were observed when comparing the unrepaired and repaired palatal muscle with control palatal muscle. Increased vacuolization, a potential indicator of cellular atrophy, was noted in the unrepaired clefted muscle. Repaired clefted muscle did not demonstrate increased vacuolization compared with control muscle. Because these findings were insignificant, histologic sections are not herein presented.

Electron Microscopy

Using transmission electron microscopy (Phillips 300 electron microscope, Phillips Electronics, Eindhoven, The Netherlands), velar muscle from both surgically repaired and unrepaired clefted goats was studied and com-

TABLE I
Palatal Motion and Ultrastructural Changes following *In Utero* Cleft Palate Repair

	Soft Palate Motion	Velopharyngeal Closure	Ultrastructural Changes
Control (unclefted)	Strong	Complete	None
Unrepaired (clefted)	None	None	Severe myofibril degeneration Severe sarcomere "scalloping" Severe Z-line and I-band degeneration
Repaired (clefted)	Strong*	Complete	Minimal myofibril degeneration Minimal sarcomere "scalloping" Minimal Z-line and I-band degeneration

* $p = 0.0022$, compared with the unrepaired group, using Fisher's exact test.

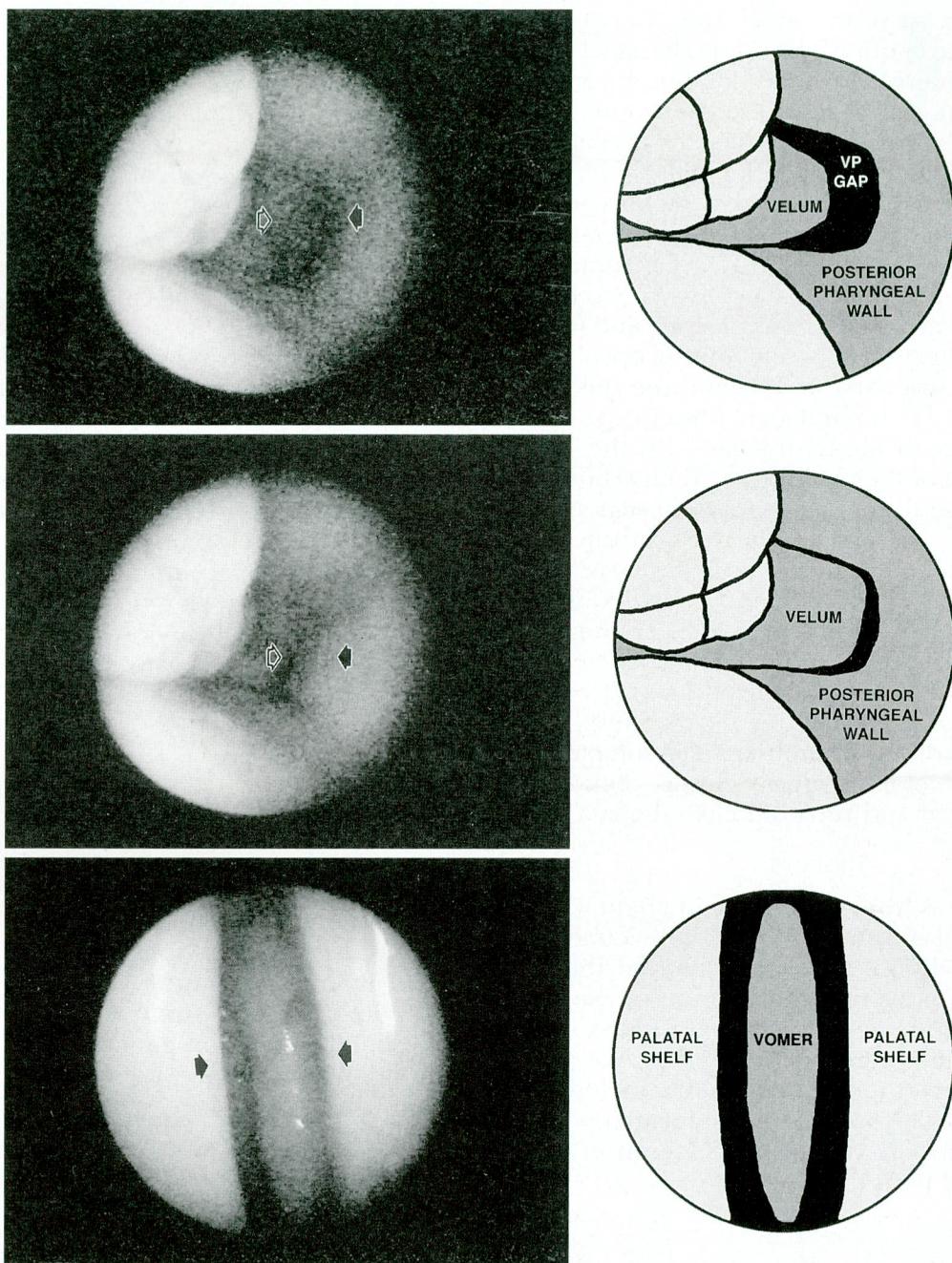


FIG. 1. Nasoendoscopy following in utero cleft repair. The control, unclefted palate (*above* and *center*) demonstrates normal motion of the soft palate with phonation, resulting in velopharyngeal closure (*above*, before phonation; *center*, during phonation; *clear arrows* demonstrate the velum and *solid arrows* demonstrate the posterior pharyngeal wall). The unrepaired cleft palate (*below*) demonstrates no evidence of palatal motion or velopharyngeal closure with phonation. The vomer is seen between the palatal shelves of the wide cleft when viewed from below (*below*). VP, velopharyngeal.

pared with muscle obtained from unclefted control goats. Ultrastructural analysis and comparison of individual sarcomeres were performed in this manner. Soft palate muscle from the unrepaired clefted group demonstrated evidence of myofibril degeneration, at-

rophy, and loss compared with unclefted control velar muscle (Fig. 3). Ultrastructural changes included sarcomere "scalloping," partial Z-line degeneration and loss, and progressive I-band degeneration and loss. Repaired clefted soft palate muscle was not indistinguish-

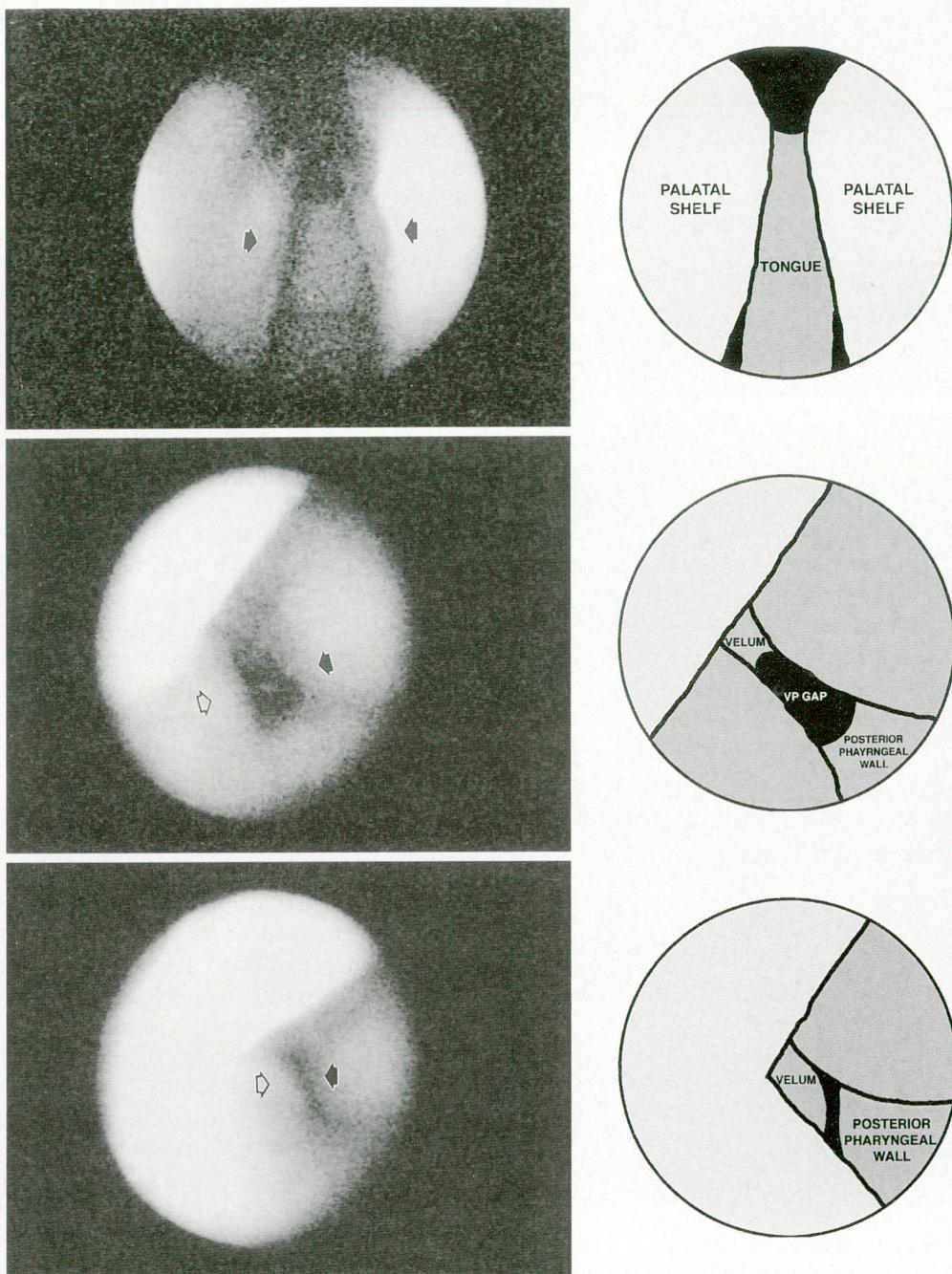


FIG. 2. (Above) The tongue is seen through the palatal defect when viewed from above (arrows demonstrate the unfused palatal shelves). The repaired cleft palate (center and below) demonstrates significant motion with phonation, similar to the unclefted palate, resulting in velopharyngeal closure (center, before phonation; below, during phonation; clear arrows demonstrate the velum and solid arrows demonstrate the posterior pharyngeal wall). VP, velopharyngeal.

able from unclefted control muscle. However, significantly less myofibril, Z-line, and I-band degeneration and loss were seen in the repaired clefted muscle compared with the un-repaired clefted muscle. In addition, there was minimal sarcomere scalloping in the repaired clefted muscle.

DISCUSSION

In utero cleft palate repair results in a functional soft palate with restoration of the ultrastructural architecture of the velum. We attribute this finding to intrauterine reconstitution of the velar muscular sling, which is disrupted in the

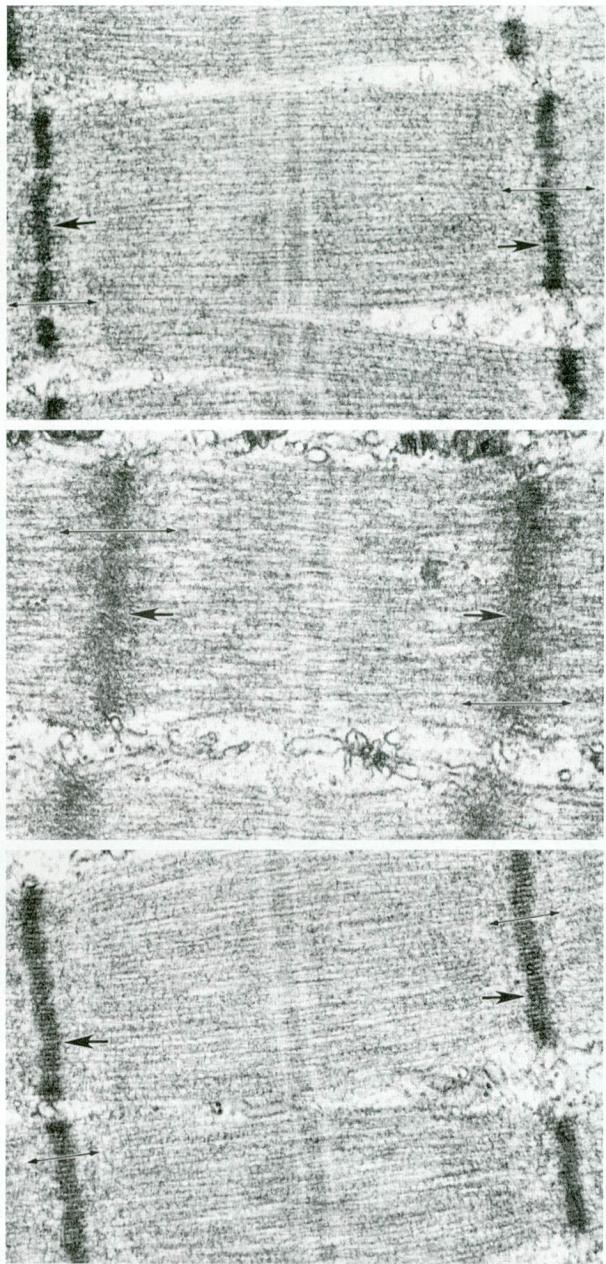


FIG. 3. Ultrastructural analysis of soft palate muscle. (Above) Control velar muscle. (Center) Unrepaired cleft velar muscle. Significant Z-line and I-band degeneration is seen. (Below) Repaired cleft velar muscle demonstrates significantly less myofibril atrophy and Z-line and I-band degeneration (original magnification, $\times 23,490$) (single-headed arrows demonstrate the Z-lines in each case; double-headed arrows demonstrate the I-band in each case.)

clefting process and remains abnormally inserted into the posterior edge of the palatal bone and along the bony cleft. Unrepaired velar muscle demonstrates significant evidence of ultrastructural sarcomeric change compared with control muscle. These ultrastructural findings are much

less pronounced in clefted muscle repaired in utero.

The "sliding filament theory" proposes that when muscle contracts, the thick (myosin) and thin (actin) filaments maintain the same length as in resting muscle, but the actin filaments move relative to the myosin filaments (Fig. 4).^{12,13} In doing so, the actin filaments slide more deeply between the A-bands (consisting of myosin molecules attached to a disklike zone represented by the M-line), and shorten the sarcomere along the entire length of the myofibril. The actin filaments are attached to a similar disklike zone represented by the Z-line. The exact manner in which actin and myosin molecules are affected in their structure or interaction on a mechanistic level because of clefting remains undetermined. Similarly, the specific effect of in utero cleft repair on subsequent interaction and function of these molecules has not yet been characterized.

"Myofibrillary degeneration" refers to the changes that affect myofilament and Z-line structure in myofibers undergoing degeneration and atrophy from various causes.¹² Such changes include "streaming of the Z-line," in which case the Z-line develops a zigzag or jagged appearance and the Z-line material extends into the I-band and A-band. An advanced stage of streaming ultimately results in disintegration of the Z-lines and the adjacent bands.

In the present work, Z-line and I-band degeneration were both associated with the unrepaired cleft palate muscle. This morphologic change likely represents a form of disuse atrophy and ultrastructural disintegration caused by the abnormal muscular insertion on the posterior edge of the clefted bony palate that prevents motion and produces a static muscular state. Although in utero palate repair does not permit specific intravelar veloplasty, as the individual muscular layer cannot be dissected free in the 85-day-old fetus, single-layer repair of the mucoperiosteal layers anteriorly and mucomuscular layers posteriorly at this gestational period does permit subsequent differentiation and development of the individual palatal layers.

Our earlier finding that intrauterine palatoplasty results in scarless healing of the mucoperiosteum and velum with subsequent development of a bilaminar palate with distinct oral and nasal mucosal layers following single-layer repair of the fetal mucoperiosteal flaps¹¹ suggests that restoration of anatomic structure and organization during gestational development can potentially minimize subsequent functional impairment.

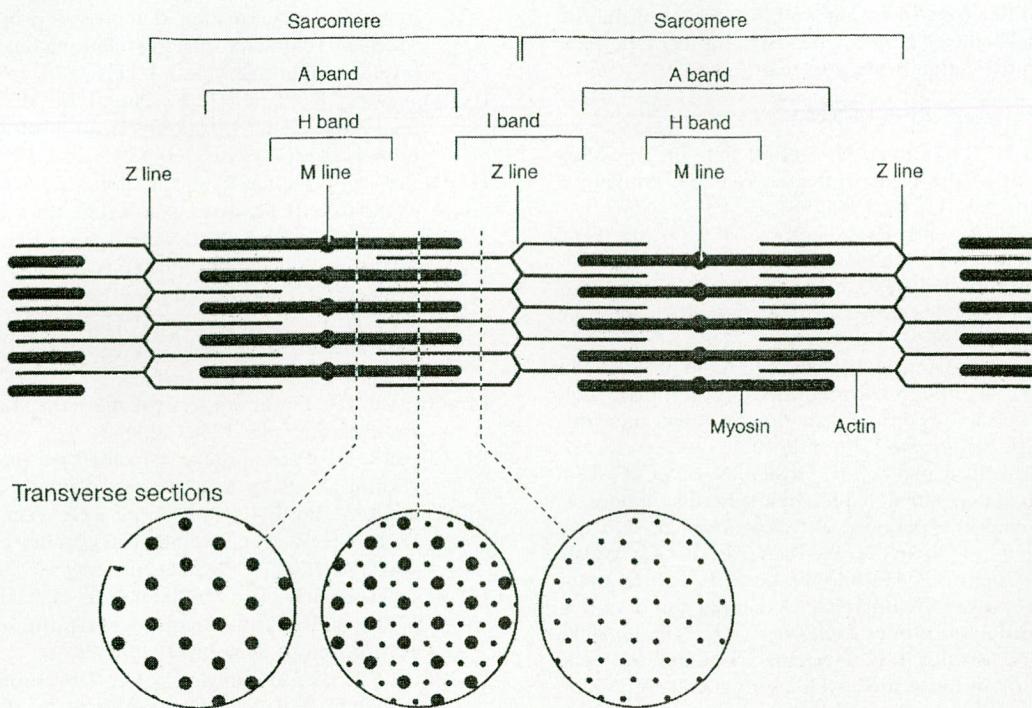


FIG. 4. Diagram of the ultrastructural architecture of the sarcomere. The I-band, an area of low electron density, is composed entirely of actin filaments without myosin filament overlap; the Z-line represents the disklike zone to which the actin filaments are attached. The H-band and M-line represent similar zones involving myosin filaments. (Modified from Burkitt, H. G., Young, B., and Heath, J. W. *Wheater's Functional Histology*, 3rd Ed. Edinburgh: Churchill Livingstone, 1993. Used with permission.)

ment. In the present study, the use of nasoendoscopy demonstrated functional palates capable of dynamic velopharyngeal closure following in utero cleft repair; this motion was similar to that observed in unclefted palates.

Dr. Christ stated that "human fetal surgery is an opportunity to decisively intervene and prevent long-term permanent anatomic deformities and/or abrogate the devastating psychological and emotional effects of these gross anatomic deformities."^{14,15} We agree. More than a decade ago, the potential ability of the fetus to heal scarlessly was established.¹⁶ However, the functional ramifications of such a finding have not been previously shown. The present work is the first to demonstrate a clear benefit of in utero cleft palate repair—the development of a functional palate in the absence of palatal scarring. Such an advantage could potentially translate to a decreased incidence of speech impairment in human cleft patients following intrauterine palatoplasty.

Despite this exciting finding, the evolution and practice of human fetal surgery must proceed prudently while technology and methods are refined to safely permit prenatal diagnosis

and intrauterine correction of anomalies that do not threaten fetal viability.¹⁷⁻²⁰ Until fetal intervention is feasible and practical for such nonlethal anomalies as cleft palate, investigations using our congenital caprine model will continue to explore a myriad of questions related to this fascinating and complex anomaly.

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