Defining the anatomy of the neonatal lingual frenulum

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Introduction:

The lingual frenulum usually achieves only a brief and vague mention in anatomy textbooks. In 1858, the first edition of *Anatomy* by Dr Henry Gray describes the lingual frenulum as a distinct fold of mucous membrane formed beneath the under surface of the tongue (Gray, 1858). There have been no significant changes to the frenulum's description in the most recent edition of this anatomy text, suggesting that there has been no increase in knowledge of frenulum structure over the intervening 150+ years (Standring, 2016). More recent

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descriptions of lingual frenulum structure in social media and specialized books have conceptualized the lingual frenulum to be a midline submucosal connective tissue structure, describing it as a string, band or "mast" (Ghaheri, 2014; Baxter, 2018), but there is no identifiable source for how this anatomical construct was developed.

Ankyloglossia is a clinical diagnosis, when the lingual frenulum is thought to be restricting tongue mobility. However, as the relationship between structure and functional limitation is still uncertain, diagnosis of ankyloglossia remains subjective. With ankyloglossia now being discussed widely in social media forums, the term "tongue tie" has gained popularity to describe both the condition of restricted tongue mobility and the lingual frenulum itself. Current popular grading systems for ankyloglossia utilize a single feature of frenulum appearance, the height of attachment of the frenulum on the ventral surface of the tongue (Kotlow, 1999; Coryllos et al., 2004). As these grading systems encompass all possible morphological variants of the lingual frenulum, they create the possibility of any frenulum being allocated a grade of ankyloglossia and therefore be considered "abnormal." The use of these grading systems, together with a paucity of knowledge of frenulum anatomical structure seem to have contributed to confusion regarding what is "normal" frenulum anatomy. As a lingual frenulum is present in almost all infants (Haham et al., 2014), the presence of a frenulum should not in itself be considered abnormal or diagnostic of ankyloglossia. Furthermore, no direct correlation has been shown between these gradings and the presence of breastfeeding difficulties or outcomes following frenotomy. This suggests that there is a wider range of anatomical variables that impact on the tongue mobility and function than this single feature of lingual frenulum appearance used in isolation.

The subjective nature of ankyloglossia diagnosis is a major dilemma for both clinical practice and research, as well as potentially creating parental confusion. It has become a source of conflict within breastfeeding support communities, when beliefs around the diagnosis of ankyloglossia and promotion of frenotomy become divergent. With a dramatically increasing rate of diagnosis and concomitant surgical intervention for ankyloglossia reported in extensive audits in a number of countries (Joseph et al., 2016; Lisonek et al., 2017; Kapoor et al., 2018), there is concern that this reflects a trend for potential over-diagnosis, such that a number of infants with a normal anatomy and tongue function are being recommended for intervention.

The popular structural concept of the lingual frenulum being a midline submucosal string, band or "mast" (Watson-Genna, 2013; Ghaheri, 2014; Baxter, 2018) has recently been replaced with the understanding that the adult lingual frenulum is formed by a layer of fascia that spans the floor of mouth (Mills et al., 2019). Tongue movement creates tension in the fascial layer, dynamically raising the fascia and overlying oral mucosa into a midline fold, recognizable as the lingual frenulum. This research has provided a structural explanation for the spectrum of lingual frenulum morphology. Variability in the relative gliding of the mucosal and fascial layers with tongue movement (together with the underlying suspended genioglossus) alters the transparency, thickness and shape of the lingual frenulum. However, there is no research or publications on comparative anatomy of the fetal, neonatal and adult in-situ lingual frenulum, therefore uncertainty remains as to whether this new anatomical construct of the lingual frenulum is applicable to infants and young children. As embryological studies have shown that the oral mucosa and associated connective tissues develop by 22 weeks gestation (Winning and Townsend, 2000), we hypothesized that the floor of mouth fascia is present at birth and the lingual frenulum structure will be similar to what was seen in adult cadavers. Using neonatal fresh tissue cadavers, this study assessed the anatomical structure of the in-situ lingual frenulum, to identify individual variations in morphology, allowing comparison with adult anatomy and discussion of potential implications for lingual frenulum surgery.

Materials and Methods:

The four fresh tissue neonatal cadavers used in this research were donated to the University of Pretoria Department of Anatomy. Ethical consent was obtained (Ethical clearance: 540/2018) from the local Research Ethics Committee from the Faculty Health Sciences with compliance for tissue use for research purposes under the South African National Health Act (2003).

Basic demographic data

The sample comprised of two male and two female neonatal cadavers, three having died at delivery and one at thirteen days post-birth. Weight, head and body length are detailed in Table 1. Gestational age was not available but was estimated based on anthropometrics. None of the infants had any dysmorphic features or craniofacial malformations. No other clinical information on the cadavers was available.

Harvesting, dissection and imaging of specimens

All dissections were performed by the first author who has 20 years of clinical experience in otolaryngologic surgery and three years' experience as a professional anatomy dissector. All dissections were performed using a Ziess Superlux 301 Microscope with a scalpel (using number 11 blades) and fine iris scissors. Images were recorded with a Canon EOS500D with a Macro EF 100mm lens with an Amaran HC100 Halo ring flash. An SPi 2000 calliper with 0.1mm graduations was used for all linear measurements, with a flexible plastic ruler with 1mm graduations used for measurement of curved surfaces.

Step 1:

Lateral (profile) and frontal photographs were captured of all cadavers to enable scaled measurement of:

- Head length (frontal image): crown to chin length
- Maxilla versus mandible position (profile image): soft tissue measurements taken at vermillion border upper lip (maxilla) and lower lip (mandible)

Step 2:

Division was made bilaterally of:

- the mandible, immediately anterior to the ramus

- all posterior extrinsic muscles at the lateral body of the tongue

- all extrinsic muscular attachments at the superior border of the hyoid

The body of the mandible, the tongue and the epiglottis were then removed en-bloc.

Step 3:

Once the mandible and tongue had been removed, images were captured of the hard and soft palate and of the excised specimen. Measurements were taken to record palate and tongue dimensions (Table 2):

The hard and soft palate: (Fig. 1)

- Hard palate length (midline, from gingival margin to junction of hard and soft palate)
- Hard palate width (inner and outer width of mid palate and outer width of posterior palate)
- Soft palate length (midline, from junction of hard and soft palate to base of uvula)

The tongue: using a flexible ruler with 1mm graduations: (Fig. 2)

- Full length (base of vallecula to tip of tongue)
- Tongue width (maximum width of dorsum)
- Anterior tongue length (ventral tongue surface: connection with floor of mouth to tip of tongue)
- Anterior “free” length (from frenulum attachment on ventral surface to tip)
- Height of attachment of fold of lingual frenulum on ventral tongue surface

Images were then captured of the floor of mouth and lingual frenulum, with tongue held elevated to place the lingual frenulum under tension. Notes were taken on the general morphology of the tongue and frenulum.

Step 4:

Using a Zeiss Microscope, dissection was then performed to remove the mucosa from the floor of mouth. Further photographs were taken with the tongue held elevated to place the frenulum under tension. The fascia was then removed (either in part, to create a “window”; or completely, to expose the whole ventral tongue surface). The relationship of genioglossus, submandibular duct and sublingual glands to the floor of mouth fascia was determined. The lingual nerve was located laterally, where it passed under the submandibular duct. It was then dissected as it passed medially, to follow the branches that passed superficially towards the midline. Photographs were captured to demonstrate the lingual nerve position.

Results:

Description of gross anatomical features of cadavers:

All of the cadavers were premature infants. Infant weight ranged from 400 to 1600g. Estimation of gestational age as outlined in Table 1, was based on median birth weight for gestational age data (Callaghan and Dietz 2010) and ranged from 21 to 30 weeks gestation.

Tongue, floor of mouth and frenulum appearance prior to dissection:

All tissues were soft and pliable, allowing passive movement of the anterior tongue for measurement, assessment of range of mobility and imaging (Fig. 3). Hard and soft palate dimensions are documented in Table 2 and tongue and lingual frenulum measurements in Tables 3 and 4.

With the tongue resting on the floor of mouth, the contour of the floor of mouth was horizontal, with no visible frenulum in any specimen. When the tongue was elevated, a visible midline fold forming the lingual frenulum was raised. The height of the fascial attachment on the ventral surface of the tongue altered the visual prominence of the frenulum when placed under tension. Variability in the relative gliding of mucosal and fascial folds also created differences in the morphology of the frenulum, illustrated in Figure 4. The submandibular ducts can be seen in all specimens, with the duct openings drawn up with the mucosa and fascia, visible being draped over the lateral sides of the fold forming the frenulum.

General description of frenulum morphology in each specimen:

- **Specimen 1** (Fig. 4A):

  A predominantly opaque frenulum, with a small crescent-shaped, transparent fold of mucosa rising a few millimetres above the fold formed by the fascia. The frenulum is broad at its base (junction of the frenulum fold with the floor of mouth) as genioglossus is drawn up into the fold. The height of attachment of the frenulum is approximately half way along the ventral surface of the tongue, raising a visually prominent fold when placed under tension. The mandibular insertion of the floor of mouth fascia is not elevated in the midline (relative to the height of insertion on either side of the midline).
- **Specimen 2** (Fig. 4B): Very similar morphology to Specimen 1, but with genioglossus drawn even higher up into the frenulum, creating an even broader base and therefore the frenulum forms a less well-defined mid-sagittal, vertical fold.
- **Specimen 3** (Fig. 4C): The midline mucosal attachment on the ventral tongue is significantly higher (closer to the tip) than the attachment of the fascial fold, creating a clearly visible transparent fold well above the opaque fold formed by the fascia. The mucosal fold is seen placed under tension between its mandibular and tongue attachments. The fascial fold is shown in the same image to be gently curved, not appearing to be under maximal tension with this manoeuvre. The floor of mouth, the mandibular attachment and base of the lingual frenulum are not visible in this image.
- **Specimen 4** (Fig. 4D): The frenulum fold is opaque, with the mucosal and fascial folds both under tension and drawn up to the full height (free edge) of the frenulum. The fascial and mucosal attachments are at the same level on the ventral surface of the tongue. Genioglossus fibers can be seen drawn up well up into the frenulum fold.

Findings following removal of the floor of mouth mucosa:

There was no discrete midline connective tissue band or cord in the floor of mouth in any specimen (Fig. 5). All four specimens had a layer of fascia spanning from the tongue across the floor of mouth and inserting around the inner arc of the mandible. In all specimens, tongue retraction and/or elevation mobilised the floor of mouth fascial layer to form a midline fold of variable prominence, with a more well-defined midsagittal vertical fold created when the frenulum fold inserted higher on the ventral surface of the tongue, which was apparent in all specimens in this study.

There was variability in the translucency of the frenulum. This was influenced by the relative height of the mucosal and fascial attachments on the ventral tongue and the relative gliding of the mucosa and fascial layers as tension is created, raising the fold of the lingual frenulum. A transparent fold was created when the mucosa was able to glide into a fold above the fascial fold, and a more opaque frenulum forming when the mucosa and fascia both rose together to the full height of the fold.

Removal of the mucosal layer in specimen 3 significantly improved the range of passive mobility of the tongue, with the floor of mouth fascia still intact. This is shown by the change in tongue tip position in Fig. 5C when compared to Fig. 4C prior to mucosal removal. Removal of the mucosa did not alter tongue mobility significantly in the other three specimens (where there was no significant discrepancy between the height of mucosal and fascial attachments on the ventral surface of the tongue), with passive tongue mobility appearing to be primarily affected by the fascial layer in these specimens. Range of passive anterior tongue mobility appeared also to be impacted by the length of the anterior tongue and the proportional length of the frenulum fold between the anterior (mandibular) and posterior (ventral tongue) attachments, which varied significantly between the individual specimens (tongue and frenulum dimensions listed in Table 3). These findings suggest that the mucosal and the fascial attachments and dimensions had the possibility of impacting on tongue mobility as independent variables, specifically when the mucosa had a higher attachment on the ventral surface of the tongue (as seen in specimen 3).

The floor of mouth fascia insertion is shown in all specimens in Figure 6 to be a continuous layer inserting around the inner surface of the mandible. Notably in specimen 4 (and less so in specimens 2 and 3) the height of fascial insertion was slightly elevated in the midline when compared with the height of attachment of the fascia either side of the midline, giving a more visually prominent anterior aspect of the frenulum.

Findings following dissection to remove a “window” in the floor of mouth fascia:

Genioglossus was suspended at a variable distance beneath the floor of mouth fascia by a vertical mid-sagittal sheet of connective tissue. The tissues forming this “suspension” appear to be a layer of transparent connective tissue, continuous with the myofascia surrounding the muscle (Fig. 7).

The lingual nerve was easily identified in a superficial location immediately beneath the floor of mouth fascia. In all specimens, large branches remained superficial on the ventral surface of the tongue from a lateral position under the lateral sides of the tongue, to a medial position as they passed towards the tongue tip. Small branches of the nerve were seen to pass towards the frenulum in all specimens (Figs. 7 and 8). There was a venous vascular plexus in the floor of mouth, located between the submandibular duct and the tongue, with some vessels extending onto the ventral surface of the tongue but always passing deep to the lingual nerve branches.

The submandibular duct openings were connected to and penetrated through the fascial layer close to the midline, and the overlying mucosa was very adherent to the fascia in this localised area. The sublingual glands were enveloped by and suspended from the deep surface of the floor of mouth fascia. They are located clustered along the length of the submandibular duct as it passes across the floor of mouth from postero-laterally towards the anterior midline of the floor of mouth (Fig. 9). The floor of mouth fascia was continuous around and under the sides of the tongue, being thickest in the anterior floor of mouth and becoming thinner and more transparent as it continues postero-laterally towards the piriform fossae. Together with the oral mucosa it forms the roof of the sublingual space.

Discussion:

This is the first study to investigate the developmental anatomy of the in-situ lingual frenulum, confirming the structural anatomy of the frenulum in late fetal development replicates the findings recently described in adults (Mills et al., 2019). These combined studies provide a new and detailed understanding of the lingual frenulum, as a dynamic structure that varies in morphology with tongue movement. With this new understanding of lingual frenulum structure, the concept of "normal" anatomy and the anatomical basis for individual variability is now established. The frenulum's relationship to neighboring structures has now been defined, providing crucial information for understanding potential risks when performing frenotomy.

Defining the structure and role of the floor of mouth fascia

In concordance with our adult study, in all of these premature infant cadavers we have demonstrated a layer of fascia that suspends the tongue within the arc of the mandible, referred to as the floor of mouth fascia. This fascia forms the "roof" of the sublingual space together with the closely connected overlying oral mucosa. It spans from its insertion around the inner surface of the mandible to merge centrally with the connective tissue on the ventral surface of the tongue. The sublingual glands and submandibular ducts are suspended from the inferior fascial surface. This fascial layer is moved passively, as tongue movement creates tension in the layer, dynamically raising it into a midline fold recognizable as the lingual frenulum.

The architectural design of the floor of mouth fascia as a "skirt" or diaphragm-like structure attached around the arc of the mandible provides suspension and stability of the tongue within the oral cavity with no energy output. It is therefore possible that an individual's FOM fascial dimensions may influence their range of tongue mobility but also the position of the tongue at times of low muscle tone, such as during sleep. From this stabilized position that the FOM fascia creates, the tongue is then mobilized within the oral cavity by contraction of extrinsic tongue muscles, with contraction of intrinsic muscles then altering the contour and shape of the tongue in a task specific complex and coordinated manner. The laxity in the floor of mouth fascial layer allows for a wide range of tongue movements before the fascia is brought under tension, potentially influencing the "end point" in range of tongue motion. Ankyloglossia can perhaps be considered an imbalance of the fascial roles, where its provision of tongue stability impacts on tongue mobility. This has potential significance in some infants, where division of the lingual frenulum (to improve tongue mobility) has potential to compromise resting tongue position and "stability." This has been reported in several infants with Pierre Robin sequence, who experienced airway compromise post frenotomy (Genther et al., 2015). Although uncommonly performed currently, infants with Pierre Robin sequence and airway compromise were historically considered for a surgical procedure that sutured to tip of the tongue to the lip, recognizing that anterior tongue position can alter the oropharyngeal airway, the apnoea/hypopnea index and oxygen saturation in infants with retrognathia (Camacho et al, 2017). As such, it should be recognized that division of the lingual frenulum may alter tongue position at rest, with potential to worsen any pre-existing airway compromise. Therefore, caution is recommended before proceeding with frenotomy in this subgroup of neonates. Biomechanical modelling of the tongue and floor of mouth fascia would provide objective insight into the potential impact of variable fascial dimensions and attachments on task specific tongue mobility and resting tongue position.

A structural explanation for variability in lingual frenulum morphology

The range in lingual frenulum morphology is created by variability (on a spectrum) of a number of factors; the height of midline attachments of the fascia (both anteriorly to the mandible and posteriorly to the ventral tongue), the length between these attachments and the relative gliding of the layers forming the frenulum fold (mucosa, fascia and suspended genioglossus). The anatomically based explanation of how these factors influence frenulum appearance, identified and outlined in our earlier adult frenulum anatomy paper, has been confirmed in this paper as an accurate structural construct to be used in the neonate (Mills et al., 2019).

The dynamically changing fold that forms the lingual frenulum is formed as tongue movement creates tension in the floor of mouth fascia. A more visually prominent frenulum fold is created when the mucosa and fascia attach higher on the ventral tongue surface, closer to the tip of the tongue, with this frenulum appearance commonly referred to as an anterior or “classical” tongue tie or labelled as a grade I or II tongue tie. When the mucosal and fascial attachments to the ventral tongue are not elevated in the midline, there is minimal or no visible frenulum fold elevated with active tongue movement. However, it is possible to palpate the tension created in the fascial layer when the tongue is passively retracted. This frenulum phenotype has commonly been referred to as a “posterior” or grade IV tongue tie, however, the structural foundation for this term is based on the limitation being caused by a midline submucosal band which this research has shown is incorrect. The biomechanical impact of division of the fascial layer in this subgroup is as yet unknown but further targeted research is warranted to objectively measure changes in tongue mobility and creation of intraoral vacuum post frenotomy, with assessment of impact for individuals with different variations in frenulum morphology.

When the frenulum is placed under tension, the anterior (mandibular) attachment of the floor of mouth fascia can create a distinctive “flared” appearance, likened to the iconic architecture of the “Eiffel Tower.” This is created when the attachment of the fascia is higher in the midline than the level of fascial attachment on either side. When the frenulum fold appears to merge with the floor of mouth anteriorly, as described in Coryllos type 3 tongue tie (Coryllos et al., 2004), the floor of mouth fascial layer still inserts into the mandible, but the height of midline attachment is not elevated from the height of fascial attachment either side of the midline.

The thickness and translucency of the frenulum fold is influenced by how the fascial and mucosal layer forming the fold glide relative to each other, as well being influenced by the variability in the height that the mucosa, fascia and genioglossus muscle are drawn up into the frenulum fold. A thin transparent frenulum is formed when the mucosal fold glides well above the fascial fold. A thicker opaque frenulum forms when the mucosa and fascia are closely adherent and mobilize together to the full height of the fold. It is possible to have the mucosal fold rising slightly above the fascial fold, which we have described as a “mixed” appearance. The thickness or bulkiness of the frenulum is also influenced by the height that genioglossus is suspended from the fascial surface. When it is suspended close to the fascial layer, as the fascia is drawn up into a fold, the muscle fibers are also drawn up into the frenulum, usually creating a broader, less well-defined “base” to the frenulum. This particular variation in morphology increases the risk of genioglossus muscle fibers being inadvertently cut during frenotomy. This would be the source of muscle fibers that have been reported in histological analysis of frenulum biopsies (Martinelli et al. 2014).

“Reframing” the concept of ankyloglossia or “Tongue Tie”

It is essential to recognize that the lingual frenulum is not formed by a discrete submucosal midline “string” or “band” but is a dynamically formed midline fold created in a layer of fascia. There is a vast spectrum of variability in many features of this fascia between individuals, but it has now been shown to be consistently present both during fetal development and in the adult. As such, the lingual frenulum should be considered a normal anatomical structure with variation in morphology existing on a spectrum. As there are no anatomical variables of the frenulum that have been shown in isolation to correlate directly with impaired tongue function, any grading systems should be considered “frenulum grading” to be used on the basis of describing appearance rather than being capable of diagnosing or categorizing a frenulum as a tongue tie. The height of the ventral tongue attachment of the frenulum can then be considered to be just one part of a larger puzzle that still has many missing pieces.

The lingual frenulum has direct connection to the anterior tongue, with potential for direct impact on active anterior and mid tongue mobility. The lingual frenulum does not have direct connection to the posterior tongue (also known as the tongue base), which is embryologically of different origin and includes the aspect of the tongue posterior to the foramen caecum. As such, we suggest that the term “posterior tongue tie” is anatomically incorrect nomenclature. A “posterior tongue tie” has an anatomical construct based on the misunderstanding of the lingual frenulum being a submucosal band or string. As such, the term “posterior tongue tie” is potentially both confusing and misleading. For clarity and anatomical integrity, we suggest the term “posterior tongue tie” should be discontinued.

Potential impact of the lingual frenulum on tongue biomechanics in breastfeeding

Ankyloglossia is a functional diagnosis that involves restriction of anterior and mid tongue movement and a direct correlation between specific features of frenulum appearance and impairment of movement has not yet been established (Suter and Bornstein, 2009; Chinnadurai et al., 2015; Francis et al., 2015; Krishnaswami and McPheeters, 2015; Walsh et al., 2017). Therefore, we emphasize that currently ankyloglossia cannot be diagnosed purely on the morphological appearance of the lingual frenulum and any grading system that allows this should not be used.

The ability of infants to alter tongue movement in response to feeding modality has been shown in studies highlighting the differences in dynamic tongue motion between bottle and breastfeeding. Clinical assessment of tongue motion during feeding is difficult, however ultrasound studies have shown milk extraction from a bottle occurs by a combination of positive pressure and peristaltic motion of the anterior tongue (Ardran et al., 1958; Weber et al., 1986; Woolridge and Baum, 1987). Breastfeeding however, has been shown to rely primarily on creation of intraoral negative pressure and sucking motion associated with en-block movement of the anterior and mid-tongue (Geddes et al., 2008a; Elad et al., 2014; Cannon et al., 2016; Geddes and Sakalidis, 2016) with tongue elevation appearing to be important in creating the intraoral vacuum. An ultrasound study has shown that patterns of tongue motions differed both in infants with ankyloglossia (with breastfeeding problems) and those without ankyloglossia, but as no anatomical variables of the lingual frenulum were included in this study, it is not possible to correlate the differences seen on ultrasound to frenulum morphology (Geddes et al., 2008b).

Our study has shown significant variance in both tongue and palatal dimensions including; mandible size and position, anterior tongue length, and the height, contour and dimensions of the arch of the hard palate. When assessing the impact of variable lingual frenulum morphology on tongue biomechanics, the impact of these other potentially confounding anatomical variables is unknown. Further, it is possible that maternal anatomical variability (nipple dimensions and tissue elasticity) could also impact on the latch and contribute to the success of milk removal and should be included in clinical assessment and be considered as a potential confounding variable in biomechanical research. No research to date has identified specific lingual frenulum anatomical variables that correlate with biomechanical dysfunction, maternal pain and effective milk removal from the breast, nor have the current grading systems used for tongue tie been shown to correlate with severity of feeding issues. Thus, there is insufficient knowledge to determine how and when the morphologic variables of floor of mouth fascial layer impact on tongue mobility to determine when ankyloglossia is present and when frenotomy is indicated. Further biomechanical research should assess different subgroups of frenulum morphology, taking into account potentially confounding anatomic variables of the palate, tongue and mandible. Longer-term assessment is also needed to account for changes that may occur in tongue mobility and the impact on the gliding of the tissue layers forming the frenulum following post frenotomy wound healing and scar maturity.

Clinical implications of this research

This study emphasizes that the lingual frenulum is a complex, layered structure that dynamically changes morphology with tongue movement. The location of its midline attachments (anteriorly on the mandible and posteriorly on the ventral tongue) and relative movement of the mucosal, fascial and muscular “layers” or the frenulum alter both its appearance and its impact on anterior tongue range of motion. Variability in these factors create a new level of complexity to be considered when evaluating an individual’s lingual frenulum anatomy and assessing the frenulum’s potential impact on task specific tongue mobility. As with the adult specimens in our earlier research, the neonatal floor of mouth fascial layer appeared to have a role in stabilizing the position of the tongue within the arc of the mandible, and appeared to be a major factor determining the range of anterior tongue mobility. Genioglossus varied in the height it was drawn up into the frenulum with tongue movement, but the muscle fibers were highly distensible and were not restricting mobility. There does not appear to be any circumstances where incision into genioglossus fibers is indicated during frenotomy.

In all specimens, lingual nerve branches passed towards the midline immediately beneath the fascia on the ventral surface of the tongue, with smaller branches continuing onto the lingual frenulum. The variable branching pattern of terminal branches of the lingual nerve on the ventral surface of the tongue has been noted previously in adult cadaveric studies (Rusu et al., 2008; Al-Amery et al., 2016). A further study in included dissection of ten adult and a single pediatric tongue, showing images of superficial lingual nerve branches on the ventral tongue surface, but no differences between the adult and pediatric anatomy was described (Paduraru and Rusu, 2013). There has not been previous recognition or description of branches from the lingual nerve extending onto the lingual frenulum. Our study has shown these nerve branches to be located superficially on the ventral tongue surface, immediately beneath the fascia, placing them at risk for direct or transmitted thermal injury during frenotomy. Risk of temporary or permanent neural injury would be higher when; the incision is deep (any incision that cuts into genioglossus), frenotomy performed with any tool that utilizes thermal energy (which is absorbed into underlying tissues), and/or any procedure that where the incision extends widely from the midline. The prevalence of sensory impairment following infant tongue post frenotomy is unknown because of the inability of non-verbal patients to self-report paresthesia and the insensitivity of any clinical testing for focal sensory impairment in this age group. In adults, altered tongue sensation following frenotomy has been reported in social media but there are no published audits that have specifically assessed the rate of temporary or permanent sensory change following the procedure. New research has shown the lingual nerve has direct connections to motor end plates of intrinsic tongue muscles, and the lingual nerve in infants is therefore likely to have a direct role in the shaping of the contour of the tongue surface in direct responsiveness to sensory input (Mu and Sanders, 2010). Thus, compromising the lingual nerve could conceivably affect tongue shaping during breastfeeding and thus impact on efficacy of latch and milk transfer or contribute to breast refusal. Our research has also shown branches passing onto the lingual frenulum, confirming that the frenulum is indeed sensate. Pain requiring treatment with analgesia has been reported as long as 13 days following frenotomy (Wilson et al., 2016). The experience of post frenotomy pain is likely to vary considerably between individuals, influenced by many factors including depth and size of the wound and the frequency and nature of any post procedure wound interventions. However, it needs to be recognized that pain associated with frenotomy and post frenotomy wound "massage" has the potential to create oral aversion in some infants, leading to a negative and prolonged impact on their feeding and oromotor development.

Strengths and limitations of the study

Access to fresh neonatal cadavers for anatomical research is a rare privilege, allowing dynamic manipulation and dissection of the tissues that is not possible in embalmed cadavers. The key strengths of this study were the possibility to remove the en-bloc specimen from the cadaver and dissection the layers of the floor of mouth to delineate and define the tissues. This provided access to and viewing of these tissues in a manner that would not be possible in live patients during any routine frenulum or floor of mouth surgery. This study's weakness is that it involved only a small number of premature infant cadavers, and therefore cannot represent the full range of infant lingual frenulum morphology.

Conclusion:

The infant lingual frenulum is a dynamic structure formed by a fold in the floor of mouth fascia. There is significant variability in morphology of the lingual frenulum relating to midline attachment of the floor of mouth fascia, and variability in how the mucosa, fascia and genioglossus are drawn into the fold of the frenulum with tongue movement. We have highlighted the superficial location of the lingual nerve branches on the ventral tongue, immediately beneath the fascia and raised concern regarding potential risk of injury during frenotomy. Lingual frenulum anatomy has now been defined, but there are still large gaps in knowledge, particularly correlating variability in structure with function. More detailed biomechanical research is required, with a more holistic approach needed to understand the potential impact of a broad range of variables in infants' oral anatomy on task specific tongue function. Until more research provides these answers, the decision-making around frenotomy remains subject to the practitioner's biases and existence of a grading system that does not allow for the presence of normal anatomy. We encourage a considered clinical approach that includes ruling out other potential causes of breastfeeding difficulties before proceeding to surgical intervention.

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FIGURE 1

MEASUREMENT OF HARD PALATE DIMENSIONS

A: Hard palate length: anterior alveolar ridge to posterior edge of hard palate
B: Maximal external width of hard palate
C: Inner width of mid hard palate: distance between internal alveolar ridges

FIGURE 2

MEASUREMENT OF TONGUE DIMENSIONS

A: Maximal width of tongue
B: Anterior free length: from frenulum attachment to tip of tongue
C: Full length of tongue: from vallecula to tip of tongue

FIGURE 3

DEMONSTRATION OF PASSIVE MANIPULATION OF FRESH TISSUES

Specimen 1: A: To demonstrate scale of specimen **B**: Tongue tip elevation **C**: Tongue tip folded posteriorly to show mandibular insertion of frenulum **D**: Anterior tongue elevation and retraction, to place frenulum under tension **E**: Lateral tongue retraction to show lateral aspect of floor of mouth fascia under tension **F**: To demonstrate fresh tissue properties

FIGURE 4

LINGUAL FRENULUM UNDER TENSION WITH TONGUE ELEVATED, PRIOR TO DISSECTION

A: Specimen 1: "Mixed" appearance: Mucosal fold visible as a small "crescent" just above fascial fold
B: Specimen 2: "Mixed" appearance: Mucosal fold visible as a small "crescent" just above fascial fold
C: Specimen 3: "Transparent" frenulum: Mucosal fold short and elevated well above fascial fold
D: Specimen 4: "Opaque" frenulum: Mucosa and fascia both at apex of fold (same height)

FIGURE 5

LINGUAL FRENULUM UNDER TENSION WITH TONGUE ELEVATED, FOLLOWING REMOVAL OF MUCOSA

A: Specimen 1 **B**: Specimen 2 **C**: Specimen 3 **D**: Specimen 4

FIGURE 6

FLOOR OF MOUTH AND MANDIBULAR INSERTION OF FRENULUM

Specimens 1 to 4: Pre (A) and post (B) dissection for each specimen

FIGURE 7

SPECIMEN 1 WITH "WINDOW" OF FASCIA REMOVED FOR VISUALISATION OF GENIOGLOSSUS AND LINGUAL NERVE

Outline of removed fascial "window" shown by continuous white line
A: Mandible **B**: Tip of tongue **Black arrow**: superior edge of genioglossus, suspended from fascia and drawn up into the fold of the frenulum **Large white arrow**: Lingual nerve **Double white arrows**: branches of lingual nerve on ventral tongue surface passing onto frenulum **Fine black line:** outlining lingual nerve branches

FIGURE 8

LINGUAL NERVE LOCATION

A: Specimen 1 **B**: Specimen 2 **C**: Specimen 3 **D**: Specimen 4
M: Mandible **Large black arrow**: Lingual nerve (main trunk) **Small black arrow**: Lingual nerve (medial branches to frenulum) **White stars**: Submandibular duct openings **Black dotted line**: midline **Fine black line:** outlining lingual nerve branches

FIGURE 9

SUSPENSION OF SUBLINGUAL GLANDS FROM FLOOR OF MOUTH FASCIA

Posterolateral floor of mouth (viewed from posteriorly) **T**: Body of Tongue (medial) **M**: Mandible (lateral) **S**: Sublingual glands: suspended from inferior surface of floor of mouth fascia (fascia passing between attachments M and T)

Table 1. Cadaver details

Cadaver	Sex	Weight (grams)	Body length (mm)	Head Length (mm)	Estimated gestational age (weeks)	Postnatal age at death (days)
1	M	600	350	85	23	0
2	F	400	300	85	21	0
3	M	800	350	75	26	0
4	F	1600	395	105	30	13

Table 2. Dimensions of Hard and Soft Palate

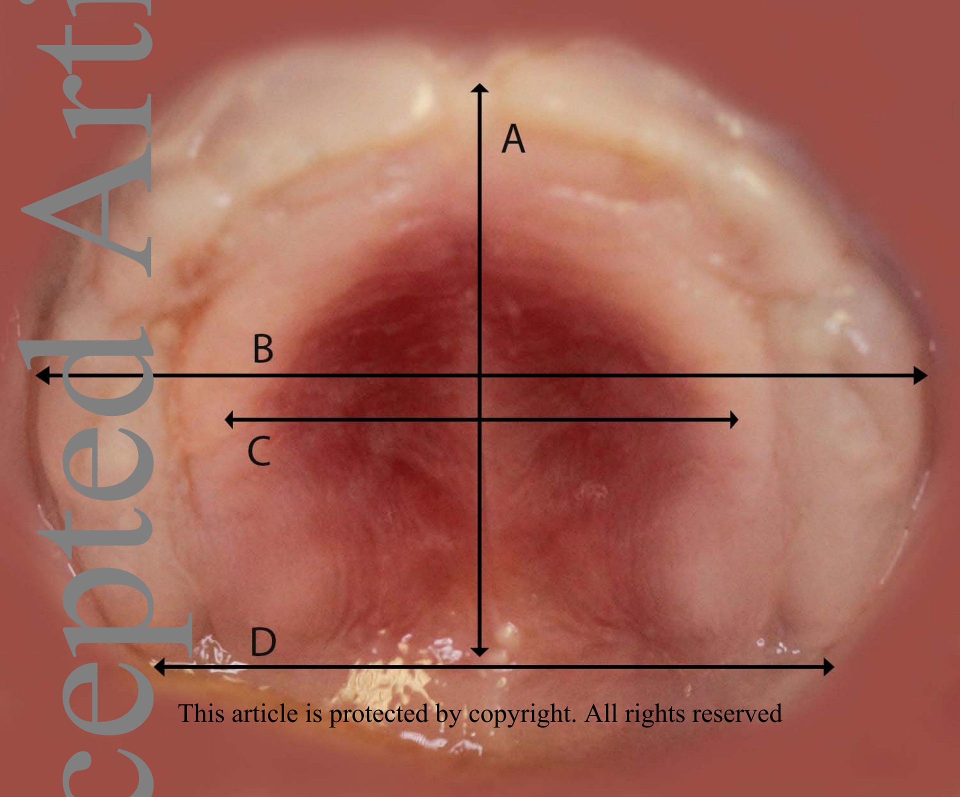
Cadaver	Mandible position relative to maxilla Degree of retrognathia (mm)	Hard Palate (mm)					Soft Palate (mm)
		Length	Internal, mid palate	External, mid palate	External, posterior	Height of arch	Length
1	8	15.1	12.0	22.8	21.5	5	19.5
2	5	14.9	13.9	25.8	24.0	7	10.1
3	3	16.1	15.2	22.3	20.8	6	10.0
4	2	20.2	16.6	16.6	29.1	5	12.2

Table 3. Tongue Dimensions

Cadaver	Tongue (mm)				
	Total Length	Width	Anterior length	"Free" anterior length: (frenulum attachment to tip)	Proportion: free length versus anterior length %
1	29	18	9	5	55
2	33	18	6	4	67
3	34	20	10	4	40
4	36	24	10	6	60

Table 4. Lingual Frenulum Dimensions

Cadaver	Frenulum (mm)				
	Height of ventral tongue attachment	Height of midline attachment on mandible	Length of frenulum: between mandibular and ventral tongue attachments	Frenulum length vs ant tongue length %	Description of appearance
1	4	Not elevated	4	44	'Mixed'
2	2	Slight elevation	6	100	'Mixed'
3	6	Slight elevation	5	50	Transparent
4	4	Moderate Elevation	7	70	Opaque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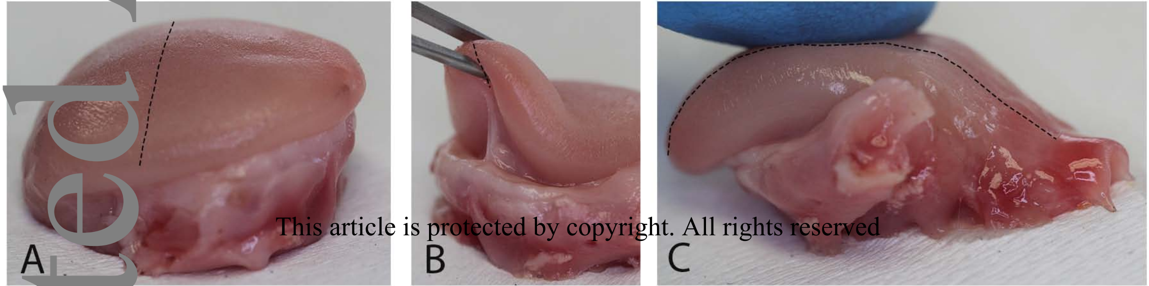
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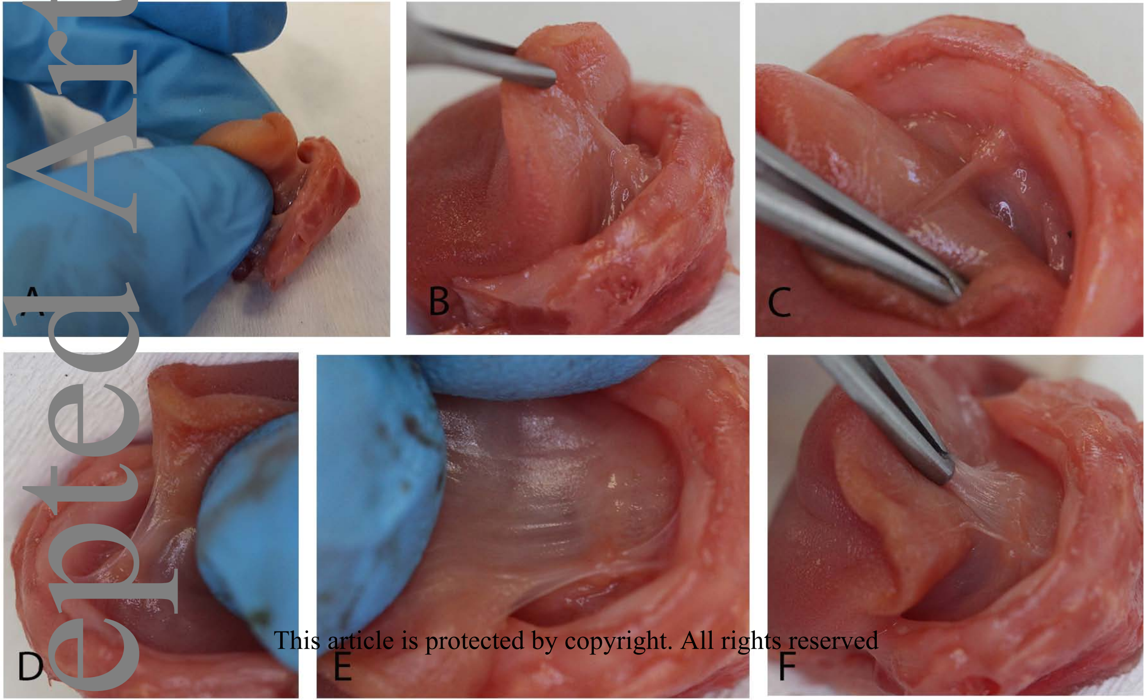
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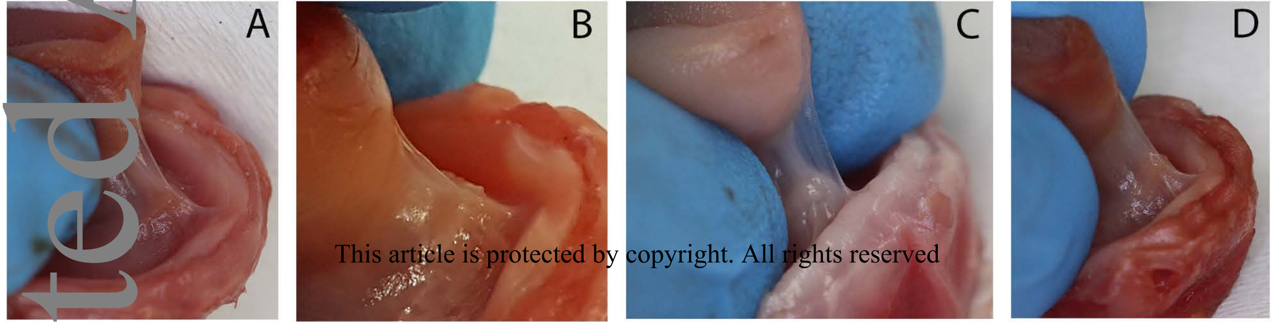
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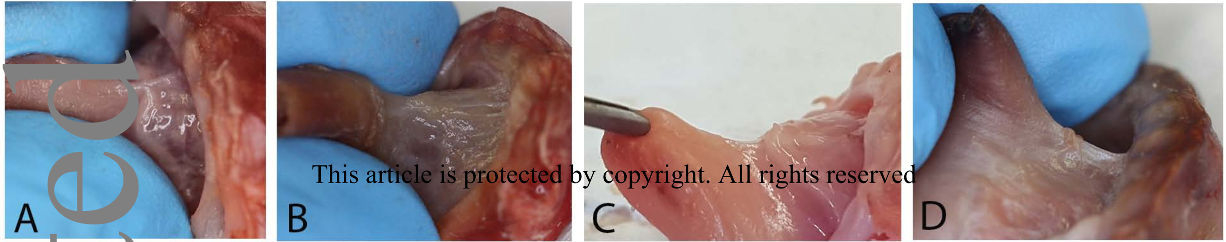
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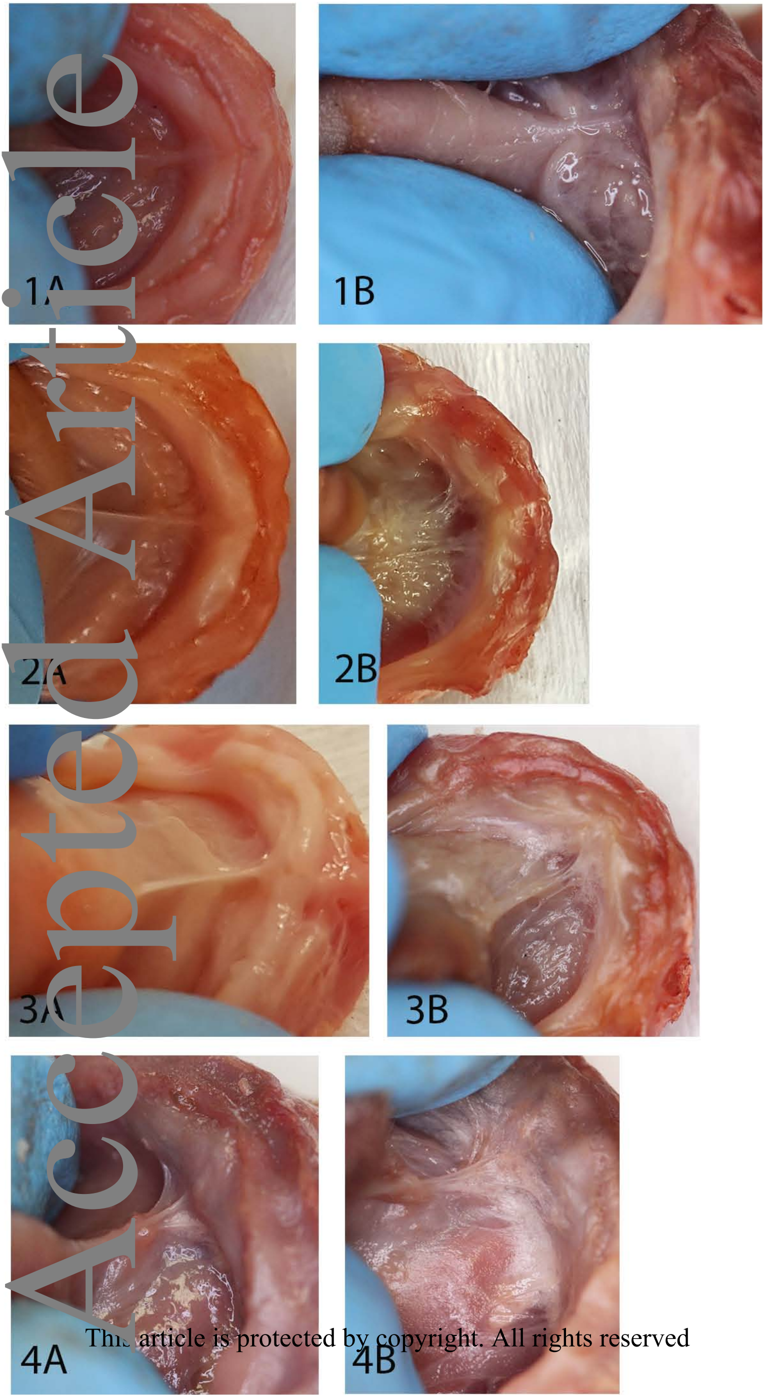
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4A
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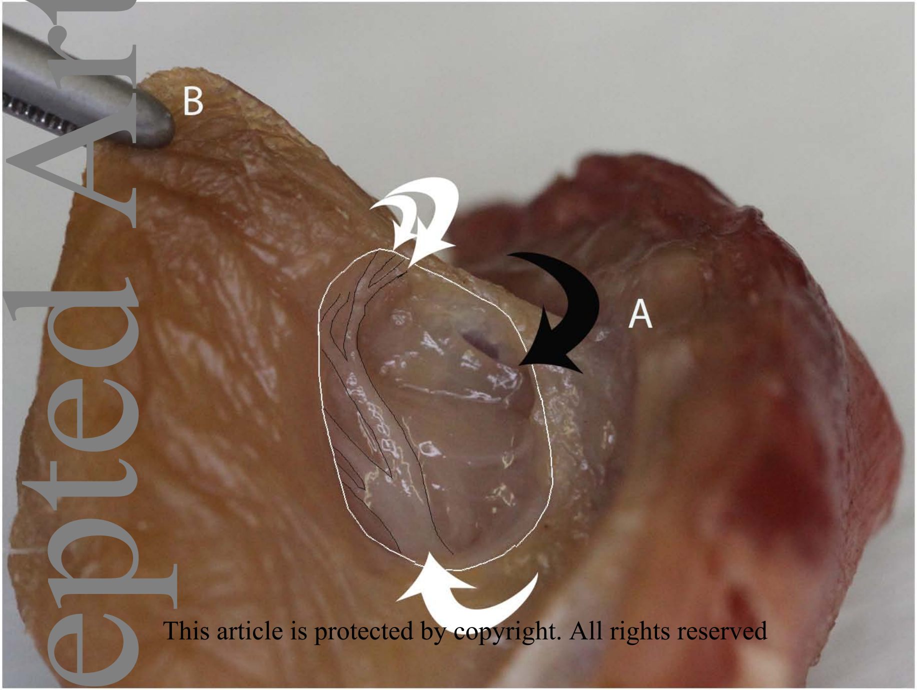
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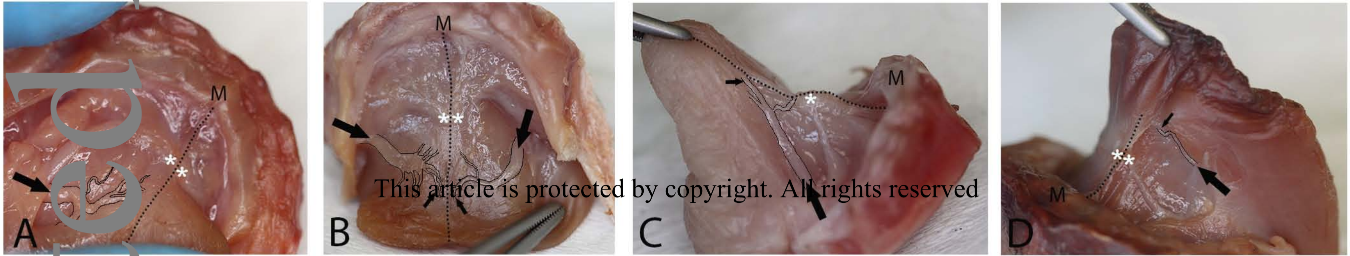
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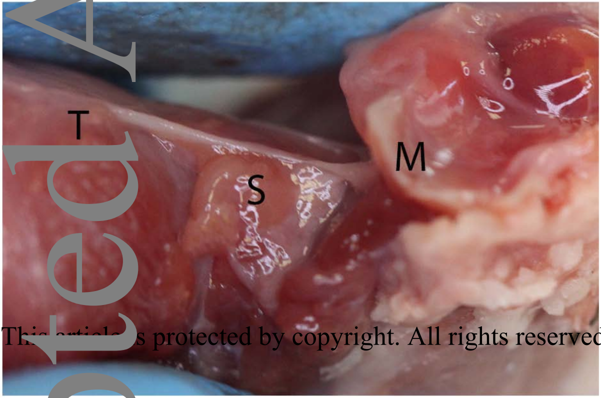
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