

Developmental and Histological Studies of Fungiform Papillae in Fetal Human Tongue

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Abstract

This study was designed to evaluate the histological development of fungiform papillae of human tongue during the prenatal period.

Lateral Sagittal sections were taken at autopsy from the anterior two-third of 18 aborted fetuses of 8, 10, 12, and 20 weeks of gestations and 2 stillborn infants at 40 weeks of gestation. Twenty specimens were prepared in paraffin and stained for histological study. The gestational ages were calculated after measuring the Crown Rump Length [C.R.L.]. It was found that the first appearance of fungiform papillae was nearly about the 10th week of gestation, before the appearance of filiform papillae, most of the developed fungiform papillae were cylindrical in shaped with appearance of nerve plexus, at 12th week they reached mushroom shaped with narrow base and broad apex and became differentiated into primary and secondary papillae, also presumptive taste buds were noticed at this age in the form of multiple local thickening of papillary epithelium. At 20th week of gestation the taste buds became more developed, the papillae appeared more vascular. At full term (40th week) the fungi form papillae reached full maturity.

It is concluded that this study suggests that the development of fungi form papillae requires an epithelial and mesenchymal interaction during morphogenesis

Introduction

Microscopic studies have been made on the prenatal developing fungiform papillae and Innervations in human fetal tongue. Their relation to vascularization and Pansky (1982) explained that the development of the tongue from the branchial arches and explained its nerve supply. The sensory nerve supply to the mucosa of almost the entire anterior two thirds of the tongue was from the lingual branch of the mandibular division of the trigeminal nerve (the nerve of the first branchial arch). Although the facial nerve was the nerve of the second branchial arch, its chorda tympani branch supplied the anterior two thirds of the tongue except for the vallate papillae.

Moore (1982) stated that around the end of the fourth week, a median somewhat triangular elevation appeared in the floor of the pharynx just rostral to foramen caecum. This elevation, the median tongue bud (tuberculum impar) gave the first

indication of tongue development. Soon, two oval distal tongue buds (lateral lingual swellings) developed on each side of the median tongue bud. The two lateral elevations rapidly increased in size, merged with each other and overgrew the median tongue bud. The merged distal buds formed the anterior two thirds or oral part of the tongue. The median tongue bud formed no recognizable portion of the adult tongue.

Romanes (1993) stated that the branchial arch mesenchyme formed the connective tissue, the lymphatic tissue and blood vessels of the tongue and probably some of its muscle fibers. Most of the tongue musculature was derived from myoblasts that migrated from myotomes of the occipital somites.

McLachlan (1994) stated that tuberculum impar arose from the caudal end of the first branchial arch and the lateral lingual swellings resulted from proliferation of mesenchyme in the ventromedial parts of

the first pair of branchial arch. Hence the mucous membrane of the anterior two-thirds of the tongue arose from the first arch.

Williams *et al.*, (1995) and Wheater *et al.*, (1996) mentioned that the fungiform papillae were located mainly on the lingual margin also irregularly on the dorsum of the anterior two-thirds of the tongue, where they might occasionally be numerous. They differed from filiform papillae by their larger size; rounded shape and deep red color, each usually bear one or more taste buds on its apical surface.

Witt and Reuters (1997) mentioned that the papillae of the tongue appeared around the post ovulatory (the 8th week of gestation). The vallate and the foliate papillae appeared first in close relationship to the terminal branches of the glossopharyngeal nerve. The fungiform papillae appeared later near the termination of the chorda tympani nerve. All the previous papillae developed later taste buds. The filiform papillae developed later after the fungiform papillae. In contrast to the large and well documented informations on the morphology of the fungiform papillae in adult human, there is insufficient reports concerning the development and differentiation of the human fungiform papillae.

The present study tries to offer more informations about the histogenesis of the prenatal development of the fungiform and their relation to vascularization and innervations in human fetal tongue.

Material and Methods

Twenty human male and female fresh fetuses (prenatal age ranged from 8th week to full term) were used in this study. The fetuses were obtained from Al-Azhar university hospitals. The CRL of each fetus was measured and then converted into weeks of menstrual prenatal ages in accordance with accurate tables of Steeper (1920) and Longman (1975). Two or three specimens represented each week of pregnancy, especially at the early prenatal ages.

The developing tongues were obtained from the fetuses and examined

macroscopically to exclude any anomalies or pathological lesions. Specimens were taken from the anterior two-thirds of the tongues. The collected specimens were fixed in 10% formal saline solution. Then dehydrated, cleared in benzene, embedded in paraffin and cut sagittal 6µm thick serial sections. Lateral sagittal sections were stained with haematoxylin and eosin to detect general histological structures of the fetal tongues (Drury and Wallington 1980). Silver stain (Rashwan *et al.* 1976) was applied for demonstrating nerve fibers, plexuses and taste buds.

Results

The tongue of human fetus with CRL of 23 mm. (8 weeks)

Microscopic examination of lateral sagittal sections from the anterior two-thirds of the tongue of human fetus with CRL of 23 mm. (8 weeks) showed that the tongue was occupied mainly by a muscular mass that was formed of thin muscular bundles arranged into different directions. The muscular mass was covered on its dorsal surface by a thick stratified gummous epithelium which thrown into multiple epithelial projections to form the primitive papillae (Fig. 1. a). These papillae had solid epithelial cores. They were cylindrical in shape with both apices and bases had nearly the same width. Their apices were covered with a single layer of flat cells. No sign of keratinization was detected (Fig. 1. b.). The subepithelial connective tissue was forming the reticulum of primitive lamina propria, where small-developed nervous elements could be detected. (Fig. 1. c).

The tongue of human fetus with CRL of 36 mm. (10 weeks)

Microscopic examination of lateral sagittal sections from the anterior two-thirds of the tongue of human fetus with CRL of 36 mm. (10 weeks) showed that the muscular mass was forming also the main bulk of the tongue but their muscular bundles were thick and apparently arranged into three different directions. Superior and inferior were longitudinal while the middle

one was transverse (Fig.2.a.). The dorsal surface of the muscular mass was covered with a stratified Squamous epithelium, which was excessively projected into different shaped lingual papillae. Most of them had narrow bases and broad apices. Each papilla showed a single connective tissue core that was not invaded with primitive capillaries. These papillae were representing the primary fungiform papillae (Fig. 2.b). The fungiform papilla was covered with thick but non-keratinized stratified squamous epithelium (Fig. 2.b). A well-developed dense nerve plexuses were detected in connective tissue core and the subepithelial layer of this papilla. Collections of dense nerve fibers were also noticed in some location in the epithelium of the papilla that represented the primitive taste bud (Fig.2.c).

The tongue of human fetus with CRL of 56 mm. (12 weeks)

Microscopic examination of lateral sagittal sections from the anterior two-thirds of the tongue of a human fetus with CRL 56mm. (12weeks) showed that the surface epithelium was very thick and had thrown into highly packed fungiform papillae primitive filiform papillae with pointed apices and broad bases could be detected (Fig.3.a).The muscle bundles of the tongue appeared very thick and well developed especially the middle transverse layer (Fig.3.a). The papillary epithelium of fungiform papilla was very thick and showed packed cells occupied the apical part of the papillary epithelium, which represented the primitive taste bud (Fig.3.b). The connective tissue core of fungiform papilla was invaded by large blood vessel (Fig.3.b.).Also rich nerve plexuses could be detected in connective tissue core, lamina propria and also in the site of primitive taste bud at the apical epithelium where the cells became packed, enlarged and associated.(Fig.3.c).

The tongue of human fetus with CRL of 160mm. (20weeks).

Microscopic examination of lateral sagittal sections from the anterior two-thirds of the tongue of a human fetus with

CRL of 160 mm. (20 weeks) showed that the fungiform papillae had developed primary and secondary types and in between few filiform papillae (Fig.4.a). The papillary epithelium of secondary fungiform papilla was very thick. This epithelium showed inward growth, intra papillary extension inside the papillary core that converting the single connective tissue core into two cores and that fungiform papilla became secondary one. The connective tissue cores of primary and secondary fungiform papillae were rich in blood vessels (Fig.4.b). Most of the fungiform papillae were acquired the typical mushroom shaped with an apical taste bud .The taste bud was oval and occupied the whole thickness of apical epithelium of the papillae. The base of taste bud rest on connective tissue and its apex had a primitive gustatory pore called taste pit (Fig.4.c). The taste bud was presumptive, as it was not fully developed as it had a thick taste pore. The transitional cells at the periphery started to be detected. The cells inside the center of the taste bud although they could be differentiated into cells with dark nuclei and others with light nuclei but they were not fully arranged yet. The subepithelial nerve plexuses and nerve fibers could be detected and also taste bud on the apex of primary fungiform papilla (Fig.4.d).

The tongue of human fetus at full term .

Microscopic examination of lateral sagittal sections from anterior two-thirds of the tongue of human fetus at full term revealed that most of the fungiform papillae appeared well developed, muscle bundles of the tongue appeared thick and ran in different directions (longitudinal, transverse and vertical) (Fig. 5.a). The fungiform papillae enlarged with broad apex and narrow base and acquired more than one taste bud on its dorsal epithelial surface. Their cores were very vascular contained numerous blood vessels (Fig. 5.b). The taste buds appeared well developed as they had more differentiated cells and taste pore in the apex of the bud and became thin liable to rupture (Fig. 5.c). The cells of taste bud were differentiated into transitional cells

which were located at the periphery and inside the taste bud, the cells had two types: (a) – dark supporting cells with dark oval nuclei and situated near the base of the bud. (b) - light gustatory cells were few in number situated near apex of the bud and had light cytoplasm and light oval nuclei. The nerve plexuses were detected at the base and the center of the papillary core (Fig. 5.d).

Plate 1 : photomicrographs of lateral sagittal sections from the anterior two-thirds of the tongue of a human fetus with C.R.L of 23 mm. (8 weeks)

Fig. 1-a: Showing multiple projections (J) arise from a thick stratified squamous epithelium (E) covers the dorsum of the tongue and Muscular mass (→), where its fibers are arranged into different directions. H&E x40

Fig.1-b: Showing two epithelial projections that form the primitive Papillae (p.p) . The papillae are cylindrical in shape with solid Core and covered with flat cells. H&E x400

Fig. 1-c: Showing few nerve fibers in sub epithelial connective tissue. Silver stain x200

Plate 2: Photomicrographs of lateral sagittal sections from the anterior Two-thirds the tongue of a human fetus with CRL of 36 mm. (10 weeks).

Fig. 2-a: Notice that most of the dorsal epithelium became differentiated into primitive fungiform papillae (f.p) and few pointed filiform Papillae (→) the muscle fibers of the tongue appeared thick and arranged into superior and inferior longitudinal layers while the middle is transverse. H&Ex40

Fig. 2-b: Showing three primitive fungiform papillae of different degrees of development. The right one (1) is the less developed one as seen at its apex and base while the two left papillae are more developed (2) as seen from their wide apices and narrow bases. H&E x400

Fig. 2-c: Showing the well-developed nerve plexus that invading the connective tissue core of the fungiform papilla. Notice also the dense collection of nerve fibers in the epithelium of its apex. Silver stain x400

Plate 3: Photomicrographs of lateral sagittal sections from the anterior Two-thirds of the

tongue of a human fetus with CRL of 56 mm. (12 weeks)

Fig. 3-a: Showing the dorsal epithelium of the tongue which is formed mainly of the fungiform papillae (f.p) and few primitive filiform (→) The muscle fibers appeared thick and well developed especially the middle layer H&Ex400

Fig. 3-b: Showing the epithelium of fungiform papilla which became thick .Its cells became packed at the apex. The connective tissue core is invaded by blood vessels (→) H&Ex400

Fig. 3-c: Showing a primary fungiform papilla with a primitive taste bud (T.B) occupies its apex. Notice the packed associate cells in the bud's epithelium. Notice also the rich nerve fibers in connective tissue core and Lamina propria. Silver stain X200

Plate 4: Photomicrographs of lateral sagittal sections from anterior Two-thirds of the tongue of a human fetus with C.R.L 160 mm. (20 weeks).

Fig.4-a: Showing well developed fungiform primary (1) and secondary (2) Types and few filiform papillae H&E x40

Fig. 4-b: Showing secondary fungiform papilla with intra-papillary extension of dorsal epithelium. The connective tissue core is rich in blood vessels (→)H&E x400

Fig. 4-c: Showing fungiform papillae which acquired the mushroom shape. Notice the taste bud (T.B) which occupied the apical epithelium and rests on the connective tissue. H&E X 200

Fig.4-d: Showing nerve fibers in connective tissue core, lamina Propria and taste bud on the dorsal surface of fungiform Papilla Silver stain x200

Plate 5: Photomicrographs of lateral sagittal sections from the anterior two-thirds of the tongue of a human fetus at a full term

Fig.5-a: Showing well developed fungiform papillae and muscle bundles which run in different directions. (→) H&E x 40

Fig.5-b: Showing a well-developed fungiform papilla with broad apex and narrow base, well-developed taste buds (T.B) on its dorsal epithelial surface. Notice the connective tissue core which became very vascular. H&E x400

Fig. 5-c: Showing two well-developed taste buds with different types of cells and taste pore is thin. (→)H&E x800

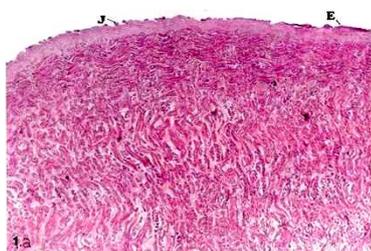


Fig. 1-a

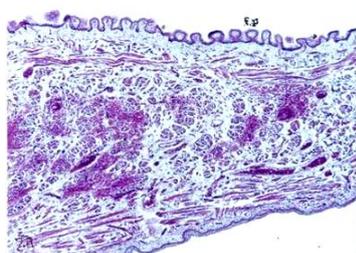


Fig.2-a

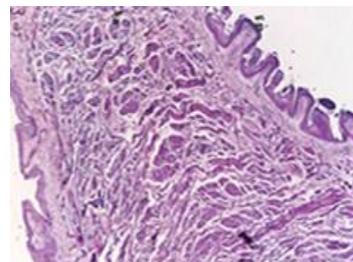


Fig. 3-a

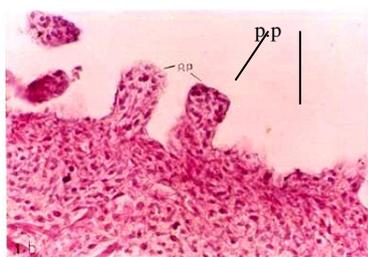


Fig. 1-b



Fig. 2-b

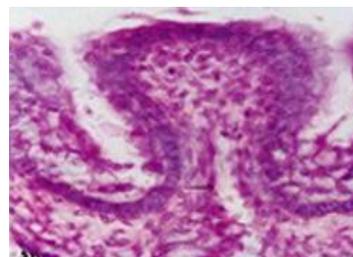


Fig. 3-b

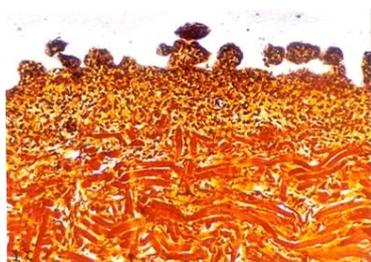


Fig. 1-c

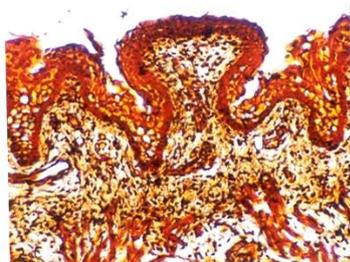


Fig. 2-c

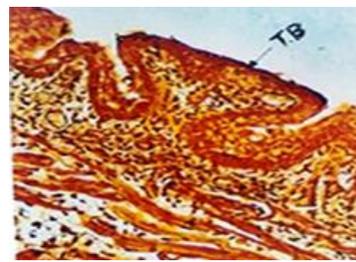


Fig. 3-c

Plate 1

Plate 2

Plate 3

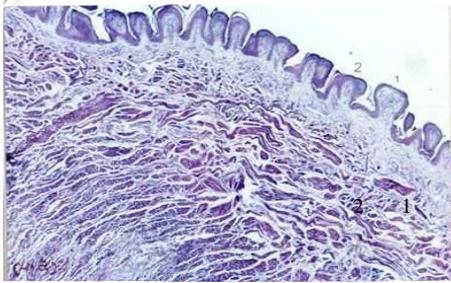


Fig. 4-a

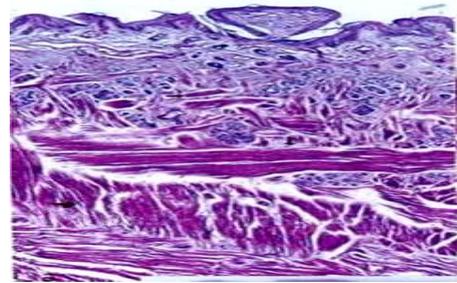


Fig.5-a

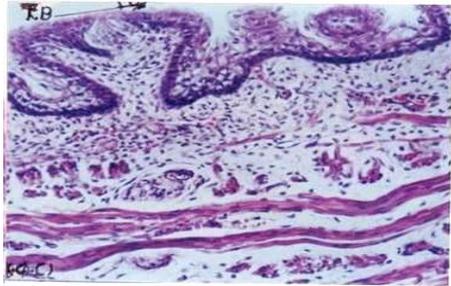


Fig. 4-b

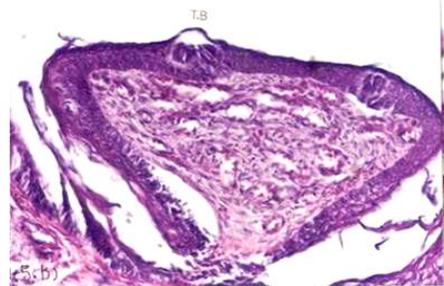


Fig. 5-b

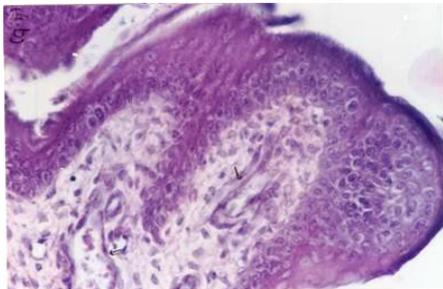


Fig. 4-c

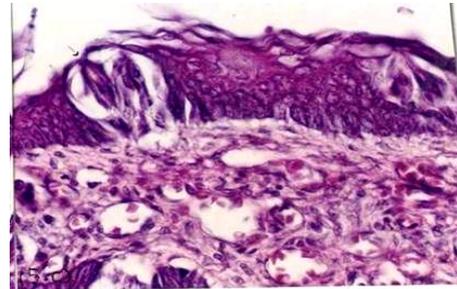


Fig. 5-c

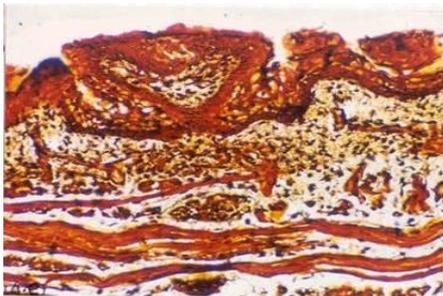


Fig.4-d
Plate 4

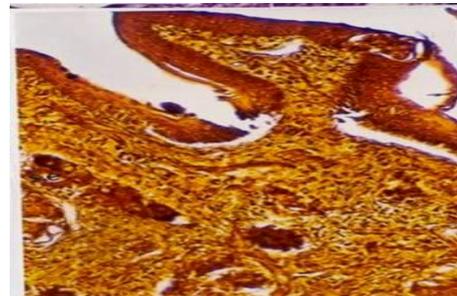


Fig. 5-d
Plate 5

Discussion

Developmental histological studies of the structural elements of the anterior two-thirds of the human tongue mainly the fungiform papillae were studied in this work. The histogenesis of the prenatal developing fungiform papillae from the 8th week of gestation till full term including

their shape, epithelial covering, vascularization and innervations were studied in this work. In addition the development of taste buds in these papillae was also studied.

In the present work, at the 8th week of gestation, the dorsal epithelium was formed of multiple epithelial projections which

were the primitive papillae covered by primitive stratified epithelium. At the 10th week, the dorsal stratified squamous epithelium became excessively projected to form primary fungiform papillae. Most of these papillae were cylindrical in shape as they had the same width in bases and apices. At 12th week of gestation the fungiform papillae became moderately developed acquired mushroom shape with narrow bases and broad apices. The filiform papillae started to appear at 12th week of gestation. These results are in agreement with those of Arey (1974) who stated that fungiform papillae were developed before filiform papillae. The fungiform papillae tend to grow between 8 and 15 week of gestation and Steven and Lowe (1998) mentioned that the adult fungiform papillae had a mushroom shape. But the result of this work was not agreed with the results of Samar *et al*, (1990) who reported that the fungiform papillae being evident at 12th week of gestation. In the present work, at the 10th week of gestation the fungiform papillae were invaded by very few, small capillaries in their connective tissue core and increased in the later ages. At full term, these capillaries were forming large capillaries network.

These results confirmed the results of Fuentz and Sanchiz (1992) who reported that there were very few capillaries making up the superficial vascular network under the epithelium of the fungiform papillae. The walls of these capillaries were progressively increased until a short time before birth. Makiyama *et al*, (1998) reported that in young rats the capillary network of the fungiform papillae had cylindrical form and later had a little fungus form.

In the present work, the muscular component of the tongue was different at 8th week of gestation and became apparently arranged into definitive layers at the 10th week of gestation. The result confirmed the results of Hamilton and Mossman (1972), Arey (1974), Moore (1982) and Sato and Sato (1992) as they noted that the tongue at 8th week was elevated and assumed prominence through the differentiation of voluntary muscle internally which arranged into three layers.

In the present study at the 10th week of gestation nerve plexuses were detected invading the connective tissue cores of the fungiform papillae. These plexuses were formed of bundles of nerve fibers. These nerve plexuses were progressively increased in size and distribution and at full term, they were detected at the bases and centers of connective tissue cores of the fungiform papillae.

In the present work the invasion of the fungiform papillae by the nerve plexuses were associated with the appearance of the presumptive taste buds and thus ensured the formative influence of the nerves to induce the development of taste buds. These results were in agreement with Arey (1974), Witt and Reuter (1998) and McDaniel (1999) as they reported that the functional relationship between the nerve and epithelium was more intimate that one might assume, since the nerve seemed to exert an organizing influence on the development of taste buds.

Witt and Reutter (1998) and McDaniel (1999) reported that there was sub epithelial nerve plexuses with ganglion cells associated with taste buds. But the autonomic and somatosensory nerves seemed not to play a key role information and maintenance of early human taste buds.

In the present work, the taste buds appeared so primitive at 12th week of gestation; they appeared as local epithelial thickening. At the 12th week, they still primitive and occupied the apical part of the fungiform papillary epithelium. They were formed of closely associated cells which could be difficulty distinguished from the surrounding epithelium.

At 20th week of gestation, it became presumptive and moderately developed taste buds were formed as they still had primitive taste pores and moderately developed cells.

At full term, the taste buds became well developed and occupied the whole epithelial thickness with well-developed taste pores and cells. These results confirmed the results of Arey (1974), Witt and Reutter (1997), and Iwasaki *et al*, (2001). As they reported that nerve fibers approached the basal membrane of the lingual epithelium around the 8th week and

invaded the basal membrane of papillae like structures at the 9th week. Local thickening of the lingual epithelium presaged the first taste buds. The basal cells of such a thickened spot lengthened and extended toward the surface of the epithelium. This produced an epithelial cluster, which in later fetal months differentiated furthermore. Some of the elements specialized into slender taste cells which ending in hair like receptive tips, while others, became columnar supporting cells supposedly non sensory function. Reflex pathway between the taste buds and facial muscles were present by week 26 to 28 of gestation.

From the present study, it could be concluded that the fungiform papillae developed before filiform and reached the adult shape at 20th week of prenatal life. There was also a vessel, nerve, taste organ relationship for development and growth of fungiform papillae.

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دراسات هستولوجية لنمو الحلمات الفطرية في لسان الإنسان في فترة ما قبل الولادة

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**كتاليست / هيئة المعونة الأمريكية

إستخدم في هذا البحث عدد ٢٠ من أجنة الإنسان تتراوح أعمارهم بين ٤٠ إلى ٤٠ أسبوع من الحمل وقد أخذت عينات عمودية من الثلثين الأماميين من اللسان لفحص الحلمات الفطرية في فترة ما قبل الولادة . وقد أوضح البحث ان أول ظهور للحلمات الفطرية كان في عمر ١٠ أسابيع من الحمل وقد ظهرت هذه الحلمات على صورة إسطوانية الشكل وفي عمر ١٢ أسبوع أصبحت شكل الفطر حيث أن لها قاعدة ضيقة وقمة واسعة مستديرة، وفي عمر ٢٠ أسبوع تميزت الحلمات إلى نوعين الإبتدائي والثانوي وقد صاحب ذلك ظهور عدد كبير من الأوعية الدموية الصغيرة في قلب النسيج الضام للحلمات والتي إزدادت بكثرة في عمر ٤٠ أسبوع وقد ظهرت الخلايا والألياف العصبية وهي تخترق قلب النسيج الضام للحلمات الفطرية لتصل الى الغشاء الطلائي وقد صاحب ذلك ظهور براعم التذوق الأولية و كانت على هيئة بقع موضعية في الغشاء الطلائي للحلمات الفطرية و كانت تتكون من مجموعة من الخلايا المتماسكة غير المتطورة و يصعب تمييزها من الخلايا المجاورة . وواصلت هذه البراعم نموها و في عمر ٢٠ أسبوع من الحمل تكونت براعم تذوق معتدلة التطور حيث إزدادت خلاياها تطوراً وقد إتخذت شكل براعم التذوق فعمر ٤٠ أسبوع من الحمل.

Developmental and Histological Studies.....