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Histogenesis of Male Gonads in Developing Human Foetuses

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Abstract:

The male Gonads or Testes are embryologically derived from "Mesoderm" except for Primordial germ cells giving rise to spermatogonia which are derived from epiblast. The true sources are the mesothelium lining the posterior abdominal wall, underlying mesenchyme & Primordial germ cells. The gross changes occurring during development of male gonads are studied in much more detail as compared to histological changes therefore very few studies available on the same.

40 aborted male human fetuses between 12- 40 weeks of gestational age with no obvious congenital anomalies were obtained for study. Testes were removed and histological slides of 5 to 7 microns sections were prepared and stained with Haematoxylin and Eosin method. Also some slides were stained with Masson's trichrome stain & studied under light microscope. The tunica albuginea & epiorchium were already differentiated at 12th week of gestational age. Tunica vasculosa appeared at 14th weeks of gestational age. At 16th week some rete tubules were seen in mediastinum. At 24th weeks of gestational age complete septae were seen, and the lobules were completely formed. At 28th week both layers of tunica vaginalis were seen. Initially dark nucleated spermatogonia were more numerous than pale nucleated spermatogonia and Sertoli cells. After 30th week pale nucleated spermatogonia were more numerous along with Sertoli cells. The findings also showed that testis at term were not assuming adult architecture. The seminiferous tubules were showing absence of **cells of** spermatogenic series. They seem to appear at puberty, and lumen in these tubules does not appear till term.

Keywords: Mesoderm, Primordial Germ Cells, Tunica Vasculosa, Rete Tubules, Spermatogonia

Introduction:

Histogenesis means differentiation of tissues, organs, various systems of whole body from a single cell. This term is basically derived from 'histogeny' (histo-cell, geny- differentiation)[1]. It is closely related with embryology & histology of an individual tissue & organ.

Testes are embryologically derived from "Mesoderm" except for germcells giving rise to spermatogonia which are derived from "Ectoderm"

i.e. epiblast[2], previously said to be derived from Endoderm of Yolk sac.

Following are the true sources from which the male gonads develop[3]:-

a) The mesothelium lining the posterior abdominal wall.
b) Underlying mesenchyme.
c) Primordial germ cells.

Although the chromosomal and genetic sex is determined at the time of fertilization; male and female morphological characteristics do not begin to develop until 7th week of Intra- uterine life. The early genital systems in the two sexes are similar (Habert et al., 2001). Thereafter differentiation into testes and ovaries occurs under the influence of some genetic and hormonal factors. Any diversion from normal growth can lead to formation of various sexual abnormalities. Ovo-testis development leads to formation of intersex individuals. When both components express themselves completely the condition is called as TrueHermaphroditismand such individual is regarded as TrueHermaphrodite, but this is very rare. Usually expression of one of the component dominates and leads to formation of various types of Pseudo-Hermaphrodites. The pure gonadal dysgenesis occurs due to failure of migration or early destruction of Germ cells[3].

The development of foetus in intrauterine life being a topic of great interest for embryologists, many studies have been carried out to know about the development of various organs. The gross developmental changes are studied in much more detail as compared to histological changes occurring during development. So the present work is undertaken to study various histological changes at different gestational ages.

Material and Methods:

Forty aborted male human foetuses between 12- 40 weeks of gestational age (Intra-Uterine Life, IUL) with no obvious congenital anomalies were obtained from the Department of Obstetrics and Gynaecology, GMC, Miraj, & PVPGH, Sangli, with the prior permission of Head of Department and consent of parents. The study was approved by the Ethical Committee. Gestational age, sex, weight and crown- rump length were noted in detail. Testes were removed from the abdomen, Scrotum and sections of right and left Testes were taken in such a way that they included testis as well as epididymis, in each single section and were fixed in 10% formalin for 48 to 72 hours. The tissue was processed and paraffin blocks were prepared and 5 to 7 μm thick sections was taken on the rotary microtome. The slides were stained with haematoxylin and eosin, masson's trichrome and observed under light microscope[4].

Observations & Results:

The observations under light microscope were done under low power and high power after staining the slides with haematoxylin and eosin, masson's trichrome stain.

At 12 Weeks of gestational age

On panoramic view 4X (**Figure1.**) tunica albuginea, mediastinum testis, mesoorchium, ill-defined primitive lobules divided by primitive septae, seminiferous tubules, primitive epididymis, & degenerating mesonephric tubules were seen.

On 10X (**Figure 2.**) low power - additionally budding intralobular vessel, fibres in tunica albuginea, newly formed seminiferous tubules more or less straight, few leydigs cells in interstitial space were seen.

On 45X (**Figure 3.**) high power - dark & pale nucleated spermatogonia, presertoli cells, peritubularmyoid cells, interstitial leydigs cells along with capillaries in interstitium were seen. Dark staining spermatogonia were more numerous than pale staining which were few in number.

Also on special staining (masson's trichrome **Figure 4.**) the constituents of tunica albuginea -epiorchium single layer of flat cuboidal epithelium lining outermost part of tunica albuginea, collagen fibres and nuclei of myofibroblasts were seen.

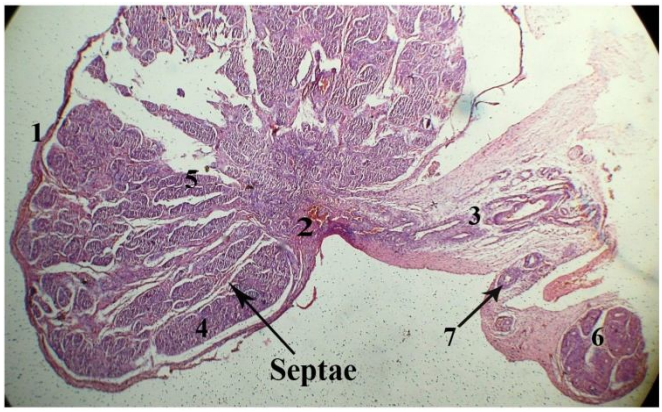

Figure 1. *12 weeks panoramic view (4X) Testis showing 1.Tunica Albuginea 2.Mediastinum testis 3.Mesoorchium 4.Primitive lobule 5.Seminiferous tubules 6.Epididymis 7.Degenerating Mesonephric tubules*

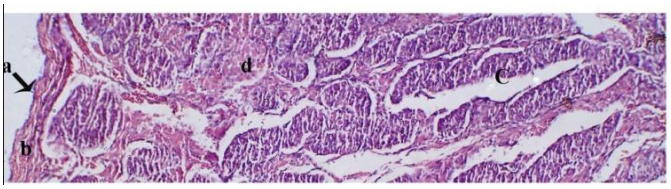

Figure 2. *12 weeks 10X low power Testis showing a. Tunica albuginea b. Fibres in tunica albuginea c. Seminiferous tubules d. Leydigs cells*

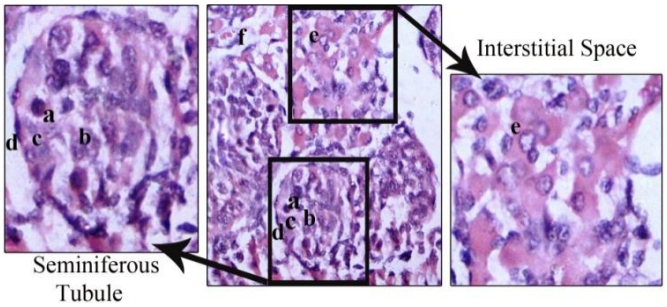

Figure 3. *12 weeks 45X high power Testis showing a. Dark & b. Pale nucleated spermatogonia c. Presertoli cells d. Peritubularmyoid cells e. Interstitialleydigs cells f. Capillary in interstitium*

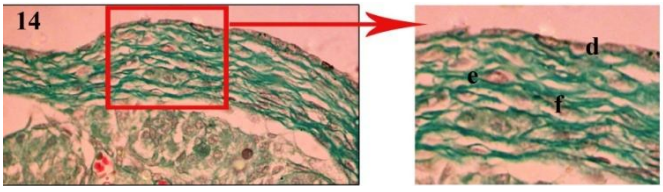

Figure 4. *12 weeks 45X high power Testis Masson's Trichrome stain showing constituents of Tunica albuginea d. Epiorchium e. Collagen fibres f. Nuclei of Myofibroblasts.*

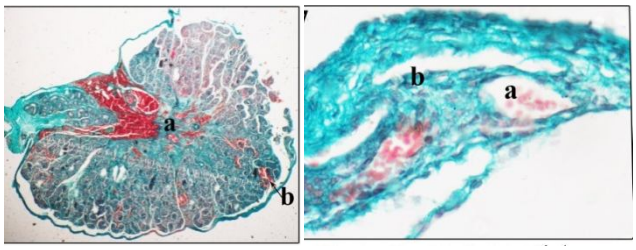

Figure 5. *14 weeks Masson's Trichrome stain Testis on left (4X) panoramic view showing increased vascularity; on right 45X high power showing a. Budding vessel in Tunica vasculosa b. other constituents of tunica albuginea.*

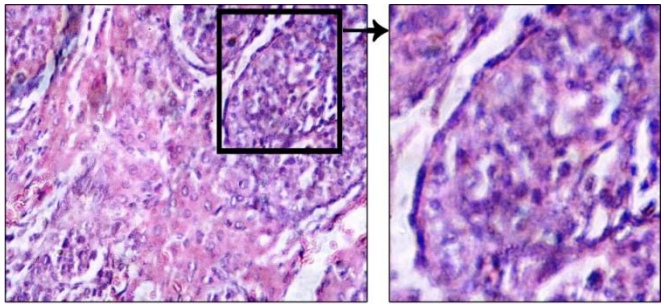

Figure 6. *14weeks high power Testis 45X showing Leydigs cells and Seminiferous tubules enlarged on right side.*

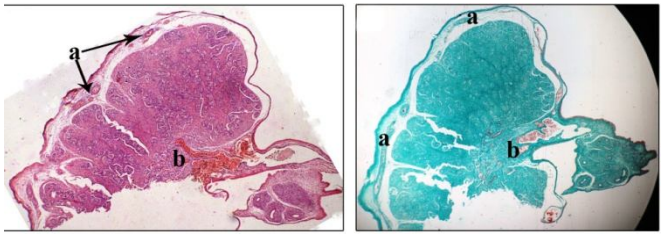

Figure 7. *16weeks 4X panoramic view Testis showing increased vascularity a. Tunica vasculosa b. Mediastinum.*

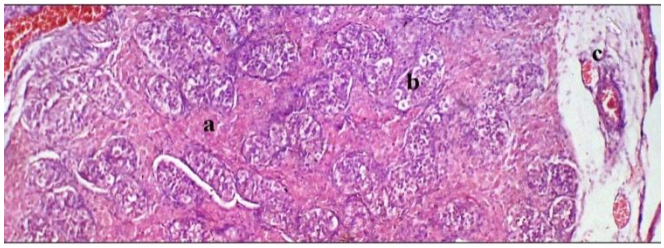

Figure 8. *16weeks 10X low power Testis showing a. Leydigs cells b. increased coiling of Seminiferous tubules c. Tunica vasculosa.*

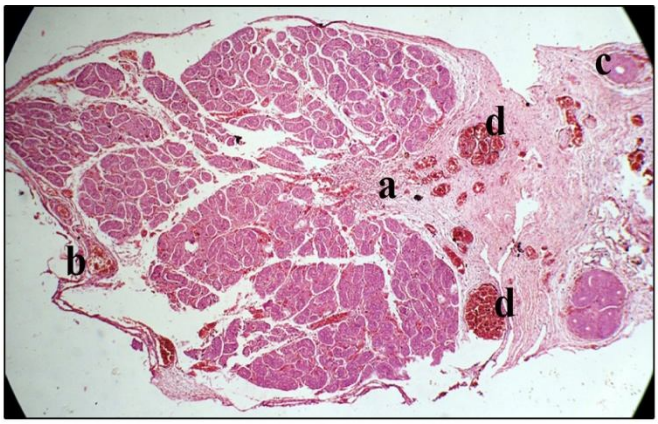

Figure 9. *18 weeks 4X panoramic view Testis a. Rete testis b. Tunica vasculosa c. Ductus deferens d. Testicular vessels.*

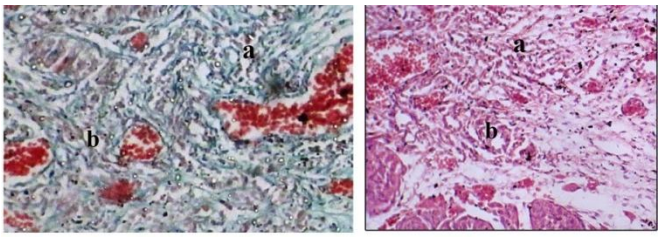

Figure 10. *18 weeks 45X high power Testis a. Rete testis b. Rete tubules lined by low cuboidal epithelium.*

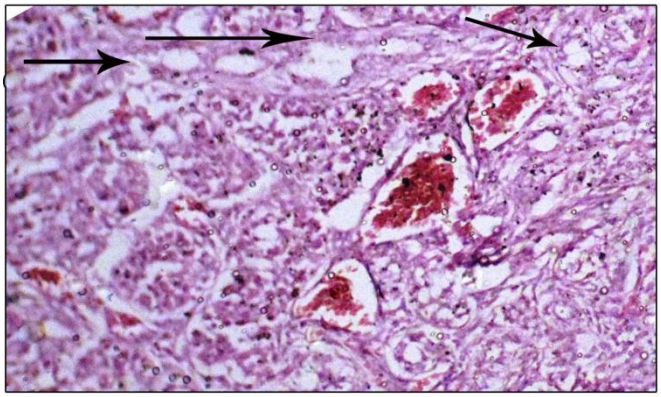

Figure 11. *22 weeks 45X high power Testis arrows showing Straight tubule (Tubuli recti)*

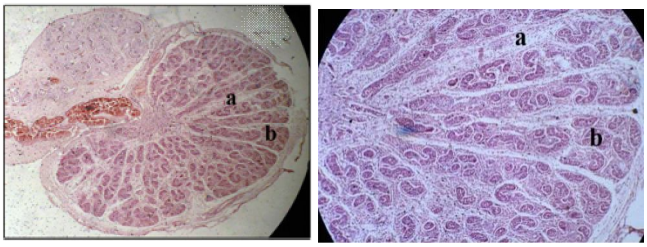

Figure 12. *24 weeks 4X panoramic view Testis right side and 10X low power view showing fully developed a. Septae running & dividing the b. Lobules clearly.*

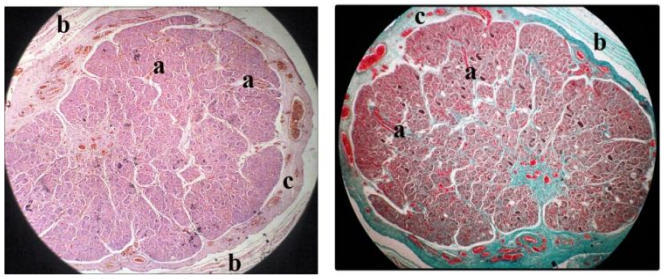

Figure 13. *30 weeks 4X panoramic view Testis showing a. Rich vascularity b. Tunica vaginalis differentiated c. Thick Tunica Albuginea*

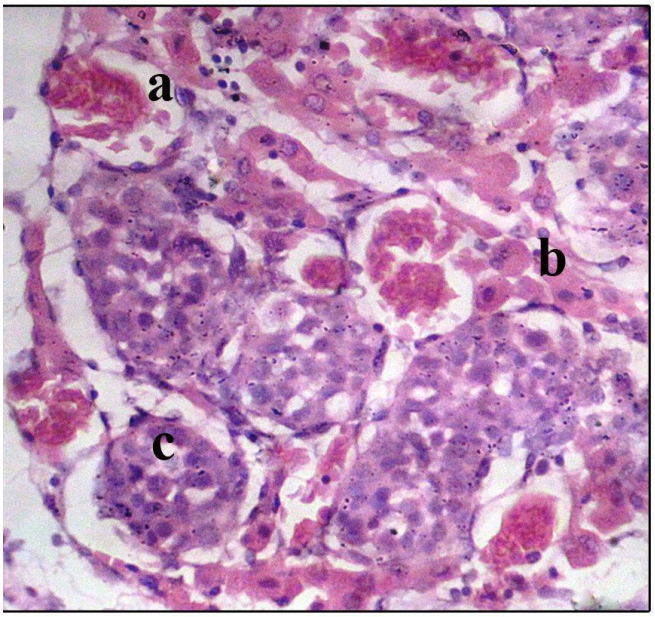

Figure 14. *30 weeks 45X high power Testis showing a. More vessels b. Leydigs cells less than c. Seminiferous tubules*

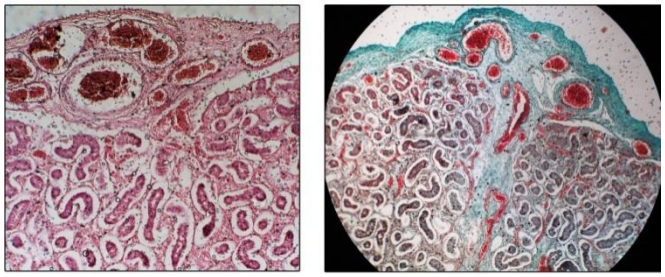

Figure 15. *At term 10X low power Testis showing many coiled Seminiferous tubules and very few Leydigs cells.*

At 14 Weeks:

The testis at 14th weeks showed increased vascularity (**Figure 5.** 4X panoramic view). Budding vessels in tunica albuginea indicated the appearance of Tunica vasculosa (**Figure 5.** 4X panoramic view). The coiling of seminiferous

tubules and amount of leydigs cells was increased **(Figure 6.***14*weeks high power 45X*)*.

At 16 Weeks:

Large vessels were seen in tunica albuginea, interstitial space indicating full differentiation of tunica vasculosa **(Figure 7.** 16weeks 4X panoramic view). Coiling of seminiferous tubules was increased. Amount of leydigs cells increased in interstitial space **(Figure 8.** 16weeks 10X low power).

At 18 Weeks:

Rete testis was seen as branching and anastomosing cords in mediastinum **(Figure 9.** 18 weeks 4X panoramic view). Rete tubules lined by low cuboidal epithelium were also seen with distinctive lumen **(Figure 10.** 18 weeks 45X high power).

From 19 to 24 Weeks:

The straight tubules, rete tubules and efferent ductules were seen with distinctive lumen at 22 weeks (**Figure 11.** 22 weeks 45X high power). At 24 weeks (**Figure 12.** 24 weeks 4X panoramic, 10X low power view) fully developed Septae running & dividing the Lobules clearly were seen. Coiling of seminiferous tubules was increased.

Leydigs cells concentration was increased and they appear to occupy the lobules more in numbers than the seminiferous tubules.

From 25 to 36 Weeks:

At 28 weeks the parietal layer of tunica vaginalis was first seen. The testis at 30 weeks (**Figure 13.** 30 weeks 4X panoramic view) showedrich vascularity, differentiated tunica vaginalis and thick tunica albuginea. The seminiferous tubules were showing (**Figure 14.** 30 weeks 45X high power) pale nucleated spermatogonia more in number than dark nucleated spermatogonia. Few Leydigs cells were seen indicating reduced number.

Testis at Term; 37 to 40 Weeks:

At term (**Figure 15.**At term 10X low power) The Cavity of testis showed highly coiled Seminiferous Tubules. The cytoarchitecture of adult Sertoli cells was not achieved. No lumen was identified inside seminiferous tubules through the developmental period. The testis at term not achieved cytoarchitecture of adult testis.

Discussion:

The observations regarding following structures were considered for study of microscopic structure Tunics (Capsules/Coverings), septae and Lobules of testes, Development of Duct system of testes in mediastinum and meso-orchium, Intralobular structures, Seminiferous Tubules development, interstitial tissue development & Blood supply of testes.

a) Tunics (Capsules/Coverings), septae and Lobules of testes:

All layers of capsule of testis were not differentiated at 12th week of gestation. The tunica albuginea & Epiorchium were already differentiated at 12th week of gestational age. Tunica vasculosa appeared at 14th weeks of gestational age. It was fully differentiated at 16th weeks of gestational age. Vascularity and thickness of capsule increased throughout the period of intrauterine life.

The lobules were primitive from 12th to 22nd weeks of gestational age, marked by abortive septae. At 24th weeks of gestational age complete septae were seen, and the lobules were completely formed. At 28th week both layers of tunica vaginalis were seen. Further differentiation of capsule continued up to term. Capsule and septae increased in thickness up to term.

Findings of present study for Tunics (Capsules/Coverings), Septae and Lobules of testes were comparable with the findings of Damayanti et al.; (2006)[5], Kye Yong Song et al. (1981)[6] and Ralf Middendorfft et al.(2002)[7], also with the findings quoted in the Textbook of Human Embryology, (Fourth Edition) Hamilton, Boyd and Mossman (1972).

b) Development of Duct system of testes in mediastinum and meso-orchium:

The duct system of testis was in developing stages at 12th week. Whereas degenerating mesonephric ductules and glomeruli were seen at this gestational age. Rete testis and tubules were not seen at 12th

week. At 16th week some rete tubules were seen in mediastinum, but rete tubules with distinctive lumen lined by flat to cubical cells were seen at 18th week only. After 18th week the Rete tubule showed changes in lining epithelium from flat to cubical epithelium up to term. The number of tubules was increased as testis approached to term. The coils of epididymis increased towards term. Duct system was fully differentiated at term.

Present study findings regarding development of duct system of testes in mediastinum and mesoorchium were comparable with the findings of Damayanti et al.; (2006)[5], Kye Yong Song et al. (1981)[6] and, also with the Textbook of Human Embryology, (Fourth Edition) Hamilton, Boyd and Mossman (1972)[8].

c) Intralobular structures i.e. Seminiferous Tubules, and Interstitial tissue development:

Intralobular structures i.e. seminiferous tubules and interstitial Leydig cells and other connective tissues were seen at 12th week. The newly formed seminiferous tubules filled the cavity of testis completely and arranged more or less straighter at 12th week. The interstitial space and cells were meagre, vascularity also minimal in lobules. The seminiferous tubules contained more dark and less pale nucleated spermatogonia along with few pre-sertoli cells. The outer lining of seminiferous tubules - peritubularmyoid cells was single layered. From 14th to 24th weeks seminiferous tubules become more coiled, and they increased in length. Interstitial Leydig cell concentration increased in interstitial space from 12th to 20th weeks. Leydig cells proliferation was the most striking feature in this period, up to 24th week they remain the most prominent structure in Intralobular space. Peritubularmyoid cells and connective tissue surrounding the seminiferous tubules increased in thickness. Further differentiation of intralobular structures showed increased coiled tubules, Leydig cells become less as testis approached to term. At term numerous coiled tubules filled the intralobular cavity; Leydig cells become very few and concentrated more nearer to vessels.

Present study findings for development of Intralobular structures, seminiferous tubule development and Interstitial tissue development were comparable with the findings of Damayanti et al.; (2006)[5], Kye Yong Song et al. (1981)[6], P. J. O'Shaughnessy et al. (2007)[9], Tessa J. Murray et al.(2000)[10],Hermo et al. (1977)[11], Hebert et al. (2001)[12] and, also with the Textbook of Human Embryology, (Fourth Edition) Hamilton, Boyd and Mossman (1972)[8], Wheater's Functional Histology – B. Young, J. W. Heath, fourth edition (2000)[13].

d) Blood supply of testes:

At 12th week the vessels were concentrated in Mesoorchium. Some branches were also approaching to mediastinum. Budding vessels were also seen nearer to mediastinum in abortive septae. With differentiation of tunica vasculosa at 14th week more vessels were localised beneath tunica albuginea. From 14th to 24th week intralobular vessels increased; mainly those surrounding Leydig cells indicating their endocrinal function. Further differentiation in vascularity continued up to term in the form of increase in number and thickness of wall of vessels.

Present study findings for development of Blood supply of testes were comparable with Damayanti et al.; (2006)[5].

Conclusions:

1. Tunica vasculosa differentiated at 14th week of gestational age, and both layers of tunica vaginalis appeared at 28th week of gestation.
2. Rete testis and tubules differentiated at 16th to 18th week of gestational age.
3. The Leydig cell proliferated between 12th to 20th weeks of gestation; they were the most striking structures in lobular cavity up to 24th week of gestational age.
4. The seminiferous tubules were less prominent up to 24th week, after 24th week

they became the most predominant structures in lobular cavity.

5. Initially dark nucleated spermatogonia were more numerous than pale nucleated spermatogonia and Sertoli cells. After 30th week pale nucleated spermatogonia were more numerous along with Sertoli cells.

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