MORPHOGENESIS OF THE NASAL SEPTUM IN WATER BUFFALO (BOS BUBALIS L.)

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ABSTRACT

Twenty one buffalo embrios and fetuses of both sexes ranging from 8 - 102 cm CVR length corresponding to 80-315 days old were collected from Mansoura abattoir. they were prepared by normal histological technique for morphometrical and light intcroscopic study. The result revealed that, nasal septum represented by a vertically oriented sheath between the developing nasul pits at 8 - 10 cm CVRL (80 -85 days-old), the mesenchymal tissue was differentiated into chondrogenic cells. At 12 cm CVRL (90 days-old), the septal cartilage showed chandroblastic condensation in the periphery, it was of hyaline type. Invasion of its basal thickening with blood vessels was observed at fetuses of 48 cm CVRL. The nasal epithelium was derived from the face ectoderm mesenchymal tissue; It was composed of cuboidal durkly stained basal cell layers and many (12-13) polyhedral cell layers. At 36 cm CVXI. (150 days-old), it was differentialed into stratified squamous epithelium in the nasal vistibile, stratified cuboidal epithelium in the transitional area and pseudostratified ciliated columnar in the nasal cavity proper. The nasal glands were observed at 12 cm CVRL (90 days-old), in the form of solid clusters with a relative volume density of 10.51%. They were numerous, isolated, solltary acint, almost canalized at 25 cm CVRL (195 days old) and constituted 12.8% of the nasul septal submucosa. It was lobulated with wide distended lumen at 68 cm CVRL (230 days-old) its volume density was 23.32%, some acini showed alclan blue positive reaction at 80 cm CVRL (260 days-old) and the others exhibited a weak PAS positive reaction it formed 25.62% of the septal submucosa, the vemeronasal gland was observed at 48 cm CVRLat the lamina propria of the vomeronasal organ, represented 12.38% of the vomeronasal organ submucosa it showed a strong PAS positive reaction. Extensive, large blood spaces mostly filled with blood, formed a cavernous tissue at the basal part of the nasal septum and at the level of the vomeronusal organs observed at 48 cm CVRL (180 days old).

INTRODUCTION

It has been believed that the nasal mucosa was involved in the thermoregulatory mechanism, offactory sense and mediation of the reproductive behavioral responses in several species of mammals. In rat (Bojsen-Moller, 1964), sheep (Khamas, 1980) and donkey (Lindsay et al., 1979). The anatomical and histological structure of the nasal cavity of domestic animals was thoroughly described by many authors such as in sheep (May, 1970), camel (Badawi et al., 1974), buffalo (Al-Ayat et al., 1981 & Abdel Axiz, 1983) and in domestic animals (Getty, 1971 & Dellmann and Browen, 1992) and in rat (Vidic, 1971), and that concerned with the structure and morphogenesis of the vomeronasal organ in domestic animals (Kratzing, 1971; Lindsay et al. 1979, Jacob et al. 1981 & Mansour et al. 2001) and in laboratory animals (Mendoza, 1986 & Szabo and Mendoza, 1988). The prenatal morphogenesis of the septum nasi was evaluated in rat (Vidic et al. 1972) and goat (Basha et al. 1994). The prenatal morphogenesis of the nasal septum of the buffalo was not evaluated. The present study is an attempt to provide information concerning the prenatal development of nasal septum and its related glands in the water buffalo.

MATERIAL AND METHODS

The present study was carried out on twenty one buffalo embryos and fetuses of both sexes ranging from 8-102 cm CVRL corresponding to 80-315 days-old. These were collected from Mansoura abattoir. The heads of fetuses were dissected, the nasal cavity was separated and nasal region including the nasal septum was isolated. fixed in Bouin's and 10% neutral buffered formal-in solutions, decalcified in 5% EDTA solution. The samples were processed by normal histological technique, sectioned at 5 _m thickness, stained with haematoxylin, eosin and alcian blue / periodic acid Schiff's reagent stains adopted by (Bancroft & Stevens 1996). Volume density(Vv) of the nasal gland was determined using double square lattice of 400 point and light micrographs with final magnification of X1300. The volume density obtained using the formula Vv. = pc/p (Weible, 1989) where pc means the number of points in a given structure and p was the number of points in reference area. The ages of fetuses were estimated according (Abdel Raouf & El-Naggar 1968). Nomencalatrue was that adopted by Nomina Anatomica Veterinaria (1983).

At 8-10 cm CVRL (80-85 days-old) the nasal septum represented by a median vertically oriented lamina between the nasal pits, it fuses with the palate in premaxillary region, where it expands laterally, the mesenchymal tissue in the nasal septum was differentiated into chondrogenic cells with rounded nuclei. The future septum nasi was covered on both sides by the subepithelial mesenchymal tissue that condensed forming the early perichondrium, which was differ-

entiated into up to five elongated cell layers (Fig. 1). The nasal epithelium was derived from the face ectoderm, which was present in stratified pattern, it was composed of cuboidal darkely stained basal cell layer with rounded eccentric nuclei and many polyhedral cell layers with lightly stained and vacuolated cytoplasm forming up to 12-13 cell layers (fig. 2 & 3).

At 12 cm CVRL (90 days-old), chondroblastic condensation was observed in the periphery of the cartilago septum has which was of hyaline type, large cells with rounded prominent nuclei and dense cytoplasm prodominate in cartilago septum has. The perichondrium was differentiated and became more dense and distinctive; it was composed of parallel bundles of collagen fibers with many fibroblasts (fig. 4). The basal cell layer of the hasal epithelium was of columnar type, followed by 7-8 polyhedral lightly stained cell layers with vacuulated cytoplasm. The most superficial cell layers were flattened with dense cytoplasm and dense elongated nuclei (fig. 5). The subepithelial tissue was more differentiated, blood vessels of different size were observed, the hasal glands were detected for the first time at this stage of development, in the form of solid clusters of densely stained cells or rode like budding from the basal cell layer (fig. 4). It had a relative volume density of 10.51 % within the subepithelial tissue (table, 2 & chart 1).

At 18 cm CVRL (105 days-old), cartilage septom has was relatively thick, the epithelium of septum has was differentiated in the hasal cavity proper. Into stratified ciliated columnar, especially at its proximal part while it was undifferentiated distally. The lamina propria was thicker, at the end of proximal third of the hasal septum; it formed a cushion like prominence projected at the hasal cavity. At this stage many blood vessels of variable size were abundant and engorged with blood in proximal part of the hasal septum (fig. 6). The perichondrium was very thick, which composed of parallel thick bundles of collagen fibers with many fibroblasts and an timer several layers of dense elongated chudroblastic cells and small blood vessels (fig. 7). The hasal glands were numerous and some actor were canalized (fig. 8). It constituted 10.86 % of the lamina propria.

At 25 cm CVRL (123 days-old), the covering epithelium was differentiated in the nasal cavity proper into pseudostratified columnar epithelium allover the nasal septum (fig. 9) except at its basal part and at the level of the vomeronasal organ it was still undifferentiated. In the lamina propria, large blood spaces were detected forming cavernous tissue like structure in the dorsal part of the nasal septum. The nasal glands were numerous in the form of isolated solitary acini. It was dispersed in the lamina propria forming 12.8 % of the subepithelial tissue, great proportion of these acini were canalized (Fig. 10).

Epithelial differentiation was complete at 36 cm CVRL (150 days) all over the nasal cavity and the nasal septum where, it was stratified squamous epithelium in the nasal vestibule, strati-

fied cuboidal epithelium in the transitional area and pseudostratified ciliated columnar in the nasal cavity proper. An aggregation of small blood vessels was observed at the basal part in the lamina propria of the nasal septum. The nasal gland arranged in groups in the lamina propria and had a wide distended lumen with arelative volume density of 12.93 % (fig. 11).

Invasion of the basal thickening of nasal septal cartilage with blood vessels was detected for the first time at fetuses of 48 cm CVRL (108 days-old). The septal cartilage was surrounded ventrally by dense packing of connective tissue which extended on both sides (fig. 12). The lamina propria was greatly differentiated, many blood vessels of different size, lymph vessels, nerve fast-cult, were observed. The nasal glands located in groups deeply in the lamina propria, its relative volume density of about 13.01%. The omeronasal glands were detected at the lateral real of lamina propria of ductus vomeronasalis for the first time, at this stage constituting 4.32% of organ vomeronasalis submucosa (table 3.), it as of serous type. Extensive, large blood spaces mostly filled with blood, forming a cavernous tissue observed at basal part of the nasal septum and at the level of the vomeronasal organs. (fig. 13).

At 54 cm CVRL (195 days-old), the nasal glands were more abundant in lamina propria of the nasal septum (fig. 14). It displayed 12.43 % it was predominantly serous, the glandular duct was lined by two layers of cuboidal epithelium and open on the surface epithelium. In the nasal vestibule, the stratified squamous epithelium in its middle was lightly stained and vacuolated with clear cell membrane, the most superfacial cell layer was dense flattened within elongated dense nuclei (fig. 15), the vomeronasal gland constituted 5.57% of the vomeronasal lamina propria. Large blood spaces mostly engorged with blood formed a cavernous tissue were present at the basal part of the nasal septum (fig. 16).

At 68cm CVRL (230 days-old), lobulated nasal glands with wide distended lumen were seen (fig. 17), the glands occupied 23.32% of the lamina propria of the nasl septum. While the volume density of the vomeronasal gland was 10.89%.

At 80cm CVRL (260 days-old), the covering epithelium at the nasal vestibule became thick and densely stained, the lamina propria formed a papillary projection into the lamina epithelialis (fig. 18). The relative volume density of nasal glands in lamina propria of the nasal septum was 25.62%.

At 102 cm CVRL (315 days-old), the volume density of the nasal glands decreased with presence of huge and large blood spaces. It constituted 25.4% of the lamina propria of the septum nast.

At 80 cm CVRL (260 days-old) and 102 cm CVRL (315 days-old), some acini showed alcian blue positive reaction and others exhibited faint PAS positive reaction (fig. 19). At 102 cm CVR

length, the vomeronasal glands extremely increased displaying 28.95 % of the vomeronasal organ submucosa (table 2&3). It showed a strong PAS positive reaction (fig. 20).

Discussion

The present study revealed that the primitive nasal septum of the buffalo fetuses 8-10 cm CVRL was represented by a vertical sheath between the developing nasal pits. It was built up from chondrogenic plate, lamina propria and thick epithelial covering. The mentioned lindings was similar to that in camel fetuses of 2 cm CRVL (Amed, 1988) and in goat of 1.5-5 cm CVRL (Basha et al., 1994).

The present investigations demonstrated sequences of osteogenesis of the septal nasal cartilage began in the prenatal life at 48 cm CVRL (180 days old). This finding was supported by studies in sheep (May, 1970) and in rat prenatal life (Wright et al., 1958 & Vidic et al., 1972). In contrast to the data previously mentioned in camel (Ahmed, 1988) in goat (Basha et al., 1994), they recorded persistence of the cartilaginous plate of the nasal septum along the prenatal life.

In rat it was stated that the epithelia of the nasal cavity differentiated rapidly following the appearance of the nasal pits (Vidic et al., 1972). This finding was confirmed in the present study, where it was completely differentiated allover the nasal cavity at 36 cm CVRL (150 days-old). The nasal septum in the buffalo fetuses was covered by stratified squamous epithelium in the nasal vestibule and pesudostratified ciliated columnar epithelium in nasal cavity proper and stratified cuboidal epithelium at the transitional zone. That was agreed with that in rat. at 17 day of gestation (Vidic et al., 1972). However, in dog (Abbas et al. 1981) and in buffalo (Abdel Aziz, 1983) it was mentioned that, the septum nasi was covered by thick stratified columnar epithelium. While, in goat (Basha et al., 1994), was claimed that the covering epithelium was stratified squumous epithelium. The flask shaped crypt which was observed post natal in the buffalo (Abdel Aziz, 1983) and in camel (Badawi et al., 1974) was not observed neither in camel fetuses (Ahmed, 1988), goat (Basha et al., 1994) nor in buffalo fetuses during the course of the present study.

The present study revealed that the first glandular primordial in the masal septum submucosa of the buffalo fetuses was observed at 12 cm CVRL (90 days-old), canalized at 25 cm CVRL (123 days-old) and increased and lobulated with advanced age. Similar findings were recorded in rat at 16 day of gestation (Vidic et al., 1972): in camel at 9 cm CVRL (Ahmed, 1988) and in goat at 12cm CVRL (Basha et al., 1994).

The present investigation recorded an alcian blue positive reaction in the nasal septal glands.

that was disagreed with that have been reported in rat (Vidic et al., 1972), while he mentioned that no affinity for the same reaction could demonstrated in the prenatal glands.

The glandular buds firstly recorded in vomeronasal organ submucosa at 48 cm CVRL (180 days-old), this finding were supported by reports in buffalo (Elmorsy, 2002) corresponding to 8 cm CVRL in goat (Abdel Aziz et al., 1991). 35 cm CVRL in came) (Abdel Aziz, 1988).

The vomeronasal glands in buffalo fetuses exhibited strongly PAS positive and alcian blue negative reactions. This finding agreed with that recorded in sheep (Kratzing, 1971), camel (Abdel Aziz, 1988), goat (Abdel Aziz et al., 1991), domestic animals (Dellmann and Browen, 1992) and in buffalo (Elmorsi, 2002) and disagreed with that reported in cat (Salazar) in horse and donkey (Lindsayet al. 1978 & Mansour et al. 2001).

The present study revealed that many blood vessels of different size and relatively large blood spaces were observed in the lamina propria in the dorsal part of the nasal septum at 25cm CVRL (123 days-old) and hug blood spaces mostly engorged with blood were detected at basal part of the nasal septum and in lamina propria of the vomeronasal organ, forming a cavernous tissue, at 48 cm CVRL (180 days-old). These finding correlated with that described in sheep, (Kratzing, 1971), bovine (Jacobs et al., 1981), mouse (Mendoza 1986), camel (Abdel Aziz, 1989), goat (Abdel Aziz et al., 1991 & Basha et al., 1994), donkey (Mansour et al. 2001) and buffalo (Elmorsi, 2002). This cavernous envelop had regarded as regulator of either inspiration and expiration of air or aspiration and expedition of fluids between the nasal cavity and the vomeronasal organ in hamster (Meredith et al., 1980), and in rat (Wysocky, 1979 & Szabo and Mendoza, 1988).

The lamina propria of nasal septum at 18 cm CVRL was thickened forming a cushion like swelling, that correlate with the observations recorded in goat (Schnorr and Hegne, 1967, Nickel, Schummer and Seiferles, 1973 & Elmorsi, 2002), sheep (Al-Ayat et al. 1981), and buffalo (Abdel Aziz, 1988).

Table (1): Estimated ages and CVRL of collected buffalo fetuses

No. of fetuses	CVRL (cm)	Estimated age (days)
2	8	80
3	10	85
1	12	90
2	18	105
3	25	123
1	36	150
2	48	180
2	54	195
2	68	230
2	80	260
1	102	315

Table (2): Volume density of the nasal glands in the nasal septum of the buffalo fetuses in relation to CVRL

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CVRL	Volume density (Vv%)
12 cm	10.51
18 cm	10.86
25 cm	12.8
36 cm	12.93
48 cm	13.01
54 cm	22.43
68 cm	23.32
80 cm	25.62
102 cm	25.4

Table (3): Volume density of the vomeronasal glands the buffalo fetuses in relation to CVRL

CVRL	Volume density (Vv%)	
48 cm	4/32	
54 cm	5.57	
68 cm	10.89	
80 cm	12.38	
102 cm	28.95	

Chart (1): Velome density of navat glands in pasat septum of

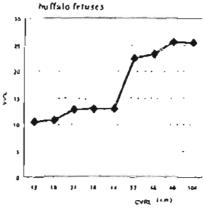
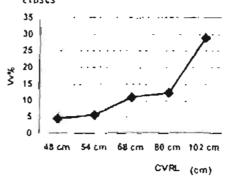


Chart (2): Volume density of vomeronasal glands in buffalo etuses



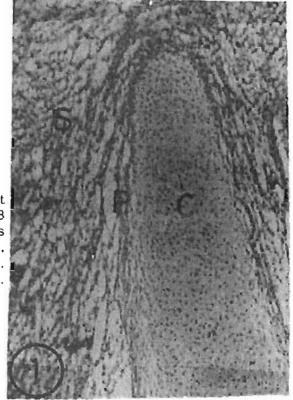
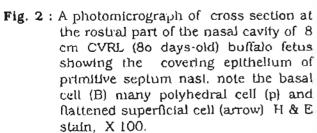
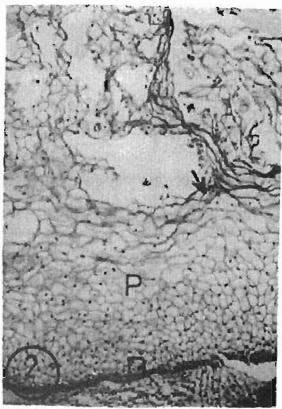


Fig. 1: Photomicrograph of cross section at the rostral part of the nasal cavity of 8 cm CVRL (80 days-old) buffalo fetus showing the chondrogenic plate (C), surrounding mesenchymal tissue (S), and the primitive perichondrium (P). H&E stain X 100.





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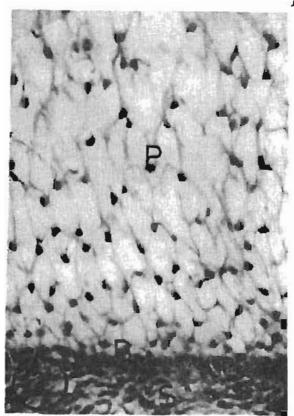


Fig. 3: Magnification of fig. (2), note the cuboldal to low columnar basal cells (B), the eccentric nuclei of the polyhedral cells (P) and the undifferentiated mesenchymal Ussue of lamina propria submucosa (S) If&E stain X 400.

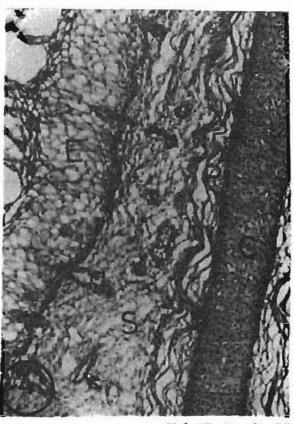


Fig. 4: A photomicrograph of cross section at the nasal vestibule of 12 cm CVRL (90 days-old) buffalo fetus showing, septal nasal cartilage (C), perichondrium (P), Covering epithelium (E), primordia of nasal gland (g) blood vessels (arrow) in lanina propria submucosa (S) H&E stain X 100.

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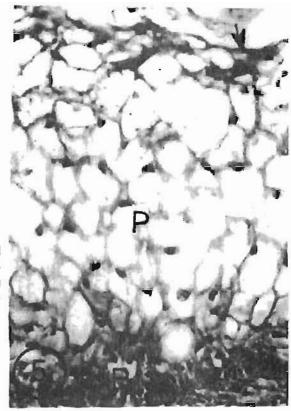


Fig. 5: magnification of fig. (4), note the basal cells (B), many lightly stained with vacuolated cytoplasm polyhedral cells (P) and superficial densely stained flattened cells (arrow) H&E stain, X 400.

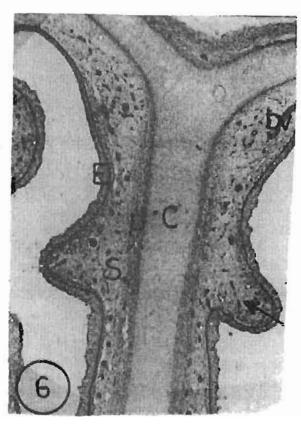


Fig. 6: A photomicrograph of cross section at the middle part of the nasal cavity of 18 cm CVRL (105 days-old) buffalo fetus showing, thick septal nasal cartilage (C), perichondrium (P), the covering epithelium (E) lamina propria (S), forming a cushion like structure (arrow) and many blood vessels almost engorged with blood (bv). H & E stain X 40.

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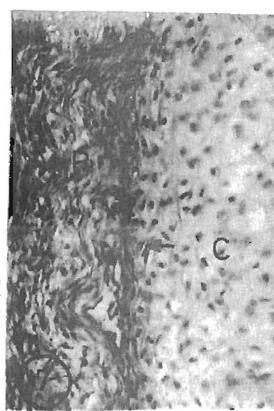


Fig. 7: Magnification of (fig. 6) showing, the septal nasal cartilage (C), inner cellular layer (arrow) of perichondrium (P) with many blood vessels (arrow heads). H&E stain X 400.

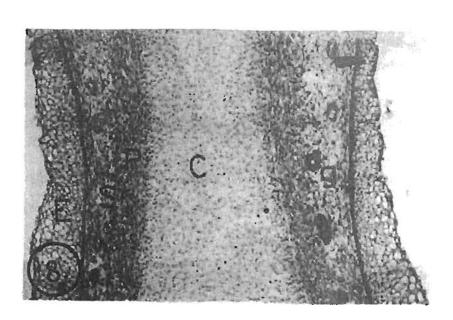


Fig. 8: A photomicrograph of cross section at the masal cavity proper of 18 cm CVRL (105 days-old) buffalo fetus showing, the basal part of the masal septum note, the masal cartilage (C), perichondrium (P) lamina propria (S), solitary actni of masal gland (g) and undifferentiated epithelium (E). H&E stain X 100.

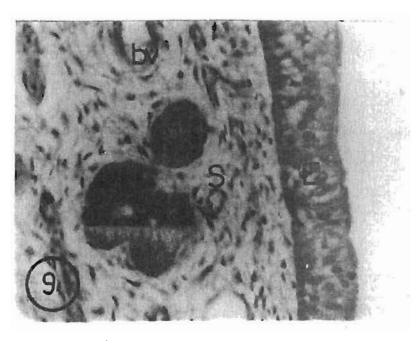


Fig. 9: A photomicrograph of cross section at the nasal cavity proper of 25 cm CVRL (123 days-old) buffalo fetus showing, the covering epithelium (E) composed of pesudostratified citated columnar epithelium, propria submucosa (S), blood vessels (bv) and nasal gland (g) H&E stain X 400.

Fig. 10: A photomicrograph of cross section at the nasal cavity proper of 25 cm CVRL (123 days-old) buffalo fetus showing, the covering epithelium (E), lamina propria submucosa (S), with many blood vessels (arrow head) and blood spaces (bs) nerve fibers (arrow), the septal nasal cartilage (C), and its perichondrium (P). H&E stain. X 100.

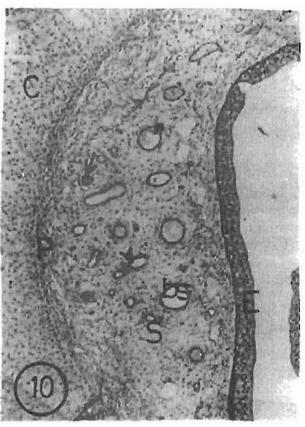


Fig. 11: A photomicrograph of cross section at the nasal cavity proper the nasal cavity of 36 cm CVRL (150 days-old) buffalo fetus showing, blood spaces of different size some of them engarged with blood (arrow head), and small blood vessels (arrow), the gland was located in clusters (g) in the lamina propria (S), note its excretory duct open on the surface of epithelium (E) and thick perichondorium (P). H & E stain X 100.

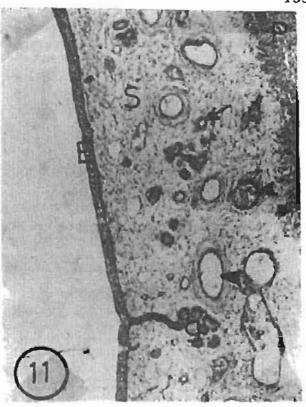
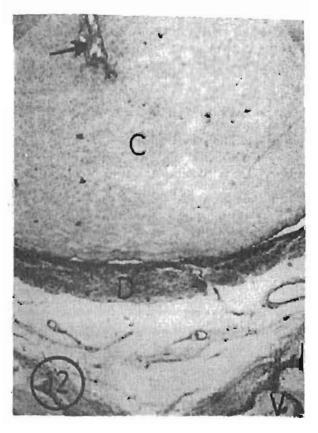


Fig. 12: A photomicrograph of cross section at the nasal cavity proper of 48 cm CVRL (180 days-old) buffalo fetus showing. Invasion of the basal part of septal cartilage (C) with blood vessels (arrow), dense packing of connective tissue (D) and ossilled vomer bone (V). H&E stain X 40.



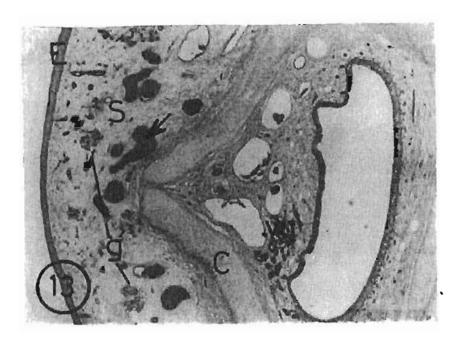
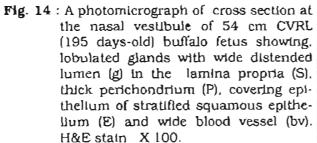
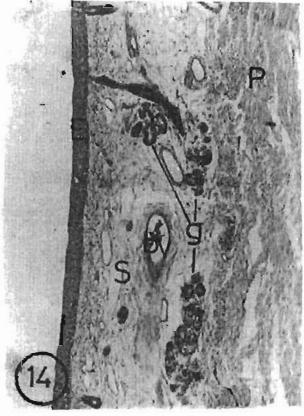


Fig. 13: A photomicrograph of cross section at the nasal cavity proper of 48 cm CVRL (180 days-old) buffalo fetus showing. the covering epithelium (E), lamin propria (S), large blood spaces (arrow), nasal glands (g), vomeronasal cartilage (C), and vomeronasal

glands (vg) H&E stain X 40.





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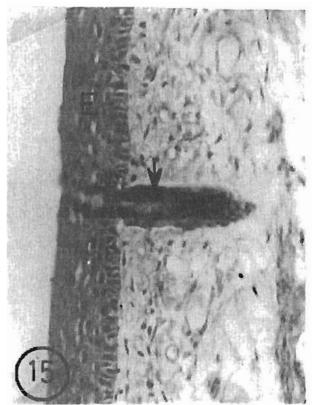


Fig. 15: A magnification of (fig. 6) showing, the duct of the nasal gland (arrow) composed of two layers of cuboidasl epithelium, and the covering stratified squamous epithelium (E) H&E stain X 100.

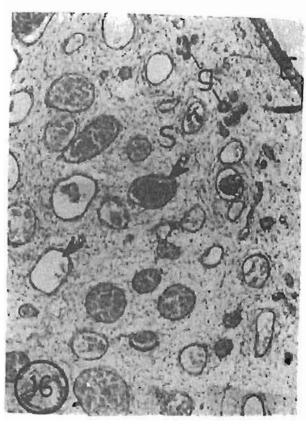
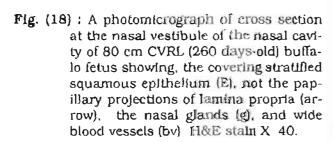
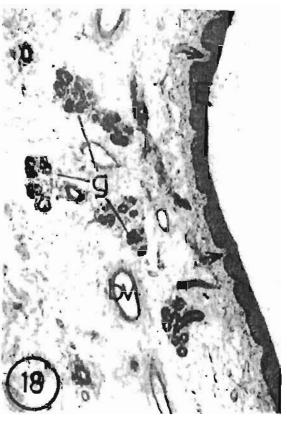


Fig. (16): A photomicrograph of cross section at the middle part septum of 54 cm CVRL (195 days-old) buffalo fetus showing, large blood spaces most of them engorged with blood (arrow head), glandular lobules (g), in lamina propria (S), and the covering epithellum (E). H&E stain X 40.

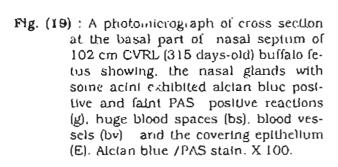


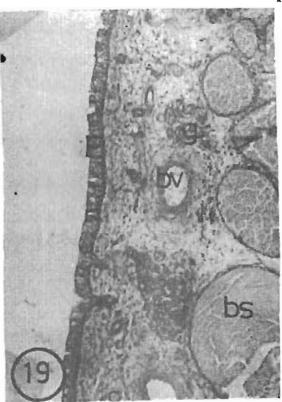
Fig. (17): A photomicrograph of cross section at the transitional area of the nasal cavity of 68 cm CVRL (230 days-old) buffalo (etus showing, large glandular lobule with distended lumen (g), wide irregular blood spaces (bs), in lamina propria (S), septal nasal cartilage (C), perichondrium (P) and its covering epithelium of stratified cuboidal epithelium (E). H&E stain X 100.

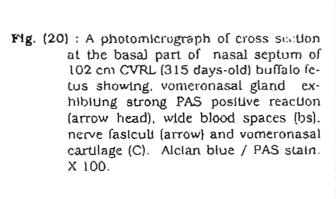


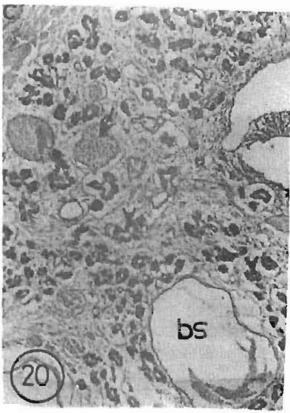


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اللخص العربي التطور الجنيني للحاجز الأنفي في الجاموس

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لقد أجريت هذه الدراسة على عدد ٢١ رأساً لأجنة جاموس تتراوح أطوالها من ٨سم إلى ٢ - ١سم بمقياس الطول٣٧٨ وأعمارها من ٨٠ يوم إلى ٣١٥ يوم وقد جهزت العينات للوصف الميكروسكوبي الضوئي وقد بينت الدراسة أن الحاجز الأنفى عند طول ٨سم (٨٠ يوماً) يتكون من نسيج رأسي رقيق من الخلايا المولدة للغضاريف، عند طول ١٢سم (٩٠ يوم) لوحظ تكثف للخلايا الغضروفية الصغيرة على جاني الغشاء الغضروفي الذي كان من النوع الزجاجي. لوحظ بعض غزو الأوعية الدموية لغضروف الحاجز الأنفي لأول مرة عند طول ٤٨سم (١٨٠ يوم) كما لوحظ بعض بؤر التعظم الصغيرة حول هذه الأوعية، عند طول ٨سم أحيط غضروف الحاجز الأنفي بخلايا طلائية مصففة تميزت عند طول ١٠٥٨سم (١٠٥ يوم) إلى خلايا عمادية كاذبة وبخاصة على الجزء العلوى من الحاجز الأنفي في التجويف الأنفي الأساسي، عند طول ٣٦سم (١٥٠ يوم) إكتمل تميز النسيج الطلاتي للحاجز الأنفى حيث أصبح نسيج طلاتي مركب مفلطح في منطقة الجيب الأنفى، نسيج عمادي كاذب في منطقة التجويف الأنفي الأساسي ونسيج مركب من الخلايا المكعبية في المنطقة الإنتقالية بينهما، ظهرت الغدد الأنفية ولوحظت لأول مرة عند طول ١٢سم (٩٠ يوم) على شكل عقد مصمته منعزلة حيث كونت ١ر٥١٪ من النسبج التحت طلاتي، عند٤٥سم (١٩٥ يوم) أصبحت الغالبية العظمي من هذه الغدد مجوفة وتوجد في مجموعات كبيرة نسبياً وكونت ٢٢ر٤٣٪ من النسيج التحت طلائي، عند طول ٨٨سم (٢٣٠يوم) كونت الغدد الأنفية فصيصات صغيرة وأصبحت ممتلئة التجاويف ومثلت ٣٢ر٢٣٪ من النسيج التحت طلاني وكونت الغدد الأنفية ٦٢. ٢٥٪ من النسيج التحت طلائي بينما شوهدت الغدد المكيعية لأول مرة عند طول ٤٨سم (١٨٠يوم) وكونت ٣٢ر٤٪ من النسيج التحت طلائي للجهاز الميكيعي عند طول ٢٠١سم (٣١٥يوم) حيث مثلت ٩٥ر٢٨٪ من النسيج التحت طلائي للجهاز الميكيعي.