HISTOGENESIS OF INTERAREOLAR REGION OF PORCINE PLACENTA

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Abstract

Progressive developmental changes in the interareolar region of porcine placenta were studied using specimens from 20 to 105 days of gestation. The placenta remained epitheliochorial throughout gestation. An enhancement in the complexity and vascularisation of maternal and foetal folds were noticed in time. The notable features were specialised, tall columnar cells in the chorionic fossae and associated endometrial ridges that enclosed abundant secretory material in between. Formation of "intra-epithelial capillaries" is another attribute. The epithelium was notably of short cuboidal type in the lateral aspect, apex chorionic ridges and associated endometrial fossa, which along with indented "intra-epithelial capillaries" reduced the diffusion barrier. Binucleated cells described in ruminants were not discernible in porcine placenta.

Key words:*Histogenesis, placenta, interareolar region, "intra-epithelial capillaries", pig*

The placenta is comprised of maternal and foetal tissues. At present, evaluation methods for ascertaining adverse effects of drugs and food supplements are firm and it includes validation of reproductive toxicity. The placenta plays a major role in these studies

to ascertain risk for mother and foetus (Furukawa, 2014). In this juncture, sound knowledge on histogenesis of porcine placenta provides information to ascertain any pathological alternations. Furthermore, an understanding of placental relationship in pig with its simple morphology and easily distinguishable components, will furnish the background for interpreting the complexity due to extensive fusion of foetal and maternal tissues. So the current study was conducted to elucidate the progressive changes in the interareolar region of porcine placenta.

Materials and methods

The specimens were collected from mixed bred pigs at authorised pig slaughter places in Bengalooru. The specimens were grouped into early (up to 40 days), mid (41-80 days) and late (above 81 days) gestational stages. Placenta from 20 to 105 days of

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gestation belonging to the groups: early (n=30), mid (n=30) and late (n=18) stages were used for the present study. The entire reproductive tract was removed immediately after slaughter and infused with 10% Neutral Buffered Formalin (NBF) pH 7.4 through the uterine artery for the immediate fixation of placenta. The fixed specimens were placed for 24 hours in 10 % NBF for further fixation. The embryos and or foetuses were located in the intact uterus by palpation and the uterine horn was incised longitudinally along its anti-mesometrial margin to examine the implantation site. The crown rump length of foetuses was noted to determine the age (Marrable, 1971). The placental tissue pieces along with uterine wall of near thickness 1 cm x 0.5 cm were collected from the central placental region at the mesometrial border and processed for routine alcohol-xylene-paraffin embedding procedures. Sections of 5 µm thickness were stained by routine stain Harris's Haematoxylin-eosin (H&E) and special stains Masson's trichrome (Luna, 1968) and Mallory's Phosphotungstic acid-Haematoxylin (PTAH) method (Singh and Sulochana, 1996).

Results and Discussion

The placental zone of porcine placenta can be divided into areolar and interareolar regions. The interareolar placenta is the largest subdivision of the placental zone of allantochorion wherein the chorionic folds from foetal side appose with the corresponding endometrial grooves to produce the epitheliochorial type porcine placenta (Fig. 1) as recorded by Dantzer *et al.* (1981). The grossly visible macroscopic folds on the chorionic surface and endometrium appeared as simple to branching villi in histology. The progressive changes occurring in this region were studied in the three different stages of gestation *viz*. early, mid and late.

Early Gestation

The embryonic trophoblast was in close proximity to the uterine epithelium at around 20 days but these epithelia were not adhered firmly. Uniform, simple, high cuboidal to columnar cells resting upon a diffuse basal lamina constituted the maternal epithelium at this period (Fig. 2). The spheroid, centrally placed vesicular nucleus of endometrial cells enclosed single nucleoli as well as some coarse chromatin granules attached to its nuclear membrane. Some cells in mitotic division resembling "binucleated cells" were also seen. The cells possessed microvilli and apical thickenings and indicated the glycocalyx as stated by Dantzer (1985) which in turn may play a role in implantation of blastocysts. The loose connective tissue of lamina propria-submucosa was oedematous and highly vascular (Fig. 2). The capillaries were abundant in the subepithelial zone that augmented by 35 days. In the loose connective tissue, fibroblasts were more abundant in the subepithelial layer than in the glandular layer. Different cells of immune system- lymphocytes, neutrophils and macrophages were also localised in the subepithelial and glandular layers (Fig. 3).

The cells of chorioallantoic membrane at around 20-25 days (Fig. 2) were simple cuboidal type, sheltered vesicular spheroid nucleus in addition to single nucleolus. Some binucleated cells were also localised in the epithelium. However giant binucleated cells described in ruminants by Klisck *et al.* (1999) and Ranjan and Singh (2013) were not discernible in the present study. A thin film of secretion was noticed between the epithelial bilayers. Highly vascular foetal mesenchyme formed the connective tissue core of this membrane. Its typical features included abundance of almost acellular homogeneous matrix, fewer stellate fibroblasts along with sparse connective tissue fibrils. Immune cells *viz.* lymphocytes, large macrophages and neutrophils were also localised in it. Another notable attribute was the presence of primitive nucleated erythrocytes (Fig. 2) having large basophilic round or oval nuclei. A gradual change to the uniformity of epithelial cells was noticed around 30 to 40 days along with the development of complex folds. During this, the epithelium in the chorionic furrows and corresponding endometrial ridges transformed to columnar type while that of chorionic ridges and corresponding endometrial fossae remained cuboidal.

Mid-gestation

The changes noticed in this group from the preceding were an enhancement in the number and extent of folds on both sides of placenta in addition to their complex branching

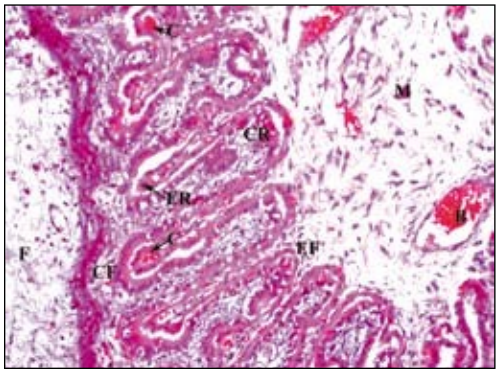

Fig. 1. The placenta at 100 days of gestation in pig. H&E. x100
M- Maternal connective tissue, F- Foetal connective tissue, CF- Chorionic fossa,
ER- Endometrial ridge, CR- Chorionic ridge, EF- Endometrial fossa, B- Blood vessel, C- Capillary

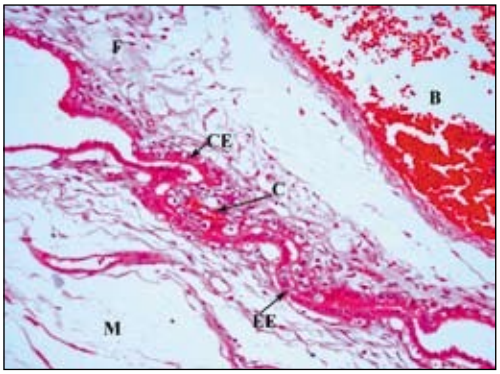

Fig. 2. The placenta of 30 days of gestation in pig. H&E. x100
M- maternal connective tissue, F-Foetal connective tissue, B-Blood vessel, CE- Chorionic epithelium, EE- Endometrial epithelium, C- Foetal capillary

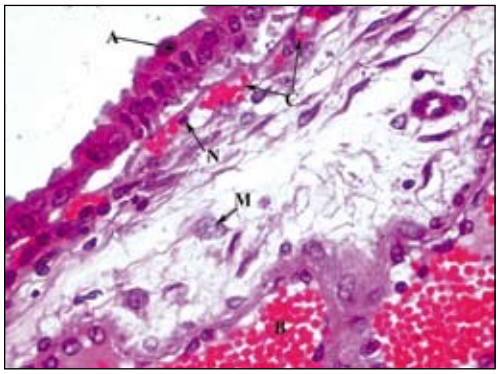

Fig. 3. The endometrium at 40 days of gestation in pig. H&E. x400
A- Apoptotic cell in the epithelium, M- macrophage, N- Neutrophil, B- Blood vessel, C- Capillary

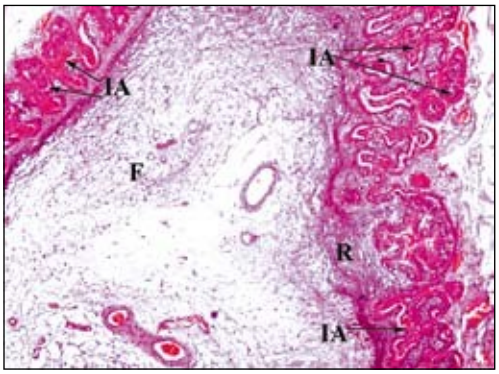

Fig. 4. The placenta of 90 days of gestation in pig. H&E. x40
F- Foetal connective tissue, RA- Regular areola, IA- Complex interareolar region

and interconnecting ridges (Fig. 4). The epithelium in the interareolar regions underwent marked specialisation (Fig.5, 6).

Chorionic Fossae and Associated Endometrial Ridges

The epithelium of the chorionic fossae (Fig. 5, 6) transformed to high columnar cells while those of its lateral aspect showed a decline in height and the chorionic apex was carpeted with cuboidal epithelium. A few lightly-stained cells with irregular outline and enclosing dense granules were noticed in the fossae (Fig. 6). In the remaining cells, apical protrusion and long apical microvilli were observed. The other characters of these cells included large basally-placed oval vesicular nucleus and distinct nucleoli. The apical surface and cytoplasm of cells contained mucus, vacuoles and granules.

All these cytological features indicated cell's absorptive function. A few apoptotic cells with irregular condensed nuclei and dense cytoplasm were also noticed. The vascularisation of this region was less compared to chorionic ridges. The cytological features of chorionic fossa cells are in partial agreement with Czarnowska (1988) who described dark cells additionally. A few darkly stained apoptotic cells distinct in the study may those described as dark cells by Czarnowska (1988).

The cells in the endometrial ridges were of columnar type but were shorter than those in the corresponding chorionic fossae (Fig. 5, 6). The subepithelial maternal capillaries possessed a distinct lumen and were separated from the epithelium by a layer of connective tissue. A thick distinct accumulation of secretion (histiotrophe) was seen at the

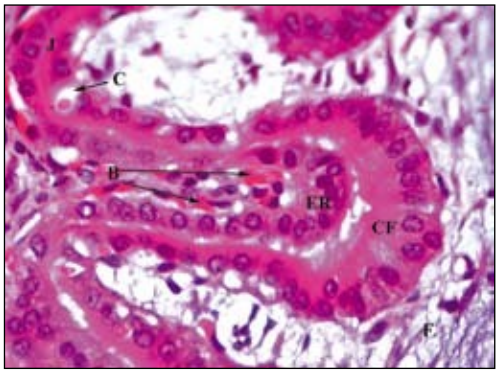

Fig. 5. The placenta of 60 days of gestation in pig showing interareolar region. H&E. x400
F- Foetal connective tissue, CF- Chorionic fossa cells, ER- Endometrial ridge cells, B-Maternal blood vessels, J- Junction of chorionic ridge to endometrial fossa, C- "Intra-epithelial capillary"

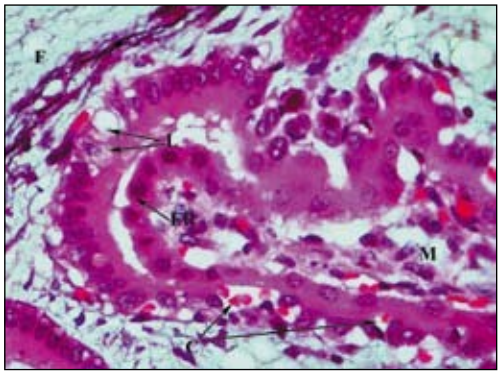

Fig. 6. The placenta of 75 days of gestation in pig. H&E. x400.
F- Foetal connective tissue, M- Endometrial connective tissue, L- Light cells in the Chorionic fossa, ER- Endometrial ridge cells, C- "Intra-epithelial capillary"

microvillar junction suggestive of secretory function of the cells, secretion being extruded to the intermicrovillar junction. The accumulated histiotrophe sealed the two surfaces at some points. However due to shrinkage of mucosa that occurred during processing of tissues, a space was seen in most places between the two epithelial cell layers.

Chorionic Ridges and Associated Endometrial Fossae

In the chorionic ridges, epithelium was uneven and cells were irregular (Fig. 5, 6). Majority cells were cuboidal with concave apical surface. The cytoplasm enclosed small vacuoles and granules. The microvilli enhanced in time and revealed interdigitations. The foetal capillaries were abundant at the sides as well as at the top of the chorionic ridges. These capillaries on the lateral sides and apex of chorionic ridges dislocated the covering chorionic epithelial cells around 60 days which were much discrete by 75 days. Such vessels were designated as "intra-epithelial capillaries" (Fig. 5, 6) which is in accordance to reports of Dantzer *et al.* (1981, 1990) in pig. Due to high degree of indentation, these capillaries were seen to be surrounded on its three superficial surfaces by chorionic cells. The cytoplasm of chorionic cells overlying such capillaries was in the form of thin membrane and the cells appeared irregular in outline. In the region of "intra-epithelial capillaries", the nuclei of trophoblast cells were shifted to the cell's lateral part.

The irregular to cuboidal cells of endometrial fossae (Fig. 5) had convex apical surface as well as almost spherical nucleus. The chorionic and the endometrial mucosa were tightly cemented at the apex and lateral surfaces of the chorionic folds, which presented thin layer of histiotrophe indicating a histiotrophic nutrition. This fusion was more marked at 50 days than at later days of this gestation. The endometrial capillaries indended the epithelial cells so that they appeared as flattened with small lumen when compared to the apex where it had a larger distinct lumen. These specialisations of chorioinic ridge cells and corresponding endometrial fossa cells greatly reduced the diffusion barrier suggesting that these sites play a major role in transport of blood borne nutrients and haemotrophic nutrition as proposed by Dantzer *et al*. (1981).

Late Gestation

Though microscopic folds and ridges were complicated by the development of interconnections orienting almost perpendicular to them resulting in a complicated structure to the interareolar regions, the epithelia remained distinct and separate (Fig. 1) as reported by Firth *et al.* (1986) in pig. The complex epithelial folds as well as apical microvilli that augmented in time not only amplified the placental exchange area but also resulted in intimate and intricate association during pregnancy. The histogenesis of the interareolar region of porcine placenta concurs with the reports of Vallet *et al.* (2009) in pig.

Chorionic Fossae and Associated Endometrial Ridges

The main changes from the preceding stage included a decline in the number of columnar cells of chorionic fossae along with an increment in number of low columnar to cuboidal cells (Fig. 1). The number of light cells showed a reduction whereas the apoptotic cells amplified especially in the complex folded regions.

Chorionic Ridges and Associated Endometrial Fossae

The features were similar to the previous group. The chorionic cells contained numerous small vesicles and granules. Very thin cuboidal cells formed the maternal epithelium of endometrial fossae. Sub epithelial as well as "intra-epithelial capillaries" were observed in plenty in this region that improved vascularity much during late stage (Fig. 1). The amplified vascularity as well as the formation of "intra- epithelial capillaries" recorded in the present study with progress of gestation, account for improved diffusion efficiency in the epitheliochorial porcine placenta. These mechanisms may be adaptation to meet the increased nutrient demands of growing foetus as been proposed by Dantzer *et al.* (1990) and Vallet *et al.* (2009) in pig. In addition to these, Friess *et al.* (1981) recorded fenestrated endothelium in foetal capillaries and Vallet *et al.* (2009) described cross-counter current flow of blood in foetal and maternal capillaries may further facilitate nutrient transfer to foetus. The high vascularity of the interareolar regions may also contribute to hold both surfaces in close approximation as postulated by Friess *et al.* (1980) and Dantzer (1984) who stated that during gestation, distended blood and tissue lymph of the interlocking folds may result in its swelling which contribute materially to hold the two surfaces in the fashion of zipper.

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