Immunolocalization of Angiotensin Converting Enzyme in Foetal and Adult Bovine Epididymis

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Abstract
The present work aimed to apply immunohistochemistry for studying the validit angiotensin converting enzyme (ACE) as a marker for differentiation and developm of the foetal bovine epididymis. Additionally, for highlighting the morphofunctic relevance of the different epididymal structures in adult males. Foetal samples w collected from foeti their ages ranged from 75 to 285 post coital day (pcd). A specimens were collected from different regions of the epididymis including eff ductules and epididymal duct. The epididymal duct was further subdivided into segments; the first three constitute the caput, the middle two make the corpus and last one represents the cauda epididymidis. Primary antibody against ACE was app on paraffin-embedded sections from the different regions from foetal and adult bo males. ACE-immunoreactivity (IR) could be seen in the epithelium of efferent duct and epididymal duct as early as at 75 pcd (10 cm CRL). Epithelium of adult bo efferent ductules showed a moderate ACE-IR. The reaction was mainly distinct in stereocilia and the apical cytoplasm in the first two segments (of caput), while it confined to the supranuclear cytoplasm of some scattered principal cells in the s (cauda) segment. Intense ACE-IR was found in the vascular endothelium. Early progressive expression of ACE in the foetal epididymis might be in synchrony with development of the foetal Leydig cells pointing to its androgen-dependency. This also reflect distinct absorptive activities of the immunoreactive epididy compartments.

Key words:
Angiotensin Converting Enzyme, Bovine, Epididymis, Immunohistochemistry.
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Introduction
ACE is a membrane-bound glycoprotein, which is detectable in all tissues and fluids of mammals (Soffer, 1976). It plays a key role in the renin-angiote aldosterone system (RAS) (Peach, 1977), which is responsible for blood pres regulation and volume homeostasis (Kondoh et al., 2005). ACE catalyses the form of the physiologically active octapeptide angiotensin II from the inert decape angiotensin I in many organs including the male reproductive tract (Wong Uchendu, 1990). Two isoforms of ACE are produced in male mammals, a sor (sACE) and testicular or germinal (gACE) isoform. The germinal isoform is exclus expressed in the male haploid germ cells (Sibony et al., 1994). There is an intrace pathway for angiotensin II synthesis and thus an endogenous or local RAS in r tissues and organs including the male (Pandey et al., 1984) and female (Lightm al., 1987) genital organs. ACE has been correlated to sperm maturation (Leung e

1999). This may be ascribed to inactivation of kinins and thus sup intraepididymal sperm motility or modifying constituents of the sp membrane (Vanha-Perttula et al., 1985). The aim of this study is to dete of ACE as a marker for differentiation and development of bovine epidi highlight the morphofunctional relevance of different segments of epididymis.

Material and Methods

Animals and tissues

Bovine epididymal specimens were obtained from 21 adult clinically healt taurus) slaughtered in the abattoir of Munich, Germany. All the specimen immediately after slaughter. The epididymis from each animal was di portions; the most proximal one represents the efferent ductules (ED). latter and according to Nicander (1958) the epididymal duct was divi segments. The first three segments constitute the head region; the 4th correspond to the body whereas the 6th segment represents the tail region male foetuses were collected from slaughtered pregnant cows. Their ag were estimated according to the crown rump length (CRL) as described b Sack (1973) and Rüsse and Sinowatz (1991).

Chemicals and methods

Bouin-fixed specimens were used for routine histological staining and for specimens were dehydrated in a graded series of ethanol, cleared embedded in paraplast and sectioned at 5μm thickness. Tissue sections we on positively charged, coated slides.

Routine histological staining:

Several conventional stains were carried out to investigate the general structure. These included Hematoxylin and Eosin, Masson and Goldner' stains, Alcian blue 8GX, Periodic acid-Schiff (PAS) reaction after Mc Toluidine blue. All staining techniques were performed according to the Romeis (1989).

Immunohistochemistry:

Immunohistochemical studies were performed using the avidin-biotin comp (ABC) according to Hsu et al., (1981). Dewaxed and rehydrated sec subjected to inactivation of endogenous peroxidases by incubation in 1% H minutes. After that the sections were washed by current tap-water for10 m by phosphate buffer saline (PBS) for 5 minutes. The sections were block containing 5% bovine serum albumin (BSA) for an hour, and then were inc the specific primary antibody (anti-ACE-IgG raised in chicken, provided by Vet. Anatomy II, LMU-Munich) as 1: 500 in antibody diluant (DAKO, Germany) for 12 hours at 4°C. The sections were washed 3 times by P minutes and incubated with the secondary antibody (biotinylated rabbit IgG, Rockland, USA) as 1: 400 in PBS for 30 minutes at room temper sections were washed by PBS for 15 minutes. Then the secondary ant detected with Vectastain ABC kit (Vector Laboratories Inc.) firstly each covered with 100 x dilution of A & B reagent in PBS (1 μl reagent A+ 1 μl re 98 μl PBS), then washed 3 times by PBS for 15 minutes and the colour was using DAB reagent (Sigma-Aldrich, St. Louis, MO, USA). Sections were cou with haematoxyline for 30 seconds, washed in water, dehydrated throu

ethanol, cleared in xylene and mounted with DPX permanent mounting media (Sig Aldrich, St. Louis, MO, USA).and photographed by light microscopy

Positive and negative controls

Immunohistochemical negative controls, where each primary or secondary antise or the ABC reagent was omitted, gave no positive staining. Positive controls were u according to the instructions provided by the manufacturers of the primary antibo For assessment of the immunolabelling a semi-quantitative subjective scoring performed by three independent observers.

Results

Foetal epididymis

At the foetal age of 75 to 80 post coital day (pcd) (10 to 13 cm CRL), the effe ductular epithelium showed a moderate ACE-IR in the apical cell membrane, whe the reactivity of the cytoplasm was weak. At the same time, the cytoplasm and apical cell membrane in the epididymal duct, expressed a weak to moderate reac (Fig. 1). The vascular endothelium exhibited a moderate to strong IR.

At the foetal age of 95 to 110 pcd (18 to 24 cm CRL), a moderate to strong react was expressed in the apical plasma membrane and cytoplasm of the efferent duct and the epididymal duct in the head region. Strongly ACE-positive, fine granules v found in the cytoplasm of the epithelial cells in these regions. A similar reaction found in the degenerating mesonephric tubules in the area opposite to the body re of the epididymis. The epididymal epithelium in the body and tail region expresse weak to moderate reaction both on the apical surface and in the cytoplasm. peritubular mesenchymal cells as well as many interstitial cells presented a wea moderate ACE-IR.

At the foetal age of 130 to 140 pcd (30 to 36 cm CRL), the apical surface of epithelium lining the ED exhibited a strong positive reaction, whereas the ap cytoplasm showed a weak to moderate reaction. The epididymal epithelium in the h region expressed a moderate reaction in the apical cytoplasm and surf Furthermore, some cells possessed a moderate reaction in their basal cytopla area. The reaction in the epithelium lining the duct in the corpus and cauda reg was similar to that in the caput region. The vascular endothelium possessed a st ACE-IR.

At the foetal age 185 pcd (56 cm CRL) and upwards, the epithelium of the ED (Fi presented strongly immunoreactive apical surface, as well as weak to mode reactive apical cytoplasm containing immunostained granules. The epithelium o epididymal duct in the caput region manifested a moderate to strong ACE-IR or apical surface and positive granules in the apical as well as in the basal cytopl The apical surface and cytoplasm in the body and tail regions expressed a mode reactivity. Apical cytoplasm of most cells presented ACE-positive granules, whe some cells possessed similar granules also in their basal cytoplasm. The vas endothelium exhibited a strong ACE-IR.

Adult epididymis:

The IR for ACE in the adult bovine ED was distinct as expressed by the apical su and the supranuclear area of many nonciliated cells (Fig. 3). Vascular endotheliu the peritubular as well as the interstitial blood vessels showed a strong ACE-IR.

The first two segments of the caput epididymidis demonstrated strongly reactive a cytoplasm and stereocilia (Fig. 4, 5). Vascular endothelium was similar to those c

ED. The epithelium of the third segment of the head region as well as body region revealed a negative reaction. Apical mitochondria-rich cells (A ACE-negative. Supranuclear cytoplasm of some scattered PCs as well as blood capillaries of the sixth segment displayed a strong immunostaining f 6).

Discussion

ACE catalyses the formation of the physiologically active octapeptide ar from the inert decapeptide angiotensin I in many organs including reproductive tracts (Wong and Uchendu, 1990). It is well-known that the an intracellular pathway for angiotensin II synthesis and thus an endogen RAS was reported in many tissues and organs including male (Pandey e and female (Lightman et al., 1987) genital organs.

Early occurrence of ACE in the epithelial cells of the mesonephros and W has already been recorded (Schütz et al., 1996). Furthermore, the protein found at the adluminal membrane of epithelial cells lining proximal n tubules and Wolffian duct and thus corresponds very early to the final expr of adults (Pauls et al., 2003). Similar findings were recorded in the current the ACE-IR appeared as early as at 75 pcd (10 cm CRL) and was restr apical cytoplasm and surfaces of ED and to the proximal portion of t epididymal duct and even in the degenerating mesonephric tubules opp epididymal body region.

Similarities in ACE-immunostaining both in growing ED and in de mesonephric tubules give support to the concept that ED arise from the cou the latter in the caput region. Since it stimulates angiogenesis in vivo (Ferna 1985) and acts as a growth factor (Naftilan et al., 1989) in cell culture sys may play a role in the development and differentiation of the extratest genital tract.

It is noteworthy that ultrastructural modifications of the apical cytopla mesonephric duct in the rat foetus were associated with absorptive activity epididymis (Flickinger, 1969). Similar findings were also recorded in foetus monkey (Alexander, 1981). Likewise, Wrobel (2001) and Wrobel and Schim reported that the epithelial cells lining the bovine ED undergo cytological dif on about the 3rd month of gestation. This differentiation proceeds in a pr direction. Consequently the columnar epithelium of the proximal portions differentiated into ciliated and reabsorptive nonciliated cells. The latter brush-border and endocytotic apparatus. These changes coincide with prol the foetal Leydig cells (Rüsse and Sinowatz, 1998) and are suggested response to an increase in androgen. It is worth noted that the epithe mesonephros-derived tissues including epididymis expresses more receptors compared to the germinal epithelium (You and Sar, 1998). Thus v that ACE is a good marker for development and morphofunctional differentia bovine extratesticular male genital tract.

Furthermore, it was concluded that the presence of the components of specific receptors for angiotensin II both in male and female reproduct supports the hypothesis that the RAS influences the reproductive functions al., 1998). The effects of angiotensin II are mediated through their paracr autocrine regulatory interactions via their receptors (Vinson et al., 1997) on t

and basal surfaces of epididymal epithelium (Leung et al., 1997). It is noteworthy th two isoforms of ACE are produced in male mammals, a somatic (sACE) and testicul or germinal (gACE) isoforms. The germinal isoform is exclusively expressed in tl male haploid germ cells (Sibony et al., 1994). Several studies described release ACE from human spermatozoa during capacitation (Köhn et al., 1995) and reported significance for fertilization process (Köhn et al., 1998; Deguchi et al., 2007; Kondoh al., 2005; Kondoh et al., 2009).

In the present work ACE-IR was localized in the vascular endothelium along the leng of ED and epididymal duct. It is notable that ACE plays a key role in the RAS (Peac 1977), which is engaged in blood pressure regulation and volume homeostasis. Thu in accord with Franke et al., (2003), the current study proposes that ACE may cont the blood flow and consequently the different metabolic activities of extratesticul male genital tract. Moreover, a strong IR was found on the luminal surface as well as the supranuclear area of many nonciliated cells lining ED. Both of apical surfaces a kinocilia of ciliated cells showed a similar reaction. Comparable findings were report in rabbit (Berg et al., 1986) and human (Vivet et al., 1987) epididymidis. The prima cellular localization of ACE in endothelial cells was found adjacent to the lumen in bo species. In addition to vascular endothelium in human male genital tract, ACE-IR w observed on the luminal surface of the epithelium of the ED, epididymis, and duct deferens especially in stereocilia (Vivet et al., 1987). Moreover, IR for ACE in t epididymis was low in the initial segment, highest in the caput and moderate in t cauda of rat epididymis (Strittmatter and Snyder, 1984; Strittmatter et al., 1985).

ACE-IR was restricted to stereocilia and the apical cytoplasm of the first two segmer of the adult bovine caput epididymidis and in supranuclear cytoplasm of scattered P in the sixth segment. The present findings go in line with those reported in the (Strittmatter and Snyder, 1984; Strittmatter et al., 1985) and in the donkey caput a cauda epididymidis (Alkafafy, 2009). Dissimilar to the current findings the stereoc exhibited negative ACE-IR in the epididymis of the buffalo bull (Alkafafy et al., 2009). humans an intense reaction has been located on the luminal surface both of corp and cauda but not of caput epididymidis. It was strictly confined to stereocilia and was not seen in the cytoplasm of the principal or the basal cells (Vivet et al., 198 The existence of RAS in the epididymis may interpret the role of ACE released epididymal spermatozoa in regulation of epididymal function and sperm maturat (Leung et al., 1999). It is assumed that ACE convert angiotensin I, locally produced epididymal epithelial cells, into angiotensin II. The latter plays a paracrine role throu regulating electrolyte and fluid transport in the epididymis (Leung et al., 19 O'Mahony et al., 2000).

The current findings support previous studies involved different mammalian spec (Levin and Marsh 1971; Goyal and Hrudka, 1980; Sinowatz, 1981; Djakiew et 1984; Hermo and Morales, 1984, Goyal, 1985; Dacheux et al., 1989; López et 1989, Alkafafy et al., 2009), which reported that most of the testicular fluid reabsorbed by the epithelium of ED and proximal segments of the epididymal du Additionally, it was suggested that the expression of ACE in the testis and epididy of rat is androgen-dependent (Wong and Uchendu, 1990); and that it plays important role in stimulating the testicular androgen production, spermatogenesis a epididymal sperm maturation (Jaiswal et al., 1985). Moreover, bradykinin is an AC substrate, which plays a role in sperm motility (Schill and Haberland, 1974). ACE I been correlated to sperm maturation (Leung et al., 1999), through inactivation of kir

and thus suppressing the intraepididymal sperm motility or modifying c the sperm plasma membrane (Vanha-Perttula et al., 1985). Moreover, t fluid is hyperosmolar to blood plasma (Levin and Marsh, 1971). Hyperc may be a factor in prolonging the survival of spermatozoa during their tra epididymal duct (Hinton and Palladino, 1995).

Dissimilar to the findings reported in the donkey epididymidis (Alkafa buffalo bull epididymidis (Alkafafy et al., 2009), the apical cells(ACs) wer ACE. It is noteworthy that carbonic anhydrase has been localized in A (Sun and Flickinger, 1980) and it has been suggested that they ar acidification of the epididymal fluid through the secretion of protons a bicarbonate resorption (Jensen et al., 1999). On the other hand, Adama (1996) reported that ACs in the rat epididymis were unreactive for carbon In accordance with Adamali and Hermo (1996), who found no reactivity anhydrase and from the present findings, it could be assumed that significant role in regulation of fluid and electrolyte movement in the bovin In conclusion early and progressive expression of ACE in the epithelia mesonephros and Wolffian duct coincides with the development of foetal This emphasizes that expression of ACE is androgen-dependent and distinct absorptive activities of male excurrent ducts. Furthermore, ACE ca in sperm maturation by regulation of trans-epithelial movement of electrol and suppression of sperm motility.

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Figure legends

Fig. 1: Immunolocalization of angiotensin converting enzyme (ACE) in efferer (ED; arrowhead) and caput epididymidis (Ep) from 13 cm long (CRL) bovine

Fig. 2: Immunolocalization of ACE in efferent ductules from 63 cm long (CR arrow heads point to markedly positive apical surface of efferent ductules. BV blood vessels.

Fig. 3: Immunolocalization of ACE in adult bovine efferent ductules. Immuno localized on apical surface (arrow) and some nonciliated cells (arrow head).

Fig. 4: Immunolocalization of ACE in segment I of adult bovine caput epi ACE- immunoreactivity is localized in strongly positive apical cytoplasm (arr stereocilia (st) and blood vessel (BV). Asterisk points to an epithe characteristic for segment I.

Fig. 5: Immunolocalization of ACE in segment II of adult bovine caput epi ACE-immunoreactivity is localized in strongly positive apical cytoplasm (arr and stereocilia (st) but not in spermatozoa (Sp).

Fig. 6: Immunolocalization of ACE in segment VI (cauda epididymidis) of the Immunoreactivity is confined to supranuclear and apical cytoplasm of some principal cells (arrow head) and to subepithelial capillaries (arrows). BV point vessels and Sp points spermatozoa.

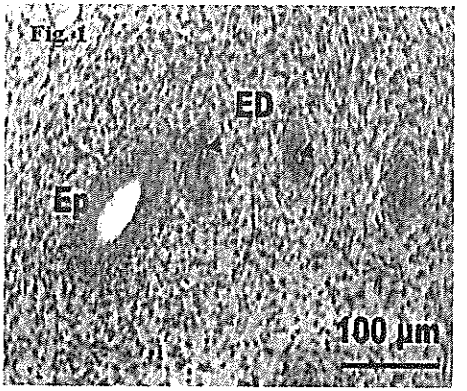
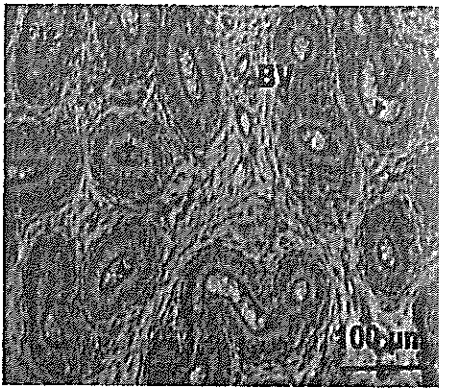


Fig. 1
ED
Ep
100 µm


BV
100 µm

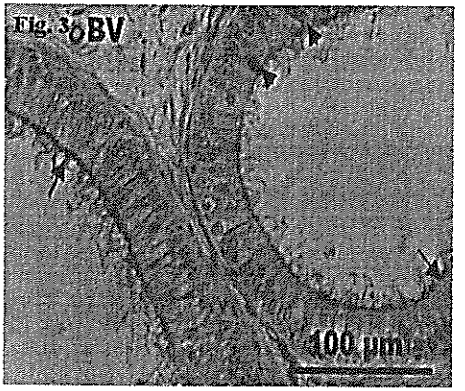


Fig. 3
BV
100 µm

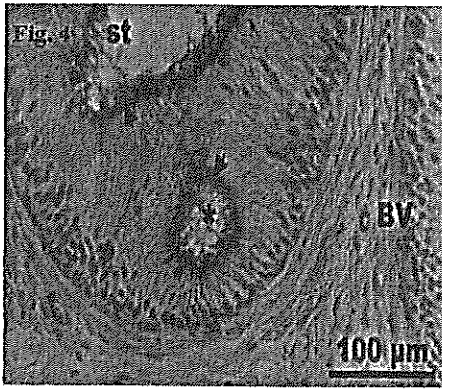


Fig. 4
st
BV
100 µm

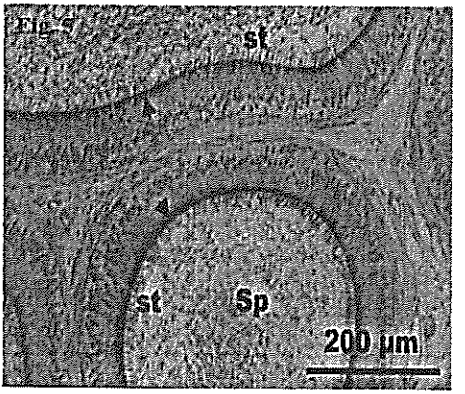


Fig. 7
st
st
Sp
200 µm

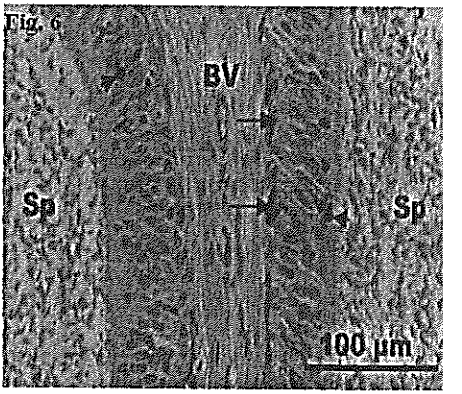


Fig. 6
BV
Sp
Sp
100 µm

Table 1: Ages of the foetal specimens.

CRL (cm)	Pcd	Gestation Month	Number of foeti
10	75	3	3
13	80	3	4
18	95	4	3
20	100	4	3
24	110	4	3
30	130	5	4
36	140	5	2
56	185	7	2
63	210	7	3
90	285	9	1

CRL = crown rump length, pcd = post-coital day.

Table 2: Distribution of ACE-binding sites in foetal efferent ductules an epididymal duct.

Gestation month	CRL (cm)	Efferent ductules (ED)					Epididymal duct		
		CC	NC	PMC	BL	BV	E	PMC	BL
3	10-13	+	+	±	-	++	±/+	±	-
4	18-24	+/+	+/+	±	-	++	+	±	-
5	30-36	+++	+++	±	-	++	++	±	-
7-9	56-90	+++	+++	±	-	++	++	±	-

BL = basal lamina, BV = blood vessels. CC = ciliated cells of ED, CRL = c length, E = epididymal epithelium, NC = non-ciliated cells of ED, PMC = muscle coat, - = negative, ± = weak, + = moderate positive, ++ = distinct po = strong positive.

Table 3: Distribution of ACE-binding sites in the adult bovine efferent du and epididymal duct.

Segment	CC	NC	BC	AC	PC	IEM	IEL	BM	PMC	CT
ED	+	+	-	0	0	-	-	-	-	-
I	0	0	+	0	+++	-	-	-	-	-
II	0	0	±	-	+++	-	-	-	-	-
III	0	0	±	-	-	-	-	-	-	-
IV	0	0	±	-	-	-	-	-	-	-
V	0	0	±	-	-	-	-	-	-	-
VI	0	0	±	0	+++	-	-	-	-	-

AC = apical cell, BC = basal cell, BM = basal membrane, BV = blood vessels ciliated cell, CT = connective tissue, IEL = intraepithelial lymphocyte, intraepithelial macrophage, NC = non-ciliated cell, PC = principal cell, VE = v endothelium. 0 = not found, ± = weak, + = moderate, ++ = distinct, +++ = reaction.

س العربي:

رالمناعي لأنزيم " الأنجيوتنسين كونفيرتنج " في بربخ الأجنة و الفحول

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الدراسة الحالية توظيف التقنية النسجوكيميائة المناعية في التحقق من صلاحية استخدام أنزيم تنسين كونفيرتنج " كدليل علي تمايز و تطور البربخ في الأجنة البقرية وكذلك إبراز الارتباط - وظيفي للبربخ في الذكور اليافعة. جمعت العينات من أجنة تراوحت أعمارها بين٧٥ و ٢٨٥ ب الإخصاب. كما تم تجميع عينات الذكور اليافعة من مناطق البربخ المختلفة؛ متضمنة القنيات و قناة البربخ. قسمت الأخيرة إلي ست نطاقات: تمثل الثلاثة الأولي منها منطقة رأس البربخ ؛ التاليان يمثلان منطقة البدن ويقابل النطاق السادس منطقة الذيل. استخدمت أجساماً مضادة لأنجيوتنسين كونفيرتنج لتحصيره مناعياً في قطاعات شمعية مُعَدّة من المناطق المختلفة في كل نة و الذكور اليافعة. وقد أمكن تحصير الأنزيم مناعياً في النسيج الطلائي المبطن لكل من لصادرة و قناة البربخ في المراحل الجنينية المبكرة (٧٥يوم عقب إخصاب؛ بما يعادل ١٠ سم ي كفلي). وفي الذكور اليافعة أظهرالنسيج الطلائي المبطن للقنيات الصادرة اصطباغاً مناعياً لشدة.أما في قناة البربخ فقد كان الاصطباغ جلياً ،علي وجه الخصوص، في سيتوبلازم الأجزاء خُميلات النسيج الطلائي المبطن للنطاقين الأول والثاني بمنطقة رأس البربخ.أما في منطقة طاق السادس) فقد كان الاصطباغ مقصوراً علي سيتوبلازم بعض الخلايا الأساسية المتناثرة. ت البطانة الوعائية اصطباغاً مناعياً قوياً في الأوعية الدموية بالمناطق المختلفة. و خلصت إلي أن التواجد المبكر والمتزايد للأنزيم في البربخ الجنيني ربما يكون متزامناً مع تطور خلايا لايا الخصية البينية) الجنينية؛ مشيراً إلي اعتماده علي الهرمون الذكري ومبرزاً القدرات صية للبربخ.