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Research Article

Anti-Ovarian Vessels Antibodies in *Peudomonas aeruginosa* Rabbit Hyper Immune Sera, an Immunohistochemical Study

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Abstract

Cyclical angiogenesis in the ovary is a unique process supporting normal folliculogenesis as well as lute genesis. In this report we investigated the reactivity of rabbit anti *Pseudomonas aeruginosa* antisera with ovarian blood vessels.

Tissues stained with anti-sera were immunohistochemically visualized using biotinylated anti rabbit immunoglobulin and peroxidase conjugated streptavidin. Positive staining sites depend on anti-stain type, however, staining was observed in endothelial cell and tunica adventitia in most cases.On the other hand, corpus luteum blood vessels showed a positive staining pattern as well. We conclude from this study that a peculiar staining pattern was seen in ovarian blood vessels stained with rabbit anti-*Pseudomonas aeruginosa* hyper immune sera, the importance of this reactivity need further investigation.

Keywords: Anti-ovarian vessels antibodies, *Pseudomonas aeruginosa*, immunohistochemical

الخلاصة

توالد الاوعية الدموية الدوري في المبيض هي عملية فريدة مساندة لتكوين الحويصلات اضافة الى تكوين الاجسام الصفراء.في هذه الدراسة تم بحث فعالية الاضداد الضادة للسيدوموناس ايروجنوزا في الارانب تجاه اوعية دم المبيض.

تمت معاينة الانسجة المصبوغة مناعيا باستخدام الاضداد الضادة لغلوبيولين الارانب الموسوم بالبايوتين والبيروكسيديز الموسوم بالستربتوافدين. يعتمد التصبيغ الموجب على الضاد الخاص بالضرب. تمت ملاحظة التصبيغ في طلائية الوعاء وفي منطقة tunica adventitia في معظم الاحيان. وفي جانب اخر فقد اظهرت اوعية الجسم الاصفر تصبيغا موجبا.

من هذه الدراسة نستنتج بوجود نمط مميز لاوعية دم المبيض المصبوغة بالاضداد الضادة للسيدوموناس ايروجينوزا. يراد دراسة اخرى فيما يخص فعالية هذه الاضداد.

Introduction

The ovary is peculiar site for cyclical angiogenesis process associated with follicular development and luteogenesis in contrast to the static nature found in the vasculature of other organs [1]. Ovarian function and follicles maturation require blood vessel network to support the endocrine function of the organ [2]. While, the angiogenesis process that accompany ovulation and CL formation [3]. Flow of ovarian blood is very necessary to provides nutrients and oxygen which is important for secretory activates and growth of follicles and corporal tea (CL) [4]. On the other hand, defective angiogenesis in the ovary will end in follicular atresia [5]. Angiogenesis refers to the generation of new blood vessels, it is necessary for normal tissue development and growth [6][7].

Todays, it is well established that angiogenesis plays a major role in various disease and tumors especially in the reproductive tract (ovary) [8][9]. Besides ovarian function is strictly dependent on new blood vessels formation and inhibitory effects to new vessels formation will ultimately impair the function of the ovary. In addition, the role of VEGF and EGF in angiogenesis is target in tumor elimination [10][11].

The present study was aimed to see the influence of rabbit anti *Pseudomonas aeruginosa* antibodies generated by hyper immunization on reaction with rabbit ovarian blood vessels, which may influence these functional, unites with subsequent probable effect on ovarian function.

Materials and Methods

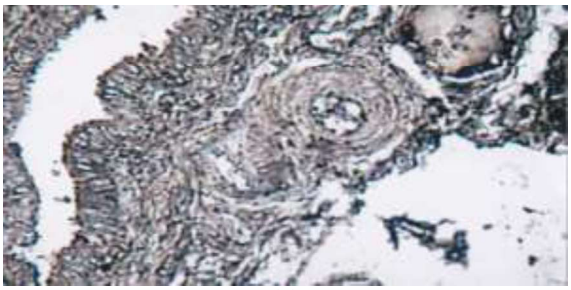
Preparation of Animals

Rabbits age ranged between 4-6 months old of both sexes (male and female) were taken and used in the preparation of anti-Pseudomonas aeruginosa antisera.
All of the animals were housed as a pair per cage and fed chew and libidium. They were ethically treated according to the established guideline.

Immunization of Animals
Rabbits were immunized essentially as following the procedure of [12], except using a subcutaneous route instead of intravenous route Sera collected were stored at -10 c° until used.

Immunohistochemical Procedure
The immunohistochemical procedure outlined in Dako cytomation labeled streptoavidin-Biotin 2 system was used.Horseradish peroxidase (LSAB-2 system HRP) ready to use detection system, code no. K0673 (CA.USA) [13][14]. Briefly, sections of female rabbit ovary 5 µm thickness was used, antisera were diluted 1: 60 with normal saline, negative controls comprissed sections of the tissues reacted with non-immune sera. The dark brown color was taken as positive results.

Results and Discussion
In the ovarian corpus luteum, the process of cyclically regulated occurs known as angiogenesis [15]. Several morphological and functional previous studies were operated on ovarian angiogenesis investigating the precence of factors of active ovarian angiogenesis. Which was related to folliculogenesis and the stimulate of gonadotropin [16][17].
The results of immunohistochemical staining of ovarian blood vessels with anti *Pseudomonas aeruginosa* antisera demonstrated a positive staining that was observed in endothelial cell, tunica adventitia of stromal blood vessels, as well as corpus leutum blood vessels.
In endothelial cell, the staining was localized to cell membrane. Positive staining of tunica adventitia of the vessels was located to area of putative connective tissue of the vessels which implicate a target in this area as seen in Figure 1.
Reactivity of the antisera is dependent on the strain of the bacterium used to prepare the antisera as shown in Table 1.
After the end of luteolysis, the highest count of blood vessels occurs in the midcycle and had rapid droping [15]. In parallel with ovary blood vessels staining, heart blood vessels used as third party control didn't show any staining of blood vessels. This results sheds some light on the target specificity for the antibodies contained in *Pseudomonas aeruginosa* hyperimmune sera.The targeting of ovary blood vessels is important.
Angiogenesis is being targeted in the treatment of various tumors [18]. Besides, the peculiar nature of the cyclical angiogenesis of ovary make antibody directed targeting fruitful in targeting the tumor of this organ [1]. Defect of angiogenesis destroy the normal ovarian cycle, express that angiogenic was rate limiting of ovulation, therefore, corpus luteum growthing [19]. None the less anti angiogenic agents including the monoclonal antibody bevacizumab with anti-VEGF activity has been used in phase II trial in recurrent epithelial ovarian cancer [20][21]. Furthermore, the overian blood vessels are target for antibody [22][23].
Because of there is no other similar or recent studies relating to this study, thatswhy, the present finding of anti ovarian blood vessel activity in selected sera of rabbits hyper-immunized with *Pseudomonas aeruginosa* requires additional studies focusing on the specificity of reaction of this antibody as well as it is real molecular target in the vessel.


(a)

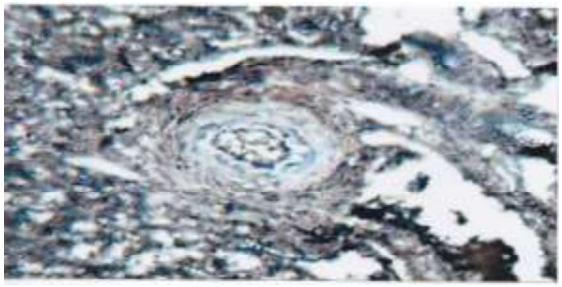

(b)

Figure 1: Endothelial cell, area of putative connective tissue of the vessels.References

Table 1: Positivity of rabbits anti-*Pseudomonas aeruginosa* antisera for ovarian blood vessels

Group	Positive no./total	
	Strain*: 1	2
Tests	1/3	3/3
Control	0/3	-

* Strain refers to bacterial isolate used to prepare the antisera.

References

[1] FraserH. M. and Duncan W.C.: SRB reproduction and fertility development award lecture 2008. Regulation and manipulation of angiogenesis in teovary and endometriam. Reproduction, Fertility and Development. 21, 377-392. 2009.

[2] Geva E. and Jaffe R.B.: Role of vascular endothelial growth factor in ovarian physiology and pathology. Fertl.Sterl.74: 429-438, 2000.

[3] Mattioli M., Barboni B., Turriani M., Galeati G., Zannoi A.: Follicle activation involves vascular endothelial growth factor production and increased blood vessel extension. Biol. Repod. 65: 1014-1019, 2001.

[4] Tamanini C. and De Ambrogi M.: Angiogenesis in developing follicle and corpus luteum. Reproduction in Domestic Animals. 39(4), 206-216, 2004.

[5] Berardinelli P., Russo V., Martelli A., Nardioncchi D., Di Giacinto O.: Colocalization of DNA Fragmentation and Caspase-3 activation during atresia in pig antral follicles. Anat. Istol. Embryo.33: 23-27, 2004.

[6] Plend J.: Angiogenesis and vascular regression in the ovary. Anat. Histol. Embroyl. 29: 257-266, 2000.

[7] Folkman J. and Klagsbrun M.: angiogenesis factors science 233.442-447, 1987.

[8] Abulafia O. and Sherer D.M.: Angiogenesis of teovary. Am. J. Obstet. Gynec. 182, 240-246, 2000.

[9] Ferrara N.: VEGF and the quest for tumor angiogenesis factors. Nat. Rev. Cancer. 2: 795-803, 2002.

[10] Shibuya M., Luo J. C., Toyoda M., Yamaguci S.: Involvement of VEGF and its receptors in ascites tumor formation. Cancer. Chemother. Pharmacol. 43: 72-77, 1999.

[11] Robinson R. S, Wood K. J, Hammond A. J, Laird M., unter M. G.: Angiogenesis and vascular function in the ovary. Reprod. 138: 869-881, 2009.

[12] Duncan N. H, Hinton N. A, Penner J. L, Duncan I. B.R.: Preparation of typing antisera specific for O antigens of *Pseudomonas aeruginosa*. J. Clin. Microbiol. 4: 124-128, 1976.

[13] Guesdon JL, Ternynck T, Avrameas S.: The use of avidin biotin interaction in immunoenzymatic techniques. J Histochem Cytochem; 27 (8): 1131-9, 1979.

[14] Giorno R.: Acomparison of two immunoperoxidase staining methods based on the avidin-biotin interaction Diag Immunol; 2 (3): 161-6, 1984.

[15] Modlich U, Kaup FJ, Augustin HG.: Cyclic angiogenesis and blood vessel regression in the ovary: blood vessel regression during luteolysis involves endothelial cell detachment and vessel occlusion; 74(4) 771-80, 1996.

[16] Fraser HM.: Regulation of the ovarian follicular vasculature. Reprod Biol Endocrinol.4: 8-22, 2006.

[17] Mattioli M., Barboni B., Turriani M., Galeati G., Zannoni A., Castellani G., Berardinelli P., Scapolo PA.: Folicle activation involves vascular endothelial growth factor production and increased blood vessel extension. Biol Repord. 65: 1014-1019, 2001.

[18] Gavalas N. G, Liontos M., Trachana S.P, Bagratuni T, Arapinis C, Liacos C, Dimopoulos M. A, Bamias A.: Angiogenesis-related pathways in the pathogenesis of ovarian cancer. Int. J. Mol. Sci.14: 15885-15909, 2013.

[19] Augustin HG.: Vascular morphogenesis in the ovary.Baillieres best pract res clin obstet Gynaecol;14 (6): 867-82, 2000.

[20] Burger R.A.: Overview of anti-angiogenic agents in development for ovarian cancer. Gynecologic Oncology 121(1): 230-238, 2011.

[21] Burger R. A, Still M. W, Monk B. J, Greer B. E, Sorosky J. I: Phase II-trial of beracizumab in persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer. A Gynecologic Oncology Group study. J. Clin. Oncol.25: 5165-5171, 2007.

[22] Mauro A., Martelli A., Berardinelli P., Ruso V., Bernabo N., Di Giacinto O., Mattioli M., Barboni B.: Effect of antiprogesterone RU486 on VEGF expression and blood vessl remodeling on ovarian follicle before ovulation-PLOSONE qie 95910. Doi: 10.1371, 2013.

[23] Smith-McCune K. K, Weidner N.: Demonstration and characterization of the angiogenic properities of cervical dysplasia. Cancer. Res. 54: 800-804, 1994.