Basrah Journal of Veterinary Research,Vol.15, No.3,2016
Proceeding of 5th International Scientific Conference,College of Veterinary Medicine
University of Basrah,Iraq

STUDY OF IMMUNOHISTOCHEMICAL TECHNIQUES FOR PLACENTA IN PLACENTA

Ruah F. Al-mayahi*; Fawzi S. AL-Asadi**

* Department of Anatomy and Histology, College of Veterinary Medicine,University of Basrah,Basrah,Iraq

Keywords: Placenta, Iummunohistochemical, sheep

ABSTRACT

The present study which include the demonstration of estrogen and progesterone receptors and demonstration of CD34 protein in placenta of pregnant sheep. Twenty one placenta from pregnant sheep were used in the present study. The immunohistochemical study of CD34 protein as marker for vascularization appear at day (27) few distribution in stroma of villi While at day (50) of gestation high density of CD34 protein in And also showed at day (120) high density. The estrogen receptor showed colour by kit at day (27) normal distribution in trophoblast cell of villus epithelium and not present in binucleate cell While at day (50) high density of estrogen receptor was showed ,And also at day (120) show high density of estrogen. The immunohistochemical study of progesterone receptor as appear at day (27) normal distribution in trophoblast cell of placentome and not present in binucleate cell While at day (50) high density, and also at day (120) .

INTRODUCTION

The bovine placental has discrete regions where is co-development of both maternal (caruncular) and fetal (cotyledonary) tissue . It is through these regions, known as placentomes that the fetus obtains oxygen and nutrient and excretes waste products, this

placentome mass to the development of the fetus has been demonstrated by carunclectomy of sheep (1). (2) showed the placental growth in sheep by measuring the diameter of cotyledons from early to mid-gestation. He reported that nutrient restriction up to 90 days of gestation did not affect placental growth , but that there was a pronounced effect on placental growth when animals were nutrient – restricted later in gestation. Taken together, such data show that the size of the placenta directly affects its capacity to transfer

nutrients which can influence the growth rate of the fetus and thus birth weight (3).

The protective role of the placenta as a barrier and for removal of end products of metabolism is vital as the fetal hepatic and renal systems have immature and insufficient metabolic and excretory capacity (4). Pregnancy is characterized by dynamic changes in multiple body systems resulting in increased basal oxygen consumption and changes in energy substrate use by different organs including the fetoplacental unit (5).

The shape of the placenta is variable due to the placental localization, so that three shapes of placentomes in ruminants : convex, flat and concave. The convex placentome is amushroom-shaped texture with a distinguish endometrial stalk, the flat placentome has a flatter shaped less convex for both of these placentomes fetal chorion coat the surface of the placentome. In contrast, in concave placentomes, maternal tissue surrounds the fetal tissue (6). In sheep and goats, concave placentomes general (7; 8),while in cattle convex placentomes general beside afew number of flat ones(9). The production of estrogen is contrast to that of progesterone, requires interaction between the fetus and the placenta (10). The formation of the placenta is regulated by hormon (11) , cytokines, growth factors and substrate that are present in the maternal and fetal circulation and bind to specific receptors on the placental surface (12,13,14)

MATERIALS AND METHODS

Twenty one placenta from pregnant sheep were collected from the local slaughterhouse of Basra city at ages of (27, 50, 120) day . Fine dissected to the uterus of pregnant sheep were removed and open the uterus and remove the fetal , then the embryo age were determines by messurement the length of fetus by using an electronic vernia , then removed number of placentomes at randomly by cutting from its location and processing for the following morphological, histological, Histochemical and immuonohistochemical studies. This work dwas done in laboratory of veterinary medicine college in basra.

Histological study for preparation section :

For the preparation of histological sections, was processed according to the following steps.

Fixation, Dehydration, Clearing, Infiltration and Embedding, Trimming and Sectioning at (4- 6) µm, Mounting: coated with mayers albumin, Staining.

Immunohistochemical study:

Estrogen receptor:

Demonstration of estrogen in placentome was done according to method (DAKO company kit (2010)). The paraffin sections at 4um were cut(use chargeable slides), then de wax the slides in ovin at 56˚C for 30min , the slides were put in xyline two jars 2min for each jar ,to Avoid slides dryness ,de hydration immediately with (100% , 90% , 80 %, 70 %, 60 %) ethanol alcohol then wash with (TBS) tris buffered saline , then the slides immerge in antigens retrieval solution target solution high PH from DAKO for 20min at 121˚C under pressure and leave slides to cool at room temperature with still in target solution , wash slides with buffered for 5min then Incubate slides with hydrogen peroxide H_2O_2 for 5min and washing with buffered for 5min ,Incubate the slides with primary antibody dilution 1/60 for 30min from (DAKO company) then washing with buffered for 5min , Incubate slides with secondary antibody one step (invasion secondary antibody) from (DAKO company) then wash with buffered for 5min, Then incubate slides with chromogen DAB (1-5)min 1.5ml from dab buffered and 20um from chromogen dab , Wash slides with buffered for 5min then stain slides with mayers haematoxylin for 5min and Wash with buffered then distill water , covering via aqueous mounting medium and incubation period 30min.

Brogesterone receptor:

Demonstration of estrogen in placentome was done according to method (DAKO company,2010). The paraffin sections were cut at 4um(use chargeable slides), then de wax the slides in oven at 56˚C for 30min , then put the slides in xyline two jars 2min for each jar ,Avoid slides dryness …do hydration immediately with (100% , 90% , 80 %, 70 %, 60 %) ethanol alcohol then wash with (TBS) tries buffered saline , put the slides in antigens retrieval solution target solution high PH from DAKO for 20min at 121˚C under pressure and leave slides to cool at room temperature with still in target solution , washing slides with buffered for 5min then Incubate slides with hydrogen peroxide H_2O_2 for 5min then wash with buffered for 5min ,Incubate slides with primary antibody dilution 1/50for 30min from (DAKO company) then wash with

buffered for 5min , Incubate slides with secondary antibody one step (invasion secondary antibody) from (DAKO company) then wash with buffered for 5min, Then incubate slides with chromogen DAB (1-5)min 1.5ml from dab buffered and 20um from chromogen dab , Wash slides with buffered for 5min then stain slides with mayers haematoxylin for 5min and Wash with buffered then distill water , covering via aqueous mounting medium and incubation period 30min.

CD34 protein:

Demonstration of CD34 protein in placentome was done according to method (DAKO company,2010), cut the paraffin sections at 4um(use chargeable slides), then de wax the slides in ovin at 56˚Cfor 30min , the put the slides in xyline two jars 2min for each jar ,Avoid slides dryness …do hydration immediately with (100% , 90% , 80 %, 70 %, 60 %) ethanol alcohol then wash with (TBS) tries buffered saline , put the slides in antigens retrieval solution target solution high PH from DAKO for 20min at 121˚C under pressure and leave slides to cool at room temperature with still in target solution , wash slides with buffered for 5min then Incubate slides with hydrogen peroxide H_2O_2 for 5min then wash with buffered for 5min ,Incubate slides with primary antibody dilution 1/100 for 30min from (DAKO company) then wash with buffered for 5min , Incubate slides with secondary antibody one step (invasion secondary antibody) from (DAKO company) then wash with buffered for 5min, Then incubate slides with chromogen DAB (1-5) min 1.5ml from dab buffered and 20um from chromogen dab , Wash slides with buffered for 5min then stain slides with mayers haematoxylin for 5min and Wash with buffered then distill water, covering via aqueous mounting medium and incubation period 30min.

Result:

Estrogen Receptor:

The immunohistochemical study of estrogen receptor showed by brown colour by kit (DAKO company) at day (27) normal distribution in trophoblast cell of villus epithelium and not present in binucleate cell (Figure.1).While showed at day (50) high density of estrogen receptor in trophoblast cell of villus epithelium and not present in binucleate cell compared to day (27) (Fig. 2) .And also at day (120) show high

density of estrogen receptor in trophoblast cell of villus epithelium and not present in binucleate cell compared to day (50) (Fig .3).

Progesterone Receptor :

The immunohistochemical study of progesterone receptor as shown by brown colour by kit (DAKO company) appear at day (27) normal distribution in trophoblast cell of placentome and not present in binucleate cell (Fig.4). While showed at day (50) high density of progesterone receptor in trophoblast cell of placentomes and not present in binucleate cell compared to day (27) (Fig. 5). And also at day (120) showed high density of progesterone receptor in trophoblast cell and not present in binucleate cell compared to day (50) (Fig. 6).

CD34 Antigen Receptor:

The immunohistochemical study of CD34 protein as marker for vascularization showed by brown colour by kit (DAKO company) appear at day (27) few distribution in stroma of villi (Fig.7). While showed at day (50) of gestation high density of CD34 protein in stromal villi compared to day (27) (Fig.8). And also showed at day (120) high density of CD34 protein in stromal villi compared to day (50) (Fig.9).

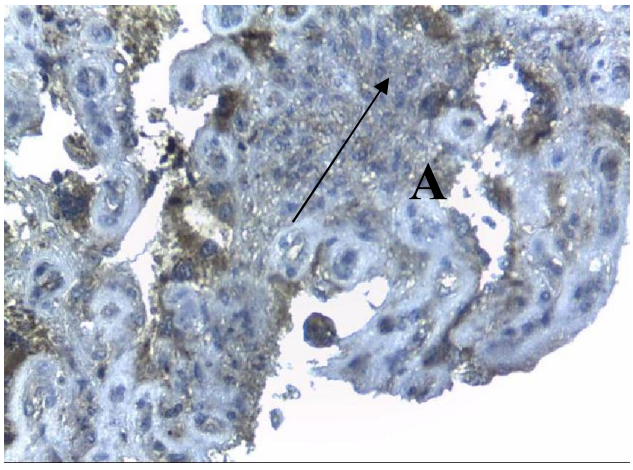


Fig.(1): Cross section of sheep placentom show: 1. Estrogen receptor ( )(brown color) at day 27 2. Binucleated cell (A) estrogen antibody 400x)

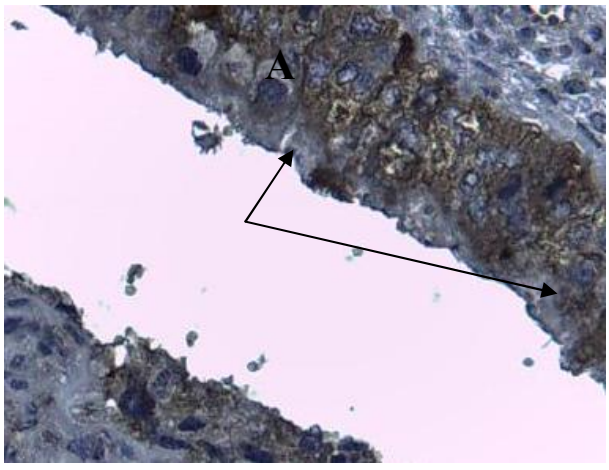


Fig.(2): Cross section of sheep placentom show: 1. Estrogen receptor ( )(brown color) at day 50 2.Binucleated cell (A) (estrogen antibody 400x)

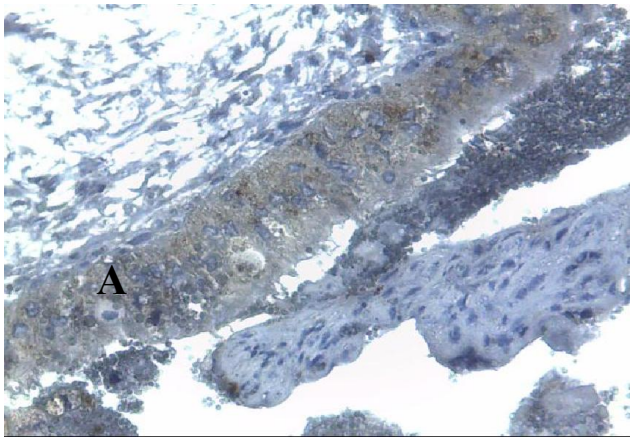


Fig.(5): Cross section of sheep placentome show:1. Progesterone receptor( )at day 50 2. Binucleate cell (A) (progesterone antibody 400x).

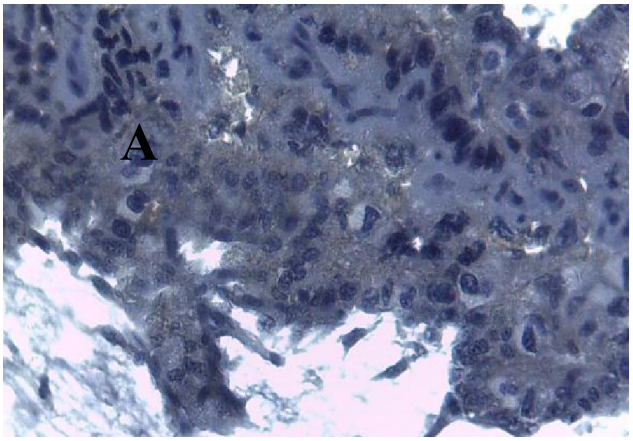


Fig.(6): Cross section of sheep placentome show:1. Progesterone receptor( )at day 120 2. Binucleate cell (A) (progesterone antibody 400x).

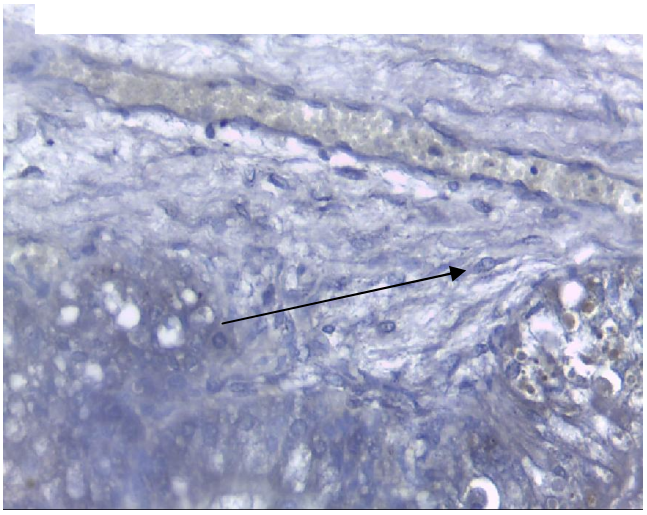
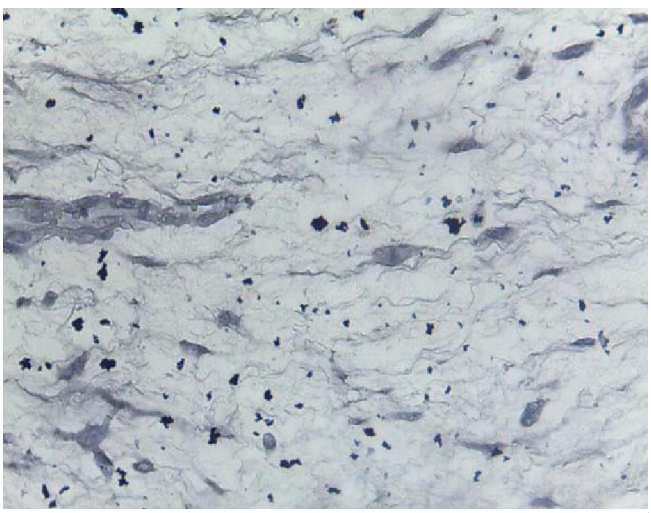


Fig.(7): Cross section of sheep placentomes show: Vascularization

( ) at day 27 (CD34 400x) .



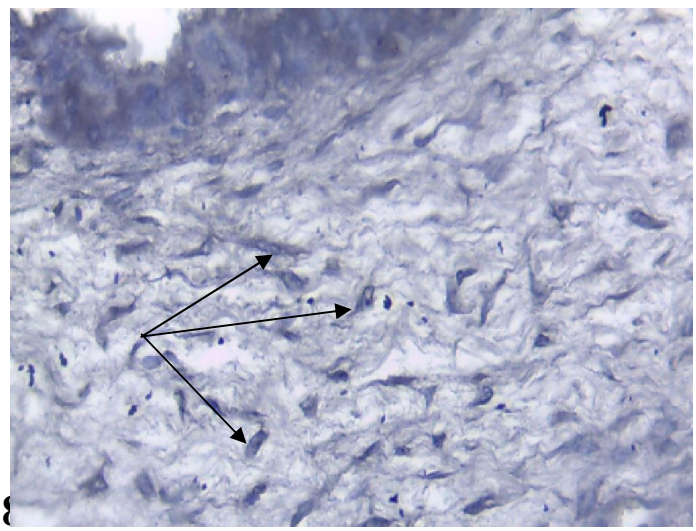


Fig. (8): Cross section of sheep placentomes show: Vascularization ( ) at day50 (CD34 400X).

Fig. (9): Cross section of sheep placentomes show: Vascularization ( ) at day120 (CD34 400X).

DISCUS

The present study showed that CD34 protein appeared as few distribution in stroma of villi at day 27 in placenta while at day 50 of gestation showed high density and also at day 120 higher density as compared to day 50 of gestation in sheep placenta . CD34 is a marker that allow to appreciate the vascular density and it is strongly correlated with angiogenesis (15) in human. The present study was agree with results of (16) who noticed that embryonic capillaries in the chorionic villous appeared between day 18 and 20 post- conception.

(17) found the immunostaining for CD34 in the endothelial cells in both cyto and synsytiotrophoblast and all villous and also the fetal blood vessels were uniformly distributed though out the chorionic villi in human (18)). (19) noticed that there were interaction between trophoblast and vascular cells of the spiral arteries. The angiogenesis is regulates by vascular endothelial growth factor, placental growth factor and angiopoietins as well as protease such as the membrane –type matrix metallo- proteinase (20) in human. The present study showed that estrogen receptor in sheep placenta showed normal distribution at day 27 of gestation while at day 50 was high density and also at day 120 showed higher density than at day 50 of gestation.

the present study show that progesterone receptor in sheep placenta showed normal density at day 27 of gestation while increase in density in day 50 and day 120 of gestation. these result may be contributed to the lack of estrogen and progesterone in maternal tissue. These result was agree with result of (21; 22,23)in rat,monkey and human respectively .

(24), (25) noticed that bovine placentomes of progesterone localized into nuclei of caruncular stromal cell and caruncular vascular pericytes suggesting that these cells are rather under the control of placental than luteal progesterone. Also (26) noticed that estrogen receptor blocker in pregnant cow had no effect on calving process . Estrogen induce and maintain female secondary gland and stimulate the

development of the endometrial lining (27) while (28) in mice noticed that estrogen function was to stimulated proliferation luminal and glandular epithelium and increase expression of progesterone receptor. The placental androgen are considered as precursors for placental estrogen synthesis so that considerable levels of androgen may also present in the uterine environment during pregnancy (29,30,31,32,33,) in human, pig and rat respectively

دراسة الكيمياء المناعية النسيجية للمشيمة في الاغنام

فوزي صدام الاسدي رؤى فاضل المياحي

فرع التشريح والانسجه كلية الطب البيطري، جامعة البصرة، البصره،العراق

الخلاصة

تضمنت الدراسة الحالية الكشف عن مستقبلات الاستروجين والبروجستيرون و بروتين CD3.استخدمت في هذه الدراسة واحد وعشرين مشيمة للاغنام الحوامل. أظهرت الدراسة المناعية النسيجية لبروتين CD34 انتشار ضئيل في لب الزغابة بعمر (27) يوم بينما بعمر (50) يوم من الحمل كثافة عالية وازداد بعمر (120) يوم .

أظهرت الدراسات الكيمياء المناعية النسيجية لمستقبلات الاستروجين بعمر (27) يوم انتشار طبيعي في خلايا التروفوبلاست وعدم وجودها في الخلايا ثنائية النواة بينما بعمر(50) يوم كانت الكثافة عالية في خلايا التروفوبلاست وكذلك عدم وجودها في الخلايا الثنائية النواة بينما بعمر (120) يوم أظهرت كثافة عالية في خلايا التروفوبلاست وعدم وجودها في الخلايا الثنائية النواة . أما الدراسات المناعية النسيجية لمستقبلات البروجسترون أظهرت بعمر (27) يوم توزيع طبيعي في خلايا التروفوبلاست وعدم وجودها في خلايا ثنائية النواة بنما بعمر (50) يوم كثافة عالية من المستقبلات في التروفوبلاست وعدم وجودها في الخلايا ثنائية النواة بينما بعمر (120) يوم أظهرت كثافة عالية من البروجسترون في خلايا التروفوبلاست وعدم وجودها ايظا في خلايا ثنائية النواة

REFERENCES

(1)Mcgeady, T.A.; Quinn, P.J.; Fitzpatrick, E.S.; Ryan, M.T. and Cahalan, S. (2006). Veterinary Embryology. Blackwell Publishing Ltd. Oxford, United Kingdom

(2)Kelly, R.W.(1992). Nutrition and placental development. *Proceedings of the Nutrition Society of Australia* 17 203-211.

(3)Fowden, A. ; Ward, J. ; Wooding, F. ; Forhead, A. and Constancia, M. (2006). Programming Placenta Nutrient Transport capacity. *The Journal of physiology* .572, 5-15.

(4)Kovo, M. and Abraham, G. (2008). In vitro models using the human placenta to study fetal exposure to drugs. Clin. Med. Repr. Heal., 2: 15-24.

(5)Casanueva, E. and Fernando, V. E. (2003). Iron and oxidative stress in pregnancy. J. nutr., 133:1700-1708. **Laven, R.A. and (6)Peters, A.R.(2001).**Gross morphometry of the bovine placentome during gestation. Reprod Domest Anim. 36(6):289-96.

(7)Igwebuike, U. M. and Ezeasor, D.N. (2013). The morphology of placentomes and formation of chorionic villous trees in West African Dwarf goat *(Capra hircus).*Veterinarski. Arhiv.; 83(3), 313-321.

(8)Vatnick, I.; Schoknecht, P.; Darrigrand, R. and Bell, A.(1991). Growth and metabolism of the placenta after unilateral fetectomy in twin pregnant ewes . *Journal of developmental physiology* 15, 351.

(9) Salafia, C. M. ; Yampolsky, M.; Misra, D.P. ; Shlakhter, O. ; Haas, D. ; Eucker, B. and Thorp, J. (2010). Placental surface shape, function, and effects of maternal and fetal vascular pathology .Placenta xxx: 1-5.

(10)Hirayama, H.; Sawai, K.; Moriyasu, S.; Hirayama, M.; Goto, Y.; Kaneko, E.; Miyamoto, A.; Ushizawa, K.; Takahashi, T. and Minamihashi, A. (2008). Excess estrogen sulfoconjugation as the possible cause for a poor sign of parturition in pregnant cows carrying somatic cell clone fetuses. Reproduction. 136(5):639-47.

(11)Haig, D. (2008). Placental growth hormone-related proteins and prolactin-related proteins. J. Placenta, 22: S36-S41.

(12) Desoye, G. and Mouzon, S. H. (2007). The human placenta in gestational diabetes mellitus. J. Diab. Care, 30(2):S120-S126.

(13)Shintaku, K.; Satoko, H.; Masayuki, T.; Hideaki, N.; Shoji, S.; Kiyomi, T.; Hitoo, N.; Tomoyuki, F.; Yuji, T.; Hisakazu, O. and Yasufumi, S.(2009). Transplacental Pharmacokinetics of Diclofenac in Perfused Human Placenta. Dru. Meta. Dispo. 37(5):962–968.

(14)Hiden, U.; Lang, I.; Ghaffari-Tabrizi, N. ; Gauster, M.; Lang, U. and Desoye, G.(2009). Insulin action on the human placental Endothelium in normal and diabetic pregnancy. J. Curr. Vasc.Pharm.,7:460-466.

(15)Manolea, M.M. ; Gavrila, O. A.; Popescu, F.C.; Novac, L. and Mateescu, G.O. (2012). The importance of immunohistochemical evalutionof the vascular changes from the decidua and placenta in recurrent pregnancy loss. *Rom. J. Morphol. Embryol* ; 53(2) : 363-368

(16) Selcer, K.W.; Difrancesca, H.M.; Chandra, A.B. and Li, P.K. (2007). Immunohistochemical analysis of steroid sulfatase in human tissues. J. Steroid Biochem. Mol. Biol. 105(1-5):115-23.

(17)Mackiewicz, Z.; Dudek, E.; Glab, G.; Kubicki, J. and Konttinen, Y.T. (2004). CD34+ stem cells in normal placenta tissues and in placenta with intrauterine growth retaradation . Acta. Medica. Lituanica ; 11(2): 34-38.

(18)Treesh, S.A. and Khair, N.S. (2015). Histological changes of the human placenta in pregnancies complicated with diabetes. J. Cytol. Histol; 6(2): 1-7.

(19)Liliana, N. (2009). *Avort recurent. Implicarea sistemului matrix metaloproteinazelor*, Ed. Medicală Universitară, Craiova, Pp.: 69–77.

(20)Carmeliet, P.(2003). *Angiogenesis in health and disease*, Nat Med, (6):653–660.

(21)Kohler, E.; Wojnorowicz , F. and Borner, K. (1975). Effects of a protein-free diet on amino acids and sex hormones of rats during the early postimplantation stages of pregnancy . *J. Reprod . Fertil.* 42:9-21.

(22)Ghosh, D.; Dhara, S.; Kumar, A. and Sengupta, J. (1999). Immunohistochemical localization of receptors for progesterone and oestradiol-17B in the implantation site of the rhesus monkey. *Human. reproduction* ; 14(2): 505-514.

(23)Schuler, G.; Wirth, C.; Klisch, K.; Pfarrer, C.; Leiser, R. and Hoffmann, B. (1999). Immunolocalization of progesterone receptors in bovine placentomes throughout mid and late gestation and at parturition. Biol Reprod. 61(3):797-801.

(24)Sherer, D.M. and Abulafia, O.(2001). *Angiogenesis during implantation, and placental and early embryonic development*, Placenta, 22(1):1–13.

(25)Boos, A.; Kohtes, J.; Stelljes, A.; Zerbe, H. and Thole, H.H. (2000). Immunohistochemical assessment of progesterone, oestrogen and glucocorticoid receptors in bovine placentomes during pregnancy, induced parturition, and after birth with or without retention of fetal membranes. J Reprod. Fertil. 120(2): 351-60.

(26)Janowski ,T.; Zdunczyk, S.; Malecki-Tepicht, J.; Baranski, W. and Ras, A.(2002). Mammary secretion of oestrogens in the cow. Domest Anim Endocrinol. 23(1-2):125-37.

(27). **Walters, K.A.; McTavish, K.J.; Seneviratne, M.G.; Jimenez, M.; McMahon, A.C.; Allan, C.M.; Salamonsen, L.A. and Handelsman, D.J.(2009).** Subfertile female androgen receptor knockout mice exhibit defects in neuroendocrine signaling, intraovarian function, and uterine development but not uterine function. Endocrinology. 150(7):3274-82.

(28)Zhou, X. (2010). Roles of androgen receptor in male and female reproduction: lessons from global and cell-specific androgen receptor knockout (ARKO) mice. J Androl. 31(3):235-43.

(29) Hsu, T.Y.; Lan, K.C.; Tsai, C.C.; Ou, C.Y.; Cheng, B.H.; Tsai, M.Y.; Kang, H.Y.; Tung, Y.H.; Wong, Y.H. and Huang, K.E. (2009). Expression of androgen receptor in human placentas from normal and preeclamptic pregnancies. Taiwan J Obstet Gynecol. 48(3):262-7.

(30)Pope, W.F. and Cardenas, H. (2006). Androgens in female pig reproduction: actions mediated by the androgen receptor. Soc Reprod. Fertil. Suppl, 62:55-67.

(31)Duda, M. and Slomczynska, M.(2007). Immunohistochemical localization of androgen receptor in two subpopulations of porcine granulosa cells in vitro. Reprod Domest Anim. 42(1):22-5.

(32)Tong, M.H.; Jiang, H.; Liu, P.; Lawson, J.A.; Brass, L.F. and Song, W.C.(2005). Spontaneous fetal loss caused by placental thrombosis in estrogen sulfotransferase-deficient mice. Nat Med. 11(2):153-9.

(33)Pelletier, G. (2002). Effects of estradiol on prostate epithelial cells in the castrated rat. J. Histochem. Cytochem . 50(11) : 1517-24.