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HISTOCHEMICAL STUDY OF THE UTERUS IN LOCAL RABBIT (Lepus cuniculus) DURING EARLY IMPLANTATION PERIOD

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Key word: Implantation, Female rabbits, Histochemistry.

ABSTRACT

The current study investigated the early stages of pregnancy (day 7 of implantation) in the uterus of rabbit by histochemical study. The study was conducted at the laboratories of the College of Veterinary Medicine of Basra University. The present study was carried out on two groups of female rabbits which included the control group (4), the implantation group (7). The histochemical analysis was carried out for the demonstration of glycogen and the activity of alkaline phosphatase enzyme. The result of this study showed a clear increase in the distribution of glycogen, which was observed in the uterine epithelium, sub- epithelial stroma and in the uterine gland, also this study revealed an increased activity of the enzyme alkaline phosphatase mainly in epithelium and site of implantation in the uterus.

INTRODUCTION

Embryo implantation is a term used to describe process of the attachment and invasion of the blastocyst to the endometrium of the uterus in placental animals. During estrus cycle, the functional layer of the endometrium undergoes specific molecular,

enzymatic, morphological, and structural changes, which are under control of ovarian hormonal changes (1).

At the time of implantation in mammals, the cell surface of luminal epithelium of endometrium undergoes changes, which terminologically named" plasma membrane transformation" (2). Epithelium of mammalian endometrial is the first site of contact between trophectoderm and maternal tissue during the period of attachment and implantation n of embryo. Both of these surfaces undergo specific molecular, morphological and ultra structural changes, which are mediated by ovarian hormones (3). In mammals, glycogen and carbohydrates are very important for embryonic nutrition in the early stages of pregnancy and the majority of these elements are produced from uterine glandular cells (4). Association between glycogen and morphogenesis has been noted by previous authors who observe association between glycogen and mitosis and differentiation of epithelia (5). Alkaline phosphatase activity in the uterus in early pregnancy was also evaluated in different animals like rat and mouse. These enzymes and components play a role in nourishment and implantation of the blastocyst (6).The aim of the current study was to perform a histochemical analysis of the distribution of glycogen and the activity of alkaline phosphatase in the uterus of the rabbit during early implantation period.

MATERIALS AND METHODS

The study was conducted at the laboratories of the College of Veterinary Medicine of Basra University including (13) domestic rabbits aged 6-8 months and weighing (2.3 - 2.6 Kg) were used. The females were killed by introducing air bubbles into the heart. Uterus was quickly dissected at the cervix and removed from the animal. The uterine cornua were carefully opened down the mesometrial side to expose the preimplantation blastocysts. The uterine tissue surrounding the area of each blastocyst was separated from the remaining tissue and the endometrium from each site was recovered for the study.

For the histochemical study, the PAS stain was used to demonstrate the glycogen (7) and on calcium phosphate method (8) to demonstrate the alkaline phosphatase activity, in comparison with the control group which represents non pregnant rabbits at (rest-stage).

RESULT

The current study showed that glycogen in the uterus of the control group was found as weak scattering in the endometriam and muscular layer (myometrium). This was shown as faint reaction with PAS (Figure1).

For the implantation period (day 7), glycogen was observed in uterine epithelium, the sub- epithelial stroma, and in the anti -mesometrial glands. The glycogen appears in the uterine secretions, in the stroma near the site of implantation, and basal parts of the uterine glands (Figure 2).

Regarding the activity of alkaline phosphatase in the control group, the results showed appositive reaction in the uterine epithelium (Figure 3).

Activity of alkaline phosphatase was seen to increase as a prominent feature in uterine epithelium of the uterus of the doe during early implantation as was compared with the control (Figure 4).

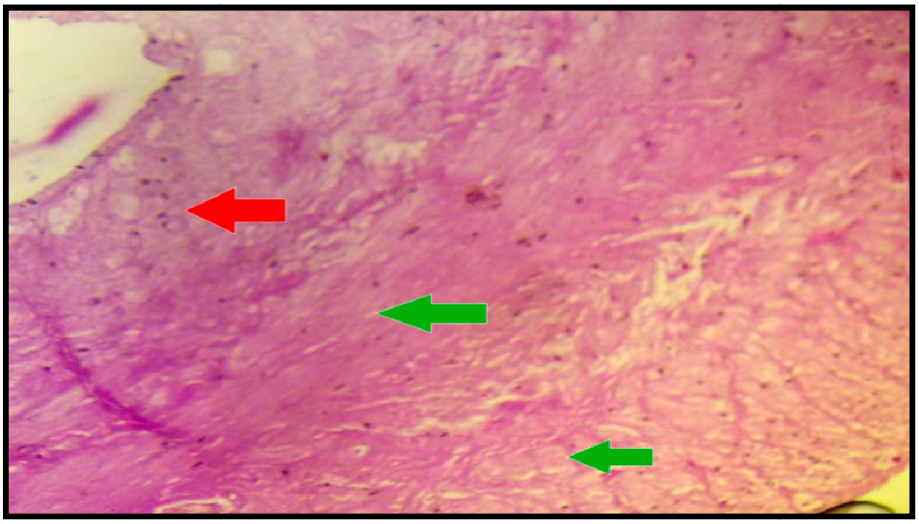
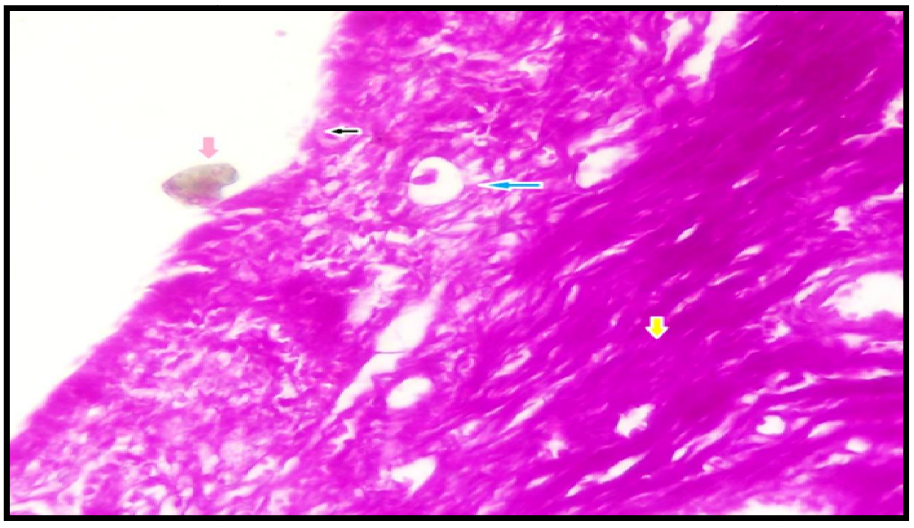


Figure (1): Section from control rabbit uterus.Was showed the little distribution of glycogens in the endometrium (red arrow), myometrium (green arrow). PAS stain.X100.



Figure(2): Section of rabbit uterus in early pregnancy (day 7 of implantation) showing distribution of glycogen in uterine epithelium (black arrow) ,uterine gland (blue arrow) and in myometrium layer (yellow arrow) in the site of implanted blastocyst (pink arrow) .PAS stain.X200.

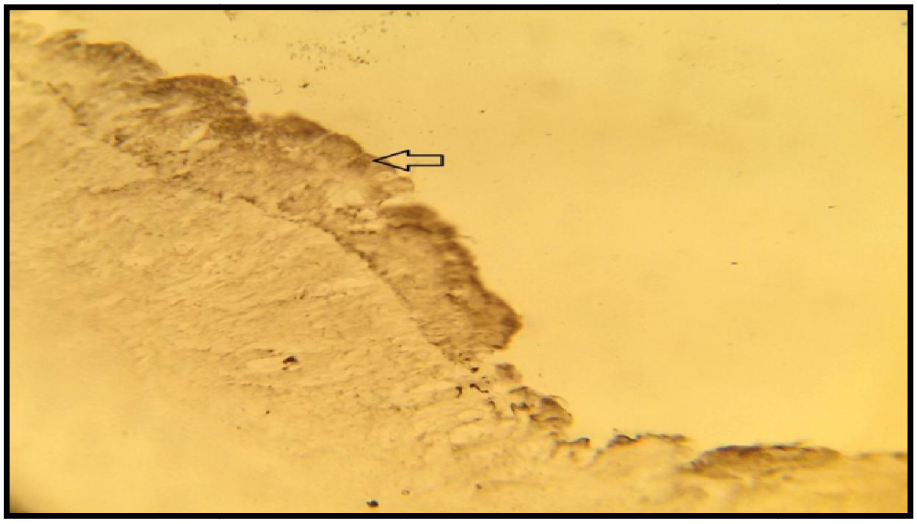


Figure (3): Uterus of control rabbit. Showed the localization of alkaline phosphatase as a weak reaction in uterine epithelium only (black arrow).Calcium Phosphate method. X200.

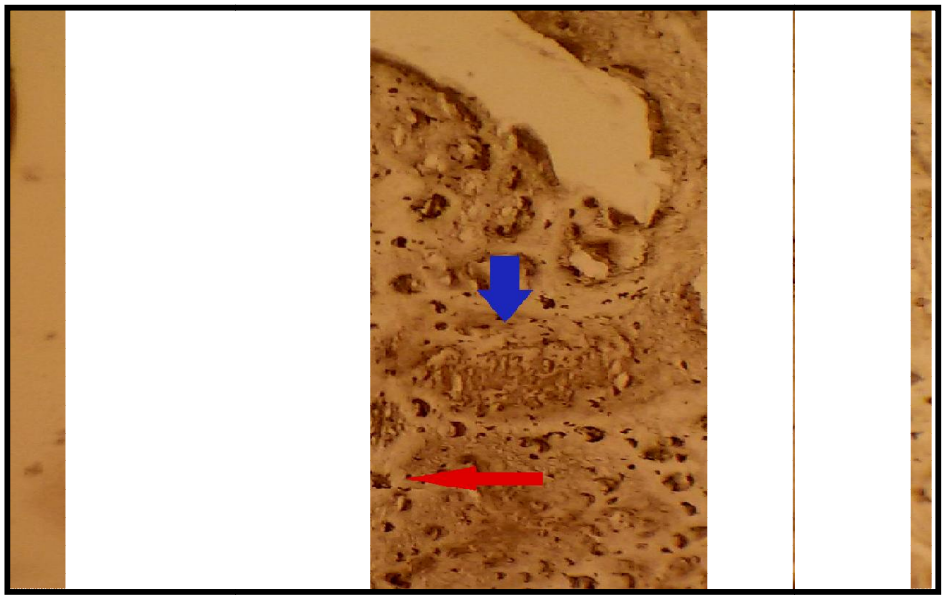


Figure (4): Uterus of rabbit in early stages of pregnancy (day 7 of implantation) demonstrate alkaline phosphatase activity in uterine epithelium (white arrow) and uterine stroma (red arrow) beside the implanted embryo (blue arrow).Calcium phosphate method.X200.

DISCUSSION

The current study showed that glycogen in early stage of implantation was seen mainly in the uterine epithelium and sub- epithelial stroma anti mesometriallys. With the progression of implantation, glycogen was shown in the uterine secretions, the stroma near the site of implantation and deep parts of the uterine glands. These findings appear as PAS positive regions.

This finding is in disagreement with (9) who mentions that glycogen is mainly seen in embryonic tissues of rabbit.

The current study found that the increase was persistent during the stage of implantation. This study is similar to (10) who compared between the rat and the rabbit and found that the glycogen in rat falls off immediately prior to degeneration, while in the rabbit there was increasing in the glycogen in the uterine epithelium.

Role of glycogen in rabbit implantation is important for use later in the implantation and placentaion processes, or as a reserve of easily available energy and this was in agreement with (11).

There are several reports which indicate that, after luteinizing hormone LH surge, glycogen begins to accumulate within the cytoplasm, initially in a sub nuclear area, but over time large aggregations are seen in the apical region of the cell by increasing progesterone (11).

The current study showed that alkaline phosphatase (ALP) activity was seen to increase in the uterine epithelium and sub –epithelial stroma during early implantation.

In fact these findings are unlike the studies of previous authors who stated that there was a fall in alkaline phosphatase activity in the inter implantation endometrium of the rabbit uterus during early pregnancy (12).

Previous studies have demonstrated that alkaline phosphatase activity is associated with the differentiate state, but not the proliferative state, of epithelial cells(13, 14).This fact was consistent with studies in the hamster which showed that the lowest (ALP)

activity in (day 2) epithelial cells when these cells were proliferating and strong AP activity in(days 3) and (day4) uterine epithelial cells when these cells are differentiating to support implantation (15) .Luminal epithelial cells show apical plasma membrane alterations such as polarity changes and flattening of microvilli with reorganization of apical molecules during epithelial cell surface preparation for blastocyst attachment in a variety of species including the hamster, mouse, rabbit, camels and human (16,17,18). These changes in the surface of epithelial cells may be associated with phosphorylation / dephosphorylation of proteins and phospholipids in their membrane lipid bilayer.

Thus, the results of the current study suggest that rabbit females may utilize the AP activity in epithelial cells to support phosphorylation status of the cell surface molecules that are helpful at the time of the uterine receptivity and blastocyst attachment.

دراسة كيمياء نسجية لرحم الأرنب المحلي خلال فترة الانغراس المبكر

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الخلاصة

الدراسة الحالية تحقق في المراحل الاولى للحمل(اليوم السابع للانغراس) لرحم الارنب بواسطة التحليل الكيمياء النسجي والكيمياء الانزيمي. تمت هذه الدراسة في مختبرات كلية الطب البيطري- جامعة البصرة.تم تنفيذ الدراسة الحالية على مجموعتين من اناث الارانب والتي تشمل مجموعة السيطرة(4) ومجموعة الانغراس (7).تم التحليل الكيمياءالنسجي لتوضيح توزيع الكلايكوجين والتحليل الكيمياء الانزيمي لتوضيح فعالية الانزيم الفوسفاتيز القلوي. الدراسة بينت زيادة واضحة في توزيع الكلايكوجين والذي شوهد في الغشاء الطلائي للرحم والسدى التحت طلائية والغدد الرحمية.بالاضافة الى ذلك اوضحت هذه الدراسة زيادة في فعالية انزيم الفوسفاتيز القلوي بصورة رئيسية في الغشاء الطلائي في موقع الانغراس في الرحم.

REFERENCES

1. **Eurell, JA.; and Frappier, BL. (2006).** Dellmann's textbook of veterinary histology. 5th Edn Christopher, J and Adam, HB (2010). The adverse effects of obesity on conception and implantation. Reproduction. 140: 347-364., UK, Blackwell. PP: 134-152.
2. **Murphy, C.R. (2001).**The plasma membrane transformation: a keyconceptin uterine receptivity. Rep. Med. Rev., 9: 197-208.
3. **Leesey, B.A. (2000**).The role of the endometrium during embryo implantation. Hum. Report. 15: 36-50.
4. **Christopher, J. and Adam, HB. (2010**). The adverse effects of obesity on Conception and implantation. Reproduction. 140: 347-364.
5. **Wang, H.; Luan, L.; Ding, T.; Brown, N.; Reese, J. and Paria, B.C.(2011).** Dynamics of zonula occludens-2 expression during preimplantation embryonic development in the hamster. The riogenology; 76:678– 686.
6. **Salgado, R. M.; Capelo, L. P.; Favaro, R. R.; Glazier, J. D. and Aplin, J.(2009) .** The estrous cycle modulates small leucine-rich proteoglycans expression in mouse uterine tissues. *Anat.Rec., 292(1)*:138-53.
7. **Luna, L.G. (1968).** Manual of histological staining methods of armed forces institute of the pathology. Third edition. New York. McGraw-Hill book Co. London. Pathol. Toxicol., 4: 183-196.
8. **Bancroft, J.D.; Stevens, A. and Turner, D.R. (1990).**Theory and Practice of Histological techniques.3rd ed. Churchill Livingstone.PP:383-385.

9. **Butte, NF. (2000).** Carbohydrate and lipid metabolism in pregnancy:normal compared with gestational diabetes mellitus. Am. J. Clin Nutr; 71:1256S–61S.

10. **Van Mourik, M.S.; Macklon, NS and Heijnen, C.J**. (2009).Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment. J. Leukoc Biol.; 85:419.

11. **Murphy, CR. and Shaw, TC. (1994).** Plasma membrane transformation: a common response of uterine epithelial cells during the peri-implantation Period. Cell. Biol. Int., 18: 1115-1128.

12. **Murdoch, RN.; Kay, DJ. and Cross, M. (1978**). Activity and sub cellular distribution of mouse uterine alkaline phosphatase during pregnancy and pseudopregnancy. J Reprod Fertil; 54:293–300.

13. **Marvin-Guy LF.; Duncan, P.; Wagniere ,S. Antille, N.; Porta, N.; Affolter, M. and Kussmann, M**. **(2008**). Rapid identification ofdifferentiation markers from whole epithelial cells by matrix-assisted laser desorption/ionisation time of flight mass spectrometry and statisticalanalysis. Rapid Commun Mass Spectrum; 22:1099–1108.

14. **Wood, S.R.; Zhao, Q.; Smith, L.H. and Daniels, C.K**. **(2003).** Altered morphology in cultured rat intestinal epithelial IEC-6 cells is associated with alkaline phosphatase expression. Tissue Cell; 35:47–58.

15. **Zhang, Q.and Paria, B.C. (2006**). Importance of uterine cell death, renewal, and their hormonal regulation in hamsters that show progesterone-dependent implantation. Endocrinology; 147:2215–2227.

16. **Bucci, M. and Murphy, C.R**. (1995). Alkaline phosphatase distribution in the plasma membrane of uterine epithelial cells is markedly altered during early pregnancy in the rat. Cell Biol. Int.; 19:921–928.

17. **Tin-Ley, U. (2000).** Relevance of endometrial pinopodes for human blastocyst implantation. Hum Report; 15(Suppl 6):67–73.

18. **Bagot, C.N.; Kliman, H.J.; Taylor, H.S**. (2001). Maternal Hoxa10 is required for pinopod formation in the development of mouse uterine receptivity to embryo implantation. Dev Dyn. ; 222:538–544.