Journal of Animal and Veterinary Advances 7 (6): 646-650, 2008
ISSN: 1680-5593

The Effect of Ligation of Right Ovarian Artery on Ovarian Follicular Function and PCOS Generation in Rats

[1]Alireza Najafpoor, [2]Mazdak Razi, [2]Golamreza Najafi, [1]Ehsan Khalilloo and [1]Golamreza Abdollalizadeh
[1]Department of Clinical Science, Faculty of Veterinary Medicine,
Islamic Azad University, Urmia Branch, Urmia, Iran
[2]Departement of Histology, Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran

Abstract: To follow this study 56 mature female rats were used, thirty days before test stars all animals separated from male rats. All rats synchronized by vaginal smir on estrus phase of menstrual cycle. Animals were kept in 7 cages with 8 rats in each, are rarred at 22±2°C temperature and 12/12light/dark conditions. The rats were divided to two treatment and control groups. On days 8, 14, 21, 30, 60 after ligation of right ovarian artery by laparatomy, all rats were scarified and ovaries were collected. histological studies did on the ovaries. Observation demonstrated that the ovary on the ligated side has significantly fewer ovulations ($p \leq 0.01$) than the ovary in the normal condition in the intact side. Observations also demonstrated cystic follicles on removed ovary (right ovary), but no polycystic follicles observed on the intact side ovaries. However, there was no corpus luteum on the removed ovary. There were no corpora albicantia observed indicating there had been no recent ovulation. But no abnormality detected on left ovary.

Key words: Polycystic follicles, ovary, corpora albicantia, corpus luteum

INTRODUCTION

In female rats, the ovaries posses a dual arterial blood supply, the ovarian artery and uterine arteries (Ginther, 1976). It is crucial to understand the contribution this 2 vessels make in supplying blood to the ovary when evaluating a treatment effect (a treatment witch result in loss of blood flow to ovary by uterine artery) has altered. For example hysterectomy failed shorten Corpus Luteum (CL) life span in guinea pigs. Rowland (1961) and deficiency on blood flowing to the ovarian and any imbalance hormone reaching can create abnormalities on ovary. For example polycystic ovary (PCOS) or cystic follicles, is not merely a disease, but is referred to the sum of some clinical findings. There are lots of dysfunctions witch collectively cause PCOS (Speroff *et al*., 1999). Cystic ovarian disease is common in dairy cattle and swine (Najati *et al*., 2006). PCOS is an endocrinologic disorder and is a common causative factor for fertility in women and some animals. And 5-10% of women effected by PCOS during their reproductive live (Najati *et al*., 2006). Study of this syndrome has been started since many years ago. This problem is studied from different points of views because it causes definite or temporary infertility and some other secondary disorders (Stain and Leventhal, 1985). Pervious studies shows that there are four crucial and important positions with disorders in PCOS cases: Ovarian, Adrenal gland, hypothalamic-hypophisis axis and insulin sensitive (responser) environmental tissues (Kabayashi, 2004; Delman and Eurell, 1998). Some disorders for examples torsion of ovarian artery in animals or in medical cases (women) can cause PCOS and it is very important to study this kind of traumatic disorders to understand possible effect of abnormal blood flowing to ovaries.

The object of this study was to determine the requirement for sustained blood flow via the right ovarian artery during normal menstrual activity source on subsequent of ovulation in the effected ovary and compariate it by the non effected ovary. But species differences between animals may be related to the presence of a local venoarterial pathway between the uterus and ovaries and it's role in regulating ovarian function (Ginther, 1976).

MATERIALS AND METHODS

Fifthy 6 female 90 day old rats (*Rattus norvegicus*) were used. The mean±SD weight of rats was 140±1.1 g. Animals were kept in 7 cages with 8 rats in each, are rarred at 22±2 temperature and 12/12 light /dark condition.

Corresponding Author: Alireza Najafpour, Departement of Clinical Science, Faculty of Veterinary Medicine,
Islamic Azad University, Urmia Branch, Urmia, Iran

All of these rats thirty days before test starts separated from male rats. They feed on special rat plate, wheat and tap water. The rats were divided into 2 groups, ie, the treatment (n = 48) and control group (n = 8) rats witch has menstrual cycle of normal length (4-5days) in their privies cycle. The perineum of this rats were observed daily for color intensity (Wehrenberg *et al*., 1977) and based on rapidand consistent decline from peak perineum color, the estimated day of ovulation was determined (Wehrenberg *et al*., 1977). Furthermore, all rats synchronized by vaginal smir. Treatment group anestesiated by ketamin Hcl 5% (Iran- razak) 40 mg kg^{-1} and xylazine 2% (Germany) 5 mg kg^{-1} intra plureal, then rats laparatomized from 1/3 middle line, 2 cm and when the site of ligation detected ovarian artery of right ovary ligated by 0-4 silk and blocked completely (Well, 1999) after surgery on days 8, 14, 21, 30 and 60, the rats were scarified by Co_2 gas in a special device. Both ovaries were dissected out and cleaned. For the histomorphologic investigations of the ovaries were fixed in 10% physiologic formalin solution and processed through paraffin embedding and cut at 5-7 μm (in the form of serial section) with ratary microtome and stained with hematoxylin eosin technique. In the groups under study, number of follicles and relation of healthy, arteries and cystic follicles were assessed appropriately. All the datas were arranged in appropriate tables.

Statistical analyses: Variance analyses of mast cell numbers were calculated by using SPSS program (version: 15) and student t-test method.

RESULTS

In this study observation determined beginning of PCOS on day 21, that type II follicles showed cystic formation. It was significantly obvious ($p \leq 0.01$) on the ligated ovary in the treatment group. No PCOS observed in control group on day 21 after ligation (Fig. 1). This reaction progressed by the time (Fig. 2 and 3). Cystic follicles formation and Artesia progression on follicles was clear on the right ovary (ligated artery) to the ovary in normal condition intact side and in the control group (Fig. 2 and 3). This study showed no corpus luteum and corpura albicantia observed indicating there had been any recent ovulation and by the time it became severs. On day 8 type II follicles showed the start of cystic formation that they had few granulosa cell layer by the vest follicular cavity, by the time passing, this reaction progressed and on day 21 observations demonstrated significantly tipic cystic follicles on the ligated ovary.

Atretic follicles were detected on ligated ovary (Fig. 4).

Furthermore, ovulation significantly ($p \leq 0.01$) declined on the ligeted ovary. There were no morphological differences observed between the ovary vascular intact side and the ovary on the ligated side. Furthermore, numerous primery, secondary, tertiary folliculles were observed by microscopic evaluation of the ovaries that had been removed from left ovary (Table 1). Atretic follicles demonstrated on the ligated side, but no atretic follicle demonstrated on the ovaries in the control group.

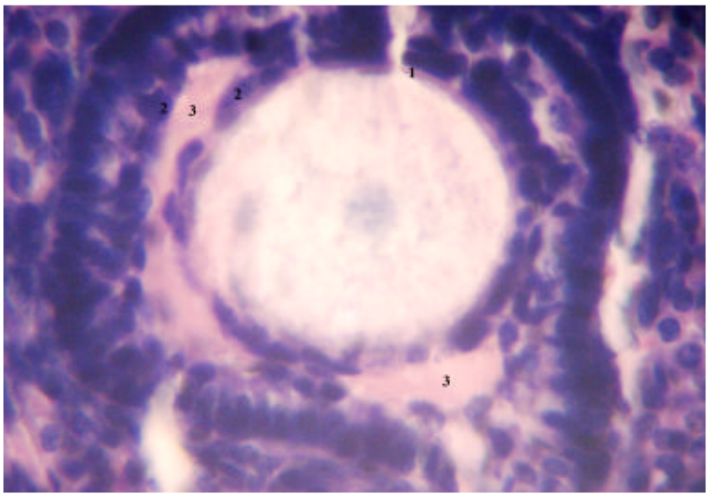

Fig. 1: Follicle type II. this follicle shows beginning of cystic formation. None continues ZP structure and obvious low cell granulosa layer.1) ZP. 2) Granolosa cell layer. 3) Follicular cavity. (Antrum)

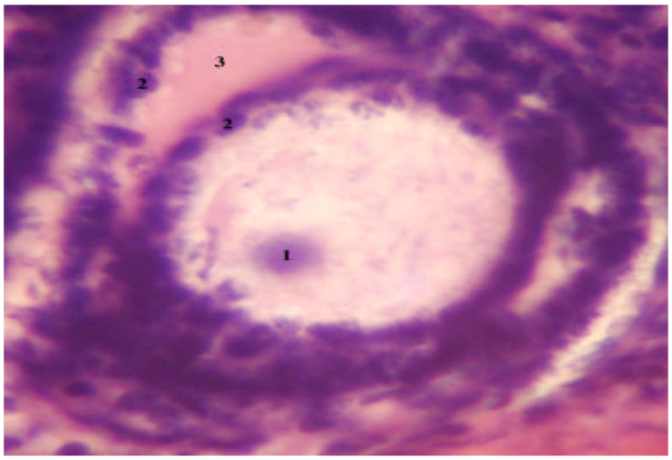

Fig. 2: Cystic follicle generation. 1) Centrifugal nucleus. 2) Granulosa cells 3) Antrum. Follicle shows centrifugal nucleus with despaired ZP and 1 layer granulosa cells. Also this follicle has vest follicular cavity

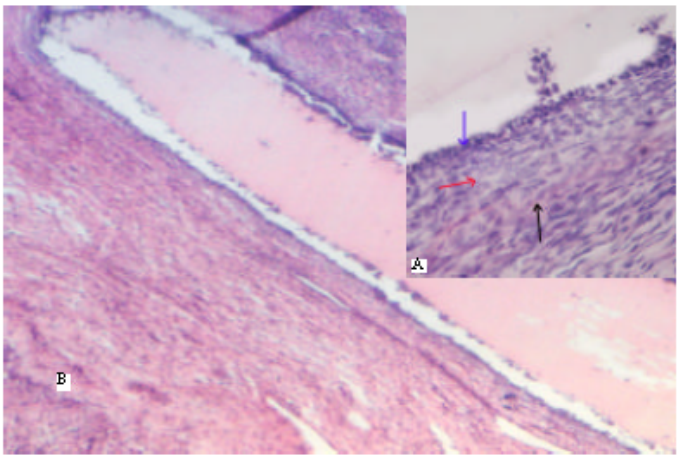

Fig. 3: Cystic follicle. A) showes the obvious low granulosa cells with vest follicular cavity. And developed inner and external tecca layer. ↓granulosa cell layer –inner tecca ↓external tecca(40×) layer B) Cystic follicle (10×).H and E technique

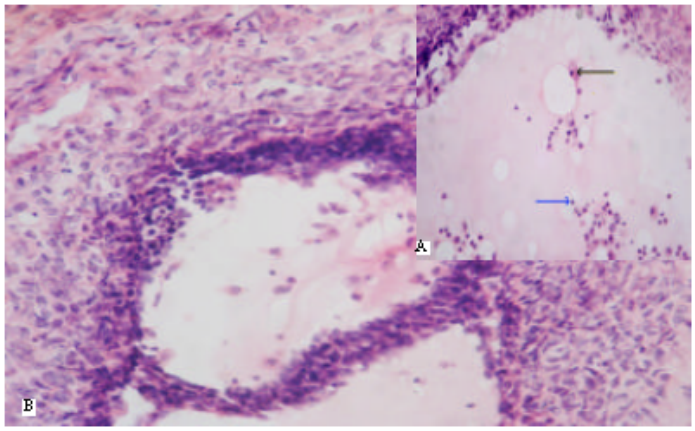

Fig. 4: Atretic follicle. A) Dissociation of granulose cells and floating of granulose cells appearance of macrophages in antrum. ← macrophage. – Granulose cell that dissociated to the antrum. Dis appearance of oocyte (40×). B) Atretic oocyte (10×). H and E technique

Table 1: Comparative means (M±SD) of normal follicles, Atretic follicles and cystic follicles on days 8, 14, 21, 30, 60

Day/Parameter	8	14	21	31	60
Normal follicles	132.05±3.42[a]	130.00±3.33	127.12±3.19[a]	119.12±2.34[a]	100.44±2.24[a]
Atretic follicles	-	-	1.20±1.47[b]	2.48±0.53	3.00±0.64[b]
Cystic follicles	-	-	2.00±0.51[c]	3.90±0.72	4.2±0.23[c]

(a) indicating significant differences between different groups on the same row ($p \leq 0.01$), (b) indicating significant differences between different groups on the same row ($p \leq 0.01$), (c) indicating significant differences between different groups on the same row ($p \leq 0.01$)

DISCUSSION

In the PCOS cases subsequent menstrual cycle, significantly has fewer ovulation in the ovary with the ligated vessel, than the ovarian the vascular intact side (Myrovych, 1975). Wehrenberg *et al.* (1977) observed Significantly fewer ovulations in the ovary with the ligated uterian artery. Our result suggest that this reaction can occur if the ovarian artery ligats completely, Because of PCOS that occurred in our animals. Women with PCOS usually present with clinical symptoms of anovulatory infertility (Atiomo *et al*., 2000). The follicular cysts affect aproximally 7-13% of dairy cows during post partum. As a result the interval parturition-conception becomes significantly longer (Carverick, 1997). This causes important losses in the dairy industry and cattle production in general. On the other hand the cysts are also accompanied by the expression of reseptive sexual behavior (estrus) (Webb, 1998). The rats in this study had not any ovulation after PCOS appearance. Also in this study animals had not any sexual behavior and intersest. The PCOS is a result of malfunction rather than a special or peripheral dysfunction (Chang, 1984; Calogera *et al*., 1987). Our study results show the same thing that if ovarian artery ligates completely cases will have PCOS so they will have anovulatory infertility.

The loss of Ovarian function on the ligated side can not be explained by cellular atrophy, as ovaries appeared normal by histological examination. This fact led us to conclude that an ovarian artery ligation can not completely evaluate the ovarian function because the uterian artery supplies 95% of the ovarian blood flow (Wehrenberg *et al*., 1977). But in the same time blood flowing from uterian artery can not Barrie from PCOS, because the results from histological study showed that on the first menstrual cycle (on day 21 after ligation) type II follicles showed cystic formation. The cystic follicles are seeing with characteristic feature such as 1-2 granulosa cell layers, increased teca cell layer and some time wecan see centrifugal nucleus of the oocyte (Liu *et al*., 1993). Observation in this study demonstrated the same reaction on the type II follicles on day 21 after ligation, that they had few granulosa cell layers by the vest follicular cavity. This follicles showes starting of cystic formation.

By the time passing, this reaction progressed. Various atretic signs such as precocious antrum formation, luteinization of follicular wall, dissociation of granulose cells and floating of granulose cells appearance of macrophages in antrum and around of oocyte, deformation and pyknosis of oocyte and it s nucleuse and in some cases dilation of nucleus of oocyte can demonstrate (Cornver *et al*., 1999). Atretic follicles obviously with these characteristic form were detected on ligated ovary. It seems that PCOS can go to atretic formed follicles. In this study observations demonstrated that rats were going to be fatter than control group because of this fact that in PCOS cases over weighting is a obvious sine that can see. Histological investigation showed that in some cases macrophages were present in the antrum and it seems that they were there to phagocyte destroyed oocyte.

No cystic follicles observed on the intact side. Complete vascular competence is also necessary in rats for normal ovarian function (Peppler, 1976). There were no morphological differences observed between the ovary vascular intact side and the ovary on the ligated side. Furthermore, numerous primery, secondary, tertiary folliculles were observed by microscopic evaluation of the ovaries that had been removed from left side and this can suggest that if blood flow to the ovary present normally so ovaries can have normal physiologic pathway for ovulation and folliculate.

CONCLUSION

This study included that ovarian artery ligation can create abnormal hormonalreaching to the ovaries, so this process can cause PCOS in rats. Ovarian artery plays critical role to reach hormones to ovary if it ligates or in medical field any torsions occurs in ragion(one of the reasons of PCOS) can cause PCOS in animals. In PCOS animals follicular physiologic pathway will be affected and there were no ovulation in this patients.

ACKNOWLEDGMENT

The authors wish to thank Prof. Rajabali Sadrkhanluo for helpful discussions. The authors thanks Dr Abas Ahnadi for preparing animals and laboratory process in this study. We also thank the staff of Histology and

Embryology, especially Mr.Karimi of the collage of Veterinary medicine of Urmia University for helping us to perform this study.

REFERENCES

Atiomo, W.U., J. Condon, O. Adekanmi, J. Friend, T.J. Wikin and A.G. Prenice, 2000. Are women with polycystic ovary syndrome resistant to activated protein C? Fertil. Steril., 74 (6): 1229-1232.

Calogera, A.E., M. Macchi, E. Vacari, A. Mongioi, V. Montanini, F. Coniglione and C. Sipione, 1987. Dynamic of plasma gonadotropin and sex steroid release in poly cystic ovarian disease after pituitary ovarian inhibitor with an analogue of gonadotropin-releasing hormone. J. Clin. Endocrinal. Metab., 64 (5): 980

Chang, R.J., 1984. Ovarian steroid secretion in poly cystic ovarian disease. Seminars Rep. Endocrinol., 2: 244.

Carverick, A.K., 1997. Ovarian follicular cysts in dairy cows. J. Dairy Sci., 80: 995-1004.

Conver, C.A., C. Oxvi, M.T. Ovargaard, M. Christiansen and L.C. Giudica, 1999. Evidence that the insulin-like growth factor binding protein-4 protease in human ovarian follicular fluid is pregnancy associated plasma protein-A. Clin. Endocrinol. Metab., 84: 5-47.

Delman, H.D. and J.A. Eurell, 1998. Text Book of Veterinary Histology, Technique. 5th Edn. Williams and Wilkins, pp: 247-270.

Fleck Well, 1999. Text Book of Small Animal Surgery and Radiology. Philadelphia Sunders. 9th Edn. pp: 506-511.

Ginther, O.J., 1976. Comparative anatomy of utero-ovarian vasculature. Scope, 20: 1-17.

Kabayashi, A.J., 2004. Laparoscopic Uterine Artery Ligation for Treatment of Symptomatic Adenomyosis. Ame. Assoc. Gynecologic Laparoscopists, 9: 293-296.

Liu, X.J., M. Malkowski, G.F. Erikson, S. Shimasaki and N. Ling, 1993. Development of antibodies to rat insulin-like growth factor binding protein (IGFBP-2-6). Analysis of IGFBP production by rat granulosa cells. Endogrinol., 132: 83-1176.

Myrovych, D.I., 1975. influence of individual and combination ligation of the uterine artery and the uterine vein on the internal genital organ and the effectiveness of the operation. Pediator Akush. Ginekol., 6: 39-43.

Najati, V., R. Sadrkhanlou and S. Hasanzadeh, 2006. Histochemical study of estradiolvalarate-indused poly cystic ovary syndrome in the rat, Iran. J. Vet. Res., 7 (17): 68-76.

Peppler, R.D., 1976. Effect of uterian artery ligation on ovulation in the rat. Anat. Rec., 184: 183-185.

Rowland, I.W., 1961. Effect of hysterectomy at different stages in the life cycleof the corpus luteom in the guinea pigs. J. Obst. Gyn., 2: 341-350.

Stain, L. and K.L. Leventhal, 1985. Influence of human menopausal gonadotropin on the recruitment of human ovarian follicles. Ferti-steri., 54: 848-852.

Speroff, L.H., R.G. Glass and N. Kase, 1999. Clinical Gynecologic Endogrinology and Infertility. 6th Edn. copy right ©Lippincott Williams and Wilkins, pp: 487-522.

Webb, R., 1998. Dynamic and etiology of ovarian follicular cysts in post-partum dairy cattles. Repro Domestic Anim., 33: 233-288.

Wehrenberg, W.B., D.P. Chaichareon, D. Dierschke, J. Rankin and O.J. Hand Ginter, 1977. Vascular dynamics of the reproductive tract in female rhesus monkeys: Relative contribution of ovarian and uterine arteries. Biol. Reprod., 17: 148-153.