Effect of Prolactin Hormone on Reproductive activity and some physiological parameters of Rabbits

Hassan F. Kashef Al-Ghetaa[1@], Rand T. Yaseen and **Amira K. Mohammed[2]**

[1]Deptartment of Veterinary Surgery and Obstetrics; [2]Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad – Iraq

kashifalkitaa@covm.uobaghdad.edu.iq

Accepted on 24/2/2013

Summary

The objective of the present study was to determine the influence of prolactin hormone on reproductive activity of female rabbit. Study was conducted on the laboratory animal house and it depended on fifteen mature rabbit does and one mature buck housed on a special cage separately. The females were divided randomly into two groups; the first group is Control group: Female of this group (n=7) were administrated 2 ml of normal saline orally /day. second group is treated group: Females of this group (n=8) were received orally 5 mg of Parlodil® (Bromocriptine) daily for 5 consecutive days then stopped one week and repeated in the same schedule. All animals were clinically observed for detect any drug reaction and signs of sexual behavior. Vaginal smears were taken to determine any changing. The results of the study revealed: Emerge of estrus at the third dose of treatment in five does (First course) and conceive with a male and three of them had been pregnant these pregnant does were aborted in post treatment period (after the end of the second course of treatment). The results of vaginal smears showed significant increase ($P<0.05$) in percentage of intermediate epithelial cells in treated group in comparison with control group simultaneously with significant decrease in superficial component. While the parabasal cells not showed differences between groups, but there was a significant decline in percentage via treating period in comparison with other study periods in treated group female. The treatment does not affect significantly on the blood parameters involved in this study except white blood cells count which decreased significantly in treated group during post treatment period while prolactin hormone concentration in treated group sera was dropped significantly immediate with starting the administration of parlodil. In conclusion, the Prolactin hormone have a role in maintenance of corpus luteum in rabbits and the treatment with dopamine agonist (Bromocriptine) leads to loses of the pregnancy. The variation of the epithelial component of the vaginal smear has no value in detection of sexual activity for the rabbits.

Keywords: Rabbit, reproduction, Bromocriptine, prolactin hormone, blood picture.

Introduction

By the end of the 19th century, rabbits were mainly considered as laboratory animals in research and in 1976 it was a new idea to consider the rabbit itself as the main subject of the studies. Rabbit breeding became a true agricultural activity with the same status as beef cattle or poultry production (1).

Prolactin is a protein hormone of the anterior pituitary gland that was originally named for its ability to promote lactation in response to the suckling stimulus of hungry young mammals. It is not only synthesized in the pituitary gland, as originally described, but also within the central nervous system, the immune system, the uterus and its associated tissues of conception, and even the mammary gland (2 and 3). Moreover, its biological actions are not limited solely to reproduction because it has been shown to control a variety of behaviors and even play a role in homeostasis. Prolactin-releasing stimuli not only include the nursing stimulus, but light, audition, olfaction, and stress can serve a stimulatory role. Prolactin is known to regulate diverse physiological functions via

its effects on cellular processes such as proliferation, differentiation, and cell survival. All these activities are mediated by the PRL receptor (PRL-R), a member of the hematopoietin cytokine receptor superfamily (4). The actions of PRL on luteal function depend on species and stage of oestrous cycle in rodents can either luteotrophic after mating or luteolytic in absence of mating stimulus. (5). PRL responsible for the maintenance of corpus luteum in association with pregnancy in rodents. On reproductive behavior, effect on reproductive behavior, e.g. PRL suppresses stereotypical male sexual behavior in rats and sheep. Prolactin act in maintain enzyme levels and protein synthesis, the effect of PRL on some enzyme is enhanced by glucocorticoids. PRL also act to prevent cell death (apoptosis) of mammary epithelium.(6 and 7).

Bromocriptine (Parlodel)® is an ergotamine derivative, a dopamine receptor agonist that prevents the release of prolactin from the anterior pituitary gland (8). Bromocriptine has been used to examine the neuroendocrine mechanism of dopamine that controls prolactin secretion in vivo (9).

The objective of the present study was to determine the influence of prolactin hormone on cyclicity of female rabbit, through the clinical observation and evaluation of the exfoliated cellular content of the vaginal lumen, and to detect the influences of bromocriptine on some physiological parameters represented by blood picture examination.

Materials and Methods

This study was conducted on fifteen mature does and one mature buck-rabbit, housed in closed controlled temperature cages. The average weight of animals was 1700gm. All animals had leaved about seven days for acclimation, and animals were examined generally to ensure clinically they were disease free. All animals were fed on concentrated and green ration, while the water was *ad libitum*.

All does were examined for pregnancy by abdominal palpation, four of does were pregnant. The buck was housed separately while all does grouped randomly into 2 groups as a follow:

Seven does were served as control animals (C) administrated orally 2 ml of normal saline only by syringe. Eight does were administrated orally a bromocriptine (P) dissolved in 4 mls of normal saline, in dose of 5mg animal^{-1} day^{-1} continual to 5 consecutive days, then the treatment arrested about 7 days, followed by repeating of an administration of bromocriptine in same schedule (10 and 11).

All does of 2 groups were exposed to the male to record emerge of estrus signs, this detection were done periodically every day. Each doe showed estrus behavior was allowed to breed with the buck. Blood samples (5 ml) were collected from all does twice, directly from the heart, each sample divided into two unequal parts once preserved in heparinized tube used to blood picture estimation, while the others preserved in non-heparinized tubes for serum harvesting which needed to investigate the effect of bromocriptin on the prolactin hormone levels in sera of treated does.

Prolactin hormone concentration was estimated by Radio-Immuno Assay method (RIA) according to (12). The vaginal swab was made for all does repeated six times during the study according to (13). The vaginal smears were examined at magnification of 100X and the cells classified into superficial, intermediate and parabasal epithelial cell as described by (14).

Erythrocytes count and Leukocytes count were determined by using hemocytometer method (15); Hemoglobin concentration (Hb) determined by Sahli's method (16) and packed cell volume (PCV) estimated by micro – hematocrit method (17).

The percentage composition of the different types of epithelial cells in each smear was presented with stack bars for individual animals in each group. The mean percentage composition of each cell type was also compared between the study groups using one way ANOVA model (18).

Results and Discussion

The clinical observation was taken daily to report any sexual behavior emerge on the animal or any reflection of the administrated drug on the animals. One pregnant doe was aborted during the first treatment course (after received 15mg of bromocriptin). In the treated group the clinical signs of estrus (catarrhal discharge, restlessness and congestion of the vaginal mucosa) were observed on five does (at the end of the first course) of treatment and allowed to conceive with the male. In the gain of treatment in a second course three of five previous conceived does were aborted during the post treatment period (third week of pregnancy). These observation come to match the findings by other studies (6) which explain that PRL responsible for the maintenance of corpus luteum in association with pregnancy in rodents and actions of PRL on luteal function depend on species and stage of estrous cycle in rodents can either luteotrophic after mating or luteolytic in absence of mating stimulus (5). This maybe explains the abortion of pregnant does.

In addition, Naloxon (Opiate antagonist) administration to lactating rabbits resulting in atonic inhibition of LH secretion leading to reducing of female reproductivity (19). In rats the suckling stimulus suppresses the number of GnRH pituitary binding sites and the degree of suppression is directly related to the intensity of the suckling stimulus (20). It has been suggested that duration and frequency of suckling are not stabilized during the first postpartum day, even in multiparous does and it could be possible that prolactin response observed on day 10 postpartum to be higher than response observed on first few days post-partum (21). Also, Theau-Clement and Roustan (22) reported similar ovulation rate in lactating and non-lactating full-fed females mated 3 days post-partum, whereas on day 10 postpartum, ovulation rate was lower in lactating does.

On other hand (23) found a particularly strong antagonism between lactation and reproductive functions in non-receptive does, the antagonistic effect represents a major problem since the intensive production in general use requires does to be inseminated during the first phase of lactation (from 0–11 days postpartum), it should be emphasized that with natural mating, the negative effect of this antagonism is hidden.

The sera levels of prolactin hormone were dropped significantly ($P<0.05$) in post treatment period in serum of treated rabbits in comparison with pretreatment period and control group at a same time (table,1).

Table, 1: Prolactin hormone levels (ng/ml) in sera of experimental groups (M±SE)

	Control group (C)	Parlodil group (P)
Pretreatment	**A 26.05±1.10**	**A 26.43±0.60**
Post treatment	**A 27.14±0.98**	**B 8.75±3.22**

The different capital letters indicate significant differences ($P<0.05$) between periods (one column).

The concentration of prolactin hormone (normal value) is agreed with that recorded by Rebollar *et al.* (24), the sharp lowering got in prolactin hormone in this study is due to the dopaminic agonist activity of bromocriptine drug which cause inhibition in prolactin hormone secretion (25).

The cytological architecture of vaginal wall composed mainly from superficial, intermediate and parabasal epithelial cells as appeared in every smear accompanied, with negligible numbers of other kinds. In each smear there are not specific pattern for the appearance of the different cell types (26). In present study the superficial cells count approximately close to other in all smears with some insignificant increases or decreases in individual cases. Only 4 of 54 smears were above 25% in bromocriptine treated group, these appeared in smears obtained two – four days post labored does and this results agree with (27).

The mean composition of the different epithelial cells under the three periods of experiment is shown in (table,2).

Table, 2: The epithelial constituent percentage of rabbit vaginal smears (M±SE)

Cell type (%) No of samples=54	Period	Control group (n=7)	Parlodil group (n=8)
Superficial	Pre-treatment	Ab13.2±0.2	Aa14.0± 0.15
	Treatment	Aab13.6± 0.18	Ab13.2± 0.22
	Post treatment	Aa15.2± 1.02	Bc11.5± 0.24
Intermediate	Pre-treatment	Aa53.3± 2.21	Bb50.1± 1.15
	Treatment	Aa54.2± 1.16	Aa53.6± 1.4
	Post treatment	Bb48.3± 2.5	Aab51.5± 1.03
Parabasal	Pretreatment	Ab33.4±1.04	Aab35.8±1.0
	Treatment	Ab32.1±0.9	Ab33.1±1.1
	Post treatment	Aa36.4±1.18	Aa36.6±1.0

The different capital letters indicate significant differences (P<0.05) between groups (one row).
The different small letters indicate significant differences (P<0.05) among periods (one column).

The three levels of contact seem to have no clear cut effect on the appearance of the superficial cells and there were only slight difference between groups. On the contrary, the mean values for intermediate and parabasal cells were significantly (P<0.05) different among three periods in treated group. The intermediate was highest value in treated group during the administration period. Also, the parabasal component of epithelial are the secondary dominate epithelial cells in the smears and majorly appeared in the post treatment period in both groups with significant (P<0.05) elevation in comparison with other previous period.

The superficial cells were in high percentage (P<0.05) in post period in smears obtained from control group while in treated group the levels of superficial were significantly (P<0.05) high levels at the pretreatment period. Unlike, in spontaneous cycler when appearance of superficial cells exceeds the parabasal cells is an indicative of behavioral oestrus, rabbit presents both epithelial cell types in the same slide. It may mean that even when the superficial cells dominate the smear they may not indicate oestrus. In rat proestrus stage is manifested by large number of parabasal cells in the smear and mating may occur at the later end of this stage during which the superficial and cornified cells will start to appear in large number (28). Rabbit mates successfully even when the vaginal lumen is devoid of identifiable epithelial cells (26).

Hematological values revealed insignificant elevation in erythrocytes count, PCV, Platelets count and Hb in blood of rabbits of treated group in comparison with control group; and WBC count were decreased significantly (P<0.05) in blood of treated group; the leucopenia maybe due to the PRL exhibits immune-stimulatory properties (table,3).

Table, 3: The hematological values of experimental groups (M±S.E)

Parameter	Time	Group (C)	Group (P)
RBC Count $X10^6$cell / ml	Pretreatment	A 5.85 ± 0.35	A 5.73 ± 0.31
	Post treatment	A 5.35 ± 0.10	A 5.30 ± 0.35
Hb Concentration gm / dl	Pretreatment	A 9.80 ± 0.40	A 9.70 ± 0.65
	Post treatment	A 9.70 ± 0.57	A 10.01 ±0.37
PCV %	Pretreatment	A 31.50 ± 0.77	A 33.10 ±0.77
	Post treatment	A 34.40 ± 0.67	A 34.0 ± 0.88
WBC Count $X10^3$cell / ml	Pretreatment	A 5.30 ± 0.20	A 5.35 ± 0.70
	Post treatment	A 5.30 ± 0.30	B 4.50 ± 0.2
Platelets Count $X10^3$cell / ml	Pretreatment	A 540.0 ± 0.10	A 539.0 ±2.20
	Post treatment	A 479.66 ± 0.20	A 480.33 ± 1.20

The different capital letters between columns indicate significant differences (P<0.05) within one row.

PRL has been shown to stimulate T cells, B cells, natural killer (NK) cells, macrophages, neutrophils and hematopoietic cells (4).

In conclusions: The administration of dopamine agonist (Bromocriptine) may have a valuable role in inducing of estrus cycle with acceptable conception rate in early lactating does, but sudden lowering of prolactin hormone levels in pregnant rabbit's sera may cause an abortion due to luteotrophic effect of prolactin. The cytological architecture of vaginal wall

doesn't give a sharp specific distribution of epithelial cells during various stages of sexual cycle. This may increase the difficulty in recognition of the estrus phases.

References

1. Lebas, F. (2006). Introduction. In: Recent Advances in Rabbit Sciences. Martens, L. and Coudert, P. (Eds.), Institution for Agricultural and Fisheries Research (ILVO), Belgium, P: 1.
2. Jabbour, H.N. and Kelly, P.A. (1997). Prolactin receptor subtypes: a possible mode of tissue specific regulation of prolactin function. Rev. Reprod., 2: 14-18.
3. Freeman, M.E.; Kanyicska, B.; Lerant, A. and Nagy, G. (2000). Prolactin: structure, function and regulation of secretion. Physiolog. Rev., 80(4): 1523-1631.
4. Yu-Lee, L. (2002). Prolactin modulation of immune and inflammatory response. Recent Progress in Hormone Res., 57: 435-455.
5. McNeilly, A. S; Glasier, A.; Jonassen, J. and Howie, P. W. (1982). Evidence for direct inhibition of ovarian function by prolactin. J. Reprod. Fert., 65: 559-569.
6. Stabenfeldt, G. and Davidson, A. (2002). Reproduction and lactation. In: Textbook of Veterinary Physiology. Cunningham, J. (Ed). 3rd Ed. Saunders Company. Philadelphia. P: 389.
7. Troedsson, M. and Madill, S. (2004). Pathophysiology of reproductive system. In: Veterinary Pathophysiology. Dunlop, R. and Malbert, C. (Eds). 1st Ed. Blackwell publishing Ltd. Iowa. USA, P: 213.
8. Howland, R.; Mycek, M.; Harvey, R. and Champ, P. (2006). Drugs affecting the C.N.S. In: Lippincott's Illustrated Reviews: Pharmacology. Howland, R.; Mycek, M.; Harvey, R. and Champ, P.(Eds). 3rd (ed). Lippincott Williams and Wilkins. Philadelphia. USA, P: 91.
9. Tindall, G.T.; Kovacs, K.; Horvath, E. and Thorner, M.O. (1982). Human prolactin – producing adenomas and bromocriptine: A histologic, immune cytochemical, ultrastructural and morphometric study. J. Clinic. Endocrin. Metabol., 55: 1178-1183.
10. Singh, M. and Ludri, M. (2000). Plasma prolactin blood metabolities and milk production in bromocriptine treated in crossbred goats. Small Rumin. Res., 35(3): 255-262.
11. Singh, M. and Ludri, M. (1999). Immediate effect of bromocriptine on plasma hormone concentrations during early lactation in crossbred goats. Small Rumin. Res.; 31 (2):141-149.
12. Berga, S. and Dainiels, T. (1991). Use of the laboratory in disorder of reproductive neuroendocrinology. J. Clinic. Immunoassay,14 : 23-38.
13. Goldman, J.M; Murr, A.S. and Cooper, R.L.(2007). The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies. Birth Defects Research (Part B), 80: 84-97.
14. Bowen, R.(1998). Classification of vaginal epithelial cells. *http://arbl.cvmbs.colostate.edu/hbooks/pahphys/reprod/vc/cells.html*.
15. Archer, R.K. (1965). Hematological techniques for use on animals; Blackwell Scientific Puplication Oxford, PP:37 – 44.
16. Coles, H.E. (1986). Veterinary Clinical Pathology; 4th Ed. Saunders Company, Pheladelphia, PP: 12 – 14.
17. Viter, F.E.; Turn, V.D. and Guzman, M.A. (1992). Normal hematological values in animal. J. Hematology, 23: 189 – 204.
18. SAS. (2010). SAS / STAT. Users Guide for personal Computers. SAS Institute Inc. Cary, N.C., USA.
19. Marongin, M. and Gulinati, A. (2008). Opioid inhibition of the pulsatile Luteinizing hormone release as assessed by naloxon treatment in the lactating rabbit. In: 9th World Rabbit Congress, Verona – Italy, P:116.
20. Smith, M. (1984). Effects of the intensity of the suckling stimulus and ovarian steroids on pituitary gonadotrophin – releasing hormone receptors during lactation. Biol. Reprod., 31: 548 – 555.
21. Coureaud, G.; Schaal, B.; Coudert, P.; Rideaud, P.; Fortun-Lamothe, L.; Hudson, R. and Orgeur, P. (2000). Immediate postnatal suckling in the rabbit: its influence on pup survival and growth. Reprod. Nutr. Develop., 40: 19 – 32.

22. Theau-Clement, M. and Roustan, A. (1992). A study on relationships between receptivity and lactation in the doe and their influence on reproductive performances. J. Appl. Rabbit Res., 15: 412 – 421.
23. Castellini, C., and Lattaioli, P. (1999). Effect of motile sperms inseminated on reproductive performance of rabbit does. Anim. Reprod. Sci., 57: 111 – 120.
24. Rebollar, P.; Milanes, A.; Esquifino, A.; Millan, P. and Lorenzo, P. (2004). Plasma oestradiol prolactin in synchronized multiparous rabbit does. Proceedings – 8th World Rabbit Congress, Puebla – Mexico, PP: 330–335.
25. Alvarez, P.; Cardinali, D.; Cano, P.; Rebollar, P. and Esquifino, A. (2005). PRL daily rhythm in suckling female rabbits. J. Circadian Rhythm, 3:1-6.
26. Kunde, M. and Proud, T. (1929). The ineffectiveness of vaginal smears in predicting the estrus cycle in the rabbit. Am. J. Physiol., 88: 446-452.
27. Ola, S. and Oyegbade, M. (2008). The influence of different contact levels with male on the vaginal cytology in rabbits under the tropical humid condition. In: 9th World Rabbit Congress. Verona, Italy, PP: 417 – 422.
28. Long, J. and Evans, H. (1922). Cited by Kunde, M. and Proud, T. (1929). The ineffectiveness of vaginal smears in predicting the estrus cycle in the rabbit. Am. J. Physiol., 88: 446-452.

تـــأثيـــــر هرمـــــون البرولاكتيـــــن في النشاط التناســـــلي وبعـض المـعايـير الفسلجيـــة للارانـــــب

حســن فلاح كاشـف الغطـاء[1] و رنـد طــه ياسيــن و اميــرة كامــل محمــد[2]
[1]فرع الجراحة والتوليد، [2]فرع الفسلجة و الادوية، كلية الطب البيطري، جامعة بغداد، بغداد – العراق

الخلاصة

أجريت الدراسة الحالية في البيت الحيواني في كلية الطب البيطري، جامعة بغداد . شملت الدراسة خمسة عشر من اناث الارانب ناضجة جنسيا و ذكر ارنب واحد ناضج جنسيا في اقفاص خاصة لتربية الارانب مع عزل الذكر في قفص خاص لوحده ، قسمت الاناث إلى مجموعتين: مجموعة السيطرة (C, n=7) عوملت اناث هذه المجموعة 2 مل من المحلول الفسلجي فقط عن طريق الفم طيلة مدة التجربة. مجموعة المعاملة (P, n=8) جرعت اناث هذه المجموعة عقار Parlodil® (Bromocriptine) وبجرعة 5 ملغم لكل حيوان يوميا ولمدة 5 ايام متوالية ثم قطع العلاج لمدة اسبوع و اعيدت الجرعة نفسها للمرة الثانية وللمدة نفسها. وضعت الاناث تحت المراقبة السريرية اليومية لتسجيل اي نشاط جنسي او تفاعل عكسي مع العقار. واخذت مسحات مهبلية من الاناث بواقع ستة مرات في الفترات قبل، أثناء و بعد العلاج لمتابعة التغيرات الخلوية الحاصلة في بطانة المهبل وعلاقتها مع حالة الانثى السريرية .اظهرت نتائج الدراسة استحداث الشبق في 5 من االاناث المعاملة بعد اليوم الثالث من بدء العلاج (الدورة الاولى). بعد التاكد من ظهور الشبق تركت الاناث مع الذكر وحصل الحمل في 3 اناث منها و بعدها اجهضت الحوامل عند معاملتها مرة ثانية بمادة البروموكربتين. اظهرت نتائج المسحات المهبلية حصول زيادة معنوية ($P<0.05$) في نسبة الخلايا الظهارية المتوسطة Intermediate في المسحات المهبلية للمجموعة المعاملة عند مقارنتها بمجموعة السيطرة في الفترة بعد انتهاء العلاج. وفي المدة ذاتها حصل انخفاض معنوي في محتوى الخلايا السطحية Superficial في مسحات مهبل الاناث المعاملة عند مقارنتها مع اناث السيطرة . اما الخلايا جنب القاعدية فلم يكن هناك اي فرق معنوي في نسبتها عند مقارنتها بين المجموعتين مع حصول انخفاض معنوي في نسبتها في المدد الاخرى لمجموعة الاناث المعاملة. استعمال البروموكربتين لم يؤثر في المعايير الدمية قيد الدراسة واقتصر على انخفاض معنوي في عدد خلايا الدم البيض للمجموعة المعاملة في الفترة بعد العلاج اما هرمون البرولاكتين فقد انخفض معنويا في مصل المجموعة المعالجة مباشرة بعد بدء التجريع بالعقار . يمكن ان نستنتج ان هرمون البرولاكتين له دور في عملية الحفاظ على الجسم الاصفر و ان المعاملة سببت بانخفاضه مما ادى إلى اجهاض الاناث الحوامل. وان زيادة محتوى الخلايا المتوسطة في المسحات المهبلية ممكن ان يشير إلى وجود نشاط جنسي لدى الانثى (شبق) وهذا يفيد في تحديد الشبق في الارانب.

الكلمات المفتاحية: ارنب، تكاثر، بروموكربتين، هورمون البرولاكتين، الصفة الدموية.