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EFFECT OF OXYTOCIN AND BROMOCRIPTINE INJECTION ON THE DURATION OF PSEUDOPREGNANCY AND THE LEVELES OF PROLACTIN, LUTEINIZING AND PROGESTERONE HORMONES IN PSEUDOPREGNANT RATS

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ABSTRACT
The present study was conducted to investigate the effect of oxytocin (OT) and Bromocriptine (BR) on duration of pseudopregnancy (pp) and the levels of prolactin (Prl), luteinizing (LH) and progesterone (P) hormones in pseudopregnant rats. OT was injected twice daily subcutaneously to pseudopregnant rats. This study involved two experiments; the first was designed to study duration of pseudopregnancy before and after treatment with: OT 500 and 600 m. u; OT+Bromocriptine (BR) (500m. u. +0. 5mg) and BR (0. 5mg) alone, at day 6 of pp for 4 days. The second experiment included treatment with the same substances and doses (except OT 600 m. u.) at day 6 of pp and scarified on day 7 (Group 1),day 8 (Group 2) and on day 11 and 12 (Group 3) which was treated with OT only. Results revealed: a significant ($P < 0.01$) increase and decrease in the length of the pp due to injection of OT (500m. u. and BR alone respectively. Hormonal changes revealed mostly a significant ($P < 0.01$) increase in prolactin and progesterone serum levels in OT treated rats, however, a significant ($P < 0.01$) reduction was seen in other treatments. LH levels showed a tendency for a reduction which was significant ($P< 0.01$) in most animals of group 1 and 2. However, a significant ($P < 0.01$) increase in LH concentration in OT treated animals of group 3. These results may indicate that OT has a stimulatory effect on rat s corpora luteal activity i. e. it's a luteotropic. This activity is mediated probably through stimulation of prolactin release from the anterior pituitary gland.

KEYWORDS: Oxytocin, Prolactin, Bromocriptin, Pseudo pregnancy, Luteinizing hormone, Progesterone. This research is apart of PhD.thesis for the first auther.

INTRODUCTION
Although investigators have made several accurate inferences regarding control of fertility and sterility in both male and female animals, several remarkable phenomena in the field of reproductive biology are still far from being clear. Regulation of corpora lutea, function, which is a central organ in female reproduction, is still an issue of controversy between scientists. Nalbandov (1976) indicated that in rodents (mice and rats) prolactin secreted by the anterior pituitary is the main luteotrophic hormone and hence it acquired its other name: luteotrophic hormone (Rothchild, 1981; Murphy et al.,1985; Ueda et al.,1985; Junqueira et al.,1986). In several other animals including domestic animals and man, LH secreted also by the anterior pituitary gland is the main Luteotrophic hormone (Boehm et al., 1980; Rothchild, 1981; Hansel and convey, 1983; Findlay, 1984). Other authors stressed on the fact that both prolactin and LH are needed for normal maintenance of corpus luteal function (De Greef and Zeilmaker, 1978; Boehm et al., 1984a; Austin and Short, 1987). An experimental model has been utilized for studying hormonal regulation of corpus luteum function is the pseudopregnancy. This phenomenon can be induced by sterile coitus, stimulation of the cervix with glass rod or suckling a foster little, in the rat, mice and other species which can be induced due to the initiation of prolonged secretion of prolactin and retention of the corpus luteum (Gunnet and Freeman, 1983; De Greef and Van der Schoot, 1985; Frye and Erskine, 1990; Ganong, 1992).

In such a model, the absence of the conceptus eliminates all hormones normally secreted by the placenta, thus help in providing presence of active corpora lutea which can be more easily studied without pregnancy hormonal complications. On the other hand, oxytocin has lately come to the forefront as a prolactin releasing agent, since its prolactin secretion decreased after the administration of oxytocin antagonists (Arey and Freeman, 1989; 1990,

Egli et al., 2004, Egli et al 2006, Kennett et al., 2009). In addition, once daily oxytocin treated mares had prolonged corpus luteum function (Vanderwall et al, 2012, Keith et al., 2013). Moreover, oxytocin was found to stimulate prolactin release by rat and porcine anterior pituitary cells (Hyde et al., 1987; 1988; Hyde and Ben-Jonathan, 1988; Mori et al., 1990) as well as the pituitary gland of monkeys (Bethea et al., 1994). In addition, the presence of prolactin releasing factor (PRF) in the posterior pituitary and its physiological role have been reported by other investigators (Averill et al., 1991; Ellerkmann et al., 1991; Samson et al., 1990; Porter and Frawley, 1992; Steinmetz et al., 1993; Steinmetz et al., 1994). Furthermore, Kennett and Mckee (2012) hypothesize that prolactin secretion is controlled by a complex network of positive (oxytocin) and negative (dopamine) feedback loops. In view of controversy concerning the actual role of both prolactin and oxytocin in controlling corpus luteal function, the present study was conducted in an attempt to shed some light on the effect of oxytocin injection and the blockade of the surge of prolactin (BR) on the luteal function in the pseudopregnant rats. Therefore, the present study included to study the effect of using. Oxytocin , prolactin inhibitor (BR) or both on the duration of pp and on the serum levels of prolactin, progesterone and LH hormones in pseudopregnant.

MATERIALS AND METHODS

Animals

Virgin female sprague-Dawley rats (Ministery of Health, Baghdad, Pharmacological control laboratory) weighting 200-230 gm (8-12 week old) were used in all the experiments. They were kept in a temperature controlled room (24-28 C) under a controlled light condition (14h. light, 10h. darkness) and received food (standard dry pellets) and tap water *ad libitum*. Vaginal smears were taken daily for at least two weeks to assess regularity of the cycle before animals were used in the experiment. Pseuopregnancy (PP) was induced by mating with vasoctomised males. Vasectomy was performed on adult male rats by mid-line incision in the lower part of the abdomen under sodium pentobarbitone anesthesia. For induction of pseudopregnancy females were kept with the male overnight in the afternoon of proestrus day, next day was considered as the first day of pseudopregnancy. Pseudopregnancy was verified when vaginal plug s was found in the vagina or in the cage. Un mated animals were kept with males at the proper (proestrus) stage of the subsequent cycle to induce pseudopregnancy.

Drugs

The following hormones or drugs were used in the study.

1. Oxytocin hormone (OT) (intertocin S, intervet, Holland) was used in all experiments of this study with a stock solution of 10U.I./ml. The stock solution was diluted with normal saline for the preparation of suitable doses given subcutaneously.
2. Bromocriptine (BR) (Pariodel, Sandoz, Basel, Switzerland). Each tablet contains 2.5 mg of the active substance. Equivalent quantity of tartaric acid (2. 5 mg) was added with few drops of ethyl alchole (70%), the mixture was dissolved under mild heating and then diluted with normal saline and kept in refrigerator before subcutaneous injection.
3. Pentobarbitone (Nembutal pentobarbitone Sodium, ABBOTT, France) was used for anesthesia, by intraperitoneal injection (35. 2 mg /kg body weight).

First experiment

Duration of pseudopregnancy. This experiment was designed to study the effect of different treatments on the duration of pseudopregnancy. We used 35 female rat divided in the following groups.

a. Normal pseudopregnancy: 10 female rats untreated and allowed to reach naturally the end of pseudopregnancy (appearance of proestrus stage). This group was used a base line for determining normal length of pseudopregnancy without any manipulation.

b. Oxitocin-treated pseudopregnant female rats: 25 females were assigned to this experiment and they were further subdivided in 5 groups (each of 5 animals). In each group the drug was injected for a period of 4 days (starting on day 6 of pseudopregnancy) twice a day (10. am and 4. pm respectively). The groups were.

1. Salin injected females (controls).
2. Oxytocin (500 m. u.) injected females
3. Oxytocin (600 m. u.) injected females
4. Oxytocin (500 m. u) + bromocriptine (0.5 mg). injected females . BR was given once a day with the morning injection of oxytocin. Saline was administred with the second injection of oxytocin.
5. Bromocriptine (0.5 mg) injected females. BR was given once a day at 10 p.m., whereas at 4 p.m. saline alone was injected.

SECOND EXPERIMENT

This experiment was designed to study the effect of different treatments on the hormones leveles. Female rats divided in the following groups.

Group I

Rats injected twice daily with following at the morning (10. am) and afternoon (4. pm) and sacrificed 24 later.

a. Subgroup (la): rats (5 animals) injected with normal saline (control).
b. Subgroup (lb): rats (5 animals) injected with oxytocin (500 m. u).
c. Subgroup (lc): rats (5 animals) injected with oxytocin (500 m. u.) + bromocriptine (0. 5 mg) given as in the first experiment.
d. Subgroup (ld): rats (5 animals) injected with bromocriptine (0. 5 mg) alone given as in the first experiment.

Group II

Rats injected twice daily with following at the morning (10. am) and afternoon (4. pm) and sacrificed 48h later.

a. Subgroup (lla): rats (5 animals) injected with normal saline (control).
b. Subgroup (llb): rats (5 animals) injected with oxytocin (500 m. u).
c. Subgroup (llc): rats (5 animals) injected with oxytocin (500 m. u) +bromocriptine (0. 5 mg) given as in the first experiment.
d. Subgroup (lld): rats (5 animals) injected with bromocriptine (0. 5 mg) alone given as the first experiment.

Group III

Rats injected twice daily with following at the morning (10. am) and afternoon (4. pm).

a. Subgroup (IIIa): rats (5 animals) injected with normal saline and sacrified 6 days after beginning of treatment (on day 11 of pseudopregnancy) and served as control.
b. Subgroup (IIIb): rats (5 animals) injected with oxytocin (500 m. u.) and sacrified 7 days after the beginning of treatment (on day 11 of pseudopregnancy).
c. Subgroup (IIIc): rats (5 animals) injected with oxytocin (500 m. u.) and sacrified 7 days after the beginning treatment (on day 12 of pseudopregnancy).

Hormonal radioimmunoassay

In serum we assayed prolactin, Luteinizing hormone and progesterone using Ameriex radioimmunoassay kit (Kodak clinical diagnostics Ltd. Amersham UK). Analyses were performed at Dr. M. Mustafa clinical laboratory. Baghdad. IRAQ.

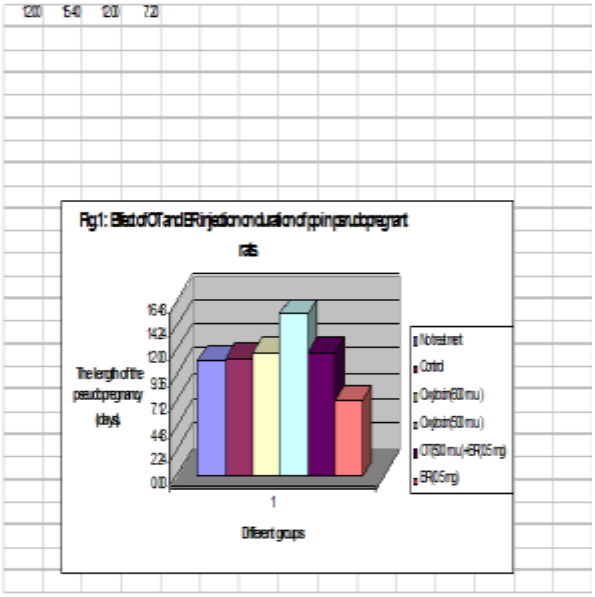
Statistical analysis

The results of this study were analyzed using the general procedures of the statistical analysis system (Gill, 1978). Complete randomized design (CRD) had been performed, and the analysis of variance and test of significance were made. Duncan's multiple ranges test and student "t" test were used to determine the differences between groups and subgroups (Duncan, 1955).

RESULTS

Duration of pseudopregnancy

It was thought, that it will be interesting to determine normal length of pseudopregnancy in the strain of rats used in the study before attempting any treatment. The results showed that this period is 11. 1±0. 875 day. Injection of oxytocin, however alone or with bromocriptine or bromocriptine alone resulted in changes of the duration of pp. Oxytocin injection (500m. u) caused a significant (p<0. 01) increase (15. 4±1. 341day). The higher oxytocin dose (600m. u) failed to induce any significant increase (12±707day) in the length of pp in comparison with control. Bromocriptine (0. 5mg) administered together with oxytocin failed to induce a significant change in the duration of pp in comparison with the control. On the other hand, bromocriptine injection alone given at the same dose caused a significant (p<0. 01) reduction (7. 2±0. 447day) in the length of pp in comparison with all the groups including the controls. Due to the fact that oxytocin injection at 600m. u. did not cause any significant changes in pp length in comparison with the control and lagged behind the lower oxytocin dose (500m. u), the latter one was used in subsequent experimental groups. Also, since administration of bromocriptine terminated pp at 7. 2±0. 447day of pp, groups given oxytocin and scarified after that day had no comparable bromocriptine treated groups. Fig 1.



Hormonal changes

1: Prolactin

Group 1

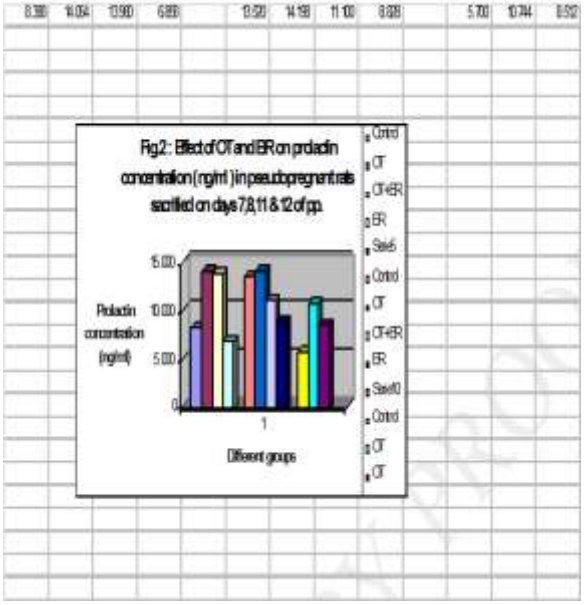
Prolactin concentrations for all groups are presented in Fig. Oxytocin injection caused (P < 0. 01) increase (14. 064 ± 1. 311 ng/ml) in Prl concentration in comparison with control (8. 388 ± 1. 295 ng/ml). BR injection both with OT caused a significant (P < 0. 01) increase (13. 980 ± 1. 281 ng/ml) in Prl concentration in comparison with the control . BR injection alone caused no significant reduction (6. 858 ± 0. 219 ng /ml) in Prl concentration in comparison with the control , and a significant (P < 0. 01) reduction in comparison with animals treated with OT or OT + BR. (Fig. 2).

Group 2

Oxytocin injection caused a non significant increase (14. 198 ± 0. 736 ng/ml) in Prl concentration in comparison with the control (13. 520 ± 0. 818 ng/ml). Oxytocin injection both with BR caused a significant (P < 0. 01) reduction (11. 100 ± 0. 732 ng/ml) in prl concentration in comparison with the control and with animals treated with oxytocin. BR injection alone caused a significant (P < 0. 01) reduction (8. 828 ± 0. 465 ng/ml) in Prl concentration in comparison with all other subgroups including the control (Fig. 2).

Group 3

Both subgroups (3 B & C) treated with OT increased (10. 744 ± 0. 300 ng/ml , 8. 512 ± 0. 786 ng/ml respectively) significantly (P < 0. 01) the Prl concentration in comparison with the control (5. 700± 0. 339 ng/ml) (Fig. 1). On the other hand, comparing animals of different groups with each other showed that the Prl concentration for the control reduced significantly (P < 0. 01) in group 3 in comparison with group 1 and 2. Prl concentration reduced significantly (P < 0. 01) in subgroup (3 c) treated with OT in comparison with the similar subgroups (3b,2b, and 1b) treated with OT . Prl concentration in animals treated with OT + BR reduced significantly (P < 0. 01) in group 2 in comparison with the same treatment in group 1. Prl concentration in animals treated with BR alone increased significantly (P < 0. 01) in group 2 in comparison with the same treatment in group 1 (Fig. 2).



2: Progesterone

Group 1

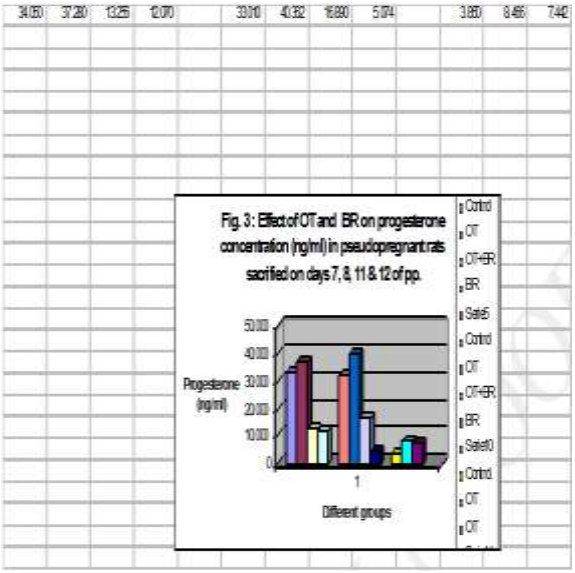
The progesterone hormone concentrations for all groups are presented in Fig 3. Oxytocin injection caused a significant (P < 0. 01) increase (37. 280 ±1. 305 ng/ml) in P concentration in comparison with the control (34. 050± 1. 212 ng/ml). BR injection both with OT or alone caused a significant (P 0. 01) reduction (13. 256 ± 1. 299 ng/ml, 12. 070 ± 0. 997 ng/ml respectively) in P concentration in comparison with the control and with animals treated with OT as shown in Fig. 3.

Group 2

Oxytocin injection caused a significant (P < 0. 01) increase (40. 362 ± 1. 057 ng/ml) in P concentration in comparison with all other subgroups including the control (33. 010 ± 1. 632 ng/ml). OT injection both with BR caused a significant (P < 0. 01) reduction (16. 890 ± 1. 50 ng/ml) in P concentration in comparison with the control and with animals treated with OT+BR injection alone caused a significant (P < 0. 01) reduction (5. 074 ± 0. 972 ng/ml) in P concentration in comparison with all other subgroups including the control as shown in Fig. 3.

Group 3

Both subgroups (3 b & c) treated with OT increased (8. 466 ± 0. 958 ng/ml, 7. 442 ± 1. 202 ng/ml respectively) the P concentration in comparison with the control (3. 850± 0. 358 ng/ml) (Fig. 3.). On the other hand, comparing animals of different groups with each other showed that the P concentration for the control reduced significantly (P <0. 01) in group 3 in comparison with the control in group 1 and 2 . P concentration in animals treated with OT increased significantly (P < 0. 01) in group 2 in comparison with the same treatment in groups 1 and 3. P concentration in animals treated with OT + BR increased significantly (P < 0. 01) in group 2 insignificantly (P < 0. 01) in subgroup (2 c) treated with BR alone in comparison with the similar subgroup in group 1 (Fig. 3).



3: Luteinizing hormone.

Group 1

LH concentrations for all groups are presented in Fig. 4. Oxytocin injection caused a significant (P < 0. 01) reduction (13. 252 ± 0. 728 unit/L) in comparison with the control (16. 662 ± 1. 264 unit/L). BR injection both with OT caused a significant (P < 0. 01) reduction (13. 088± 0. 517 unit / L) in LH concentration in comparison with the control. However, this reduction was not significant in comparison with OT treated animals (1 b). BR injection alone caused a significant (P < 0. 01) reduction (12. 456± 1. 258 unit /L) in LH concentration in comparison with the control , and this reduction was not significant in comparison with subgroups (1 b & c) treated with OT and OT + BR respectively (Fig. 4).

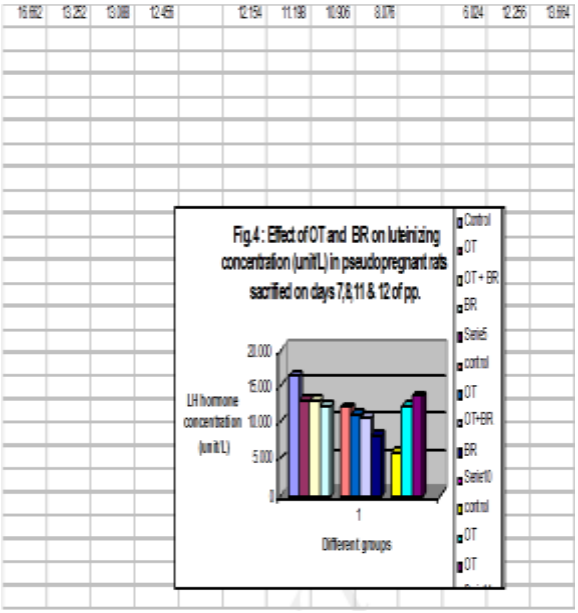
Group 2

oxytocin injection caused a non significant reduction (11. 198 0. 993 unit /L) in LH concentration in comparison

with the control (12. 154± 0. 782 unit / L). Also, a non significant reduction (10. 906 ± 0. 784 unit / L) in animals treated with OT + BR in comparison with the control. BR injection alone caused a significant (P < 0. 01) reduction (8. 076 ± 0. 617 unit / L) in LH concentration in comparison with all other subgroups including the control (Fig. 4).

Group 3

Both subgroups (3 b & c) treated with OT increased (12. 256 ± 0. 735 unit/L, 13. 664 ± 1. 033 unit/L respectively) significantly (P <0. 01) the LH concentrations in comparison with the control (6. 024 0. 603 unit/L) as shown in Fig. 4. On the other hand, comparing animals of different groups with each other showed that the LH concentration for the control reduced significantly (P < 0. 01) in group 3 in comparison with the control in group 1 and 2. LH concentration in animals treated with OT (3 c) in group 3 increased significantly (P < 0. 01) in comparison with similar subgroup treated with OT (2b) and not significantly in comparison with other subgroups treated with OT (3 b & 1 b) LH concentration in animals treated with OT + BR or BR reduced significantly (P < 0. 01) in group 2 in comparison with the same treatment in group 1. (Fig. 4).



DISCUSSION

Duration of normal pp obtained in the study (11. 100± 0. 875 day) is comparable to length of pp reported in other strains of rats which last 12-14 day. (Gunnet and Freeman, 1983). Concerning the effect of different treatments on pp duration, since OT, for instance at a dose level of 500 m. u. caused a significant increase in pp (which is the lower dose) was adopted as the dose utilized in this study. The reason behind the difference in response are not known. However, since the higher dose was less effective than the lower dose, one may speculate that higher dose could have induced desensitization or to down regulation as may be seen in several hormonal treatments . (Yen and Jaffe, 1986). The prolongation of pp as a result of OT injection at a dose of a level of 500 m. u may indicate that it has played luteotrophic role. Other authors arrived to a similar conclusion concerning oxytocin luteotrophic role in human and bovine. (Tan et al, 1982 a, b) and in pseudopregnant rats and mice. (Shaher, 1987; Al-Khadi, 1989). On the other hand, Brinkley and Nalbandov (1963) were unable to alter the length of rat pp by OT injection. Hormonal picture obtained in this study indicates that prolactin serum level (Fig. 2) increased significantly (P < 0. 01) 24 h after OT administration in the level of this hormone was not significant in comparison with the control. Although values of this day are slightly higher than the previous day. The reason behind that, is the fact that prolactin serum level of the control normally increase during this period. Moreove, prolactin concentration increased during the pp in the rat (Garland et al; 1987). The prolactin changes clearly indicate that all results discussed so for are due to OT treatment i. e. prolongation of pp . Also De Greef and Zellmaker (1978) concluded that the disappearance of Prl surges at the end of pp probably caused by the decline in luteal activity in pseudopregnant rats. The end result of this delay in corpora luteal regression is a prolongation of pp. Changes in serum prolactin level obtained in OT treated animals on day 11 and 12 give further support to this assumption since prolactin level in this critical period (coincided with normal termination of pp) is significant higher in OT treated animals in comparison with control. (Fig. 1). Our findings in this study agree with the result of the previous several studies reported about the stimulatory role of oxytocin in prolactin secretion. (Steinmetz et al; 1993, Bethea et al; 1994). However, other authors are unable to confirm the relationship between OT and Prl secretion. (Page et al; 1990). Further support for the prolactin stimulating effect of OT came from the result of BR administration alone , since prolactin level decreased significantly 24h and 48h after its administration in comparison with OT+BR and control. In other words the stimulatory effect of OT on prolactin secretion offset the inhibitory effect of BR. Since prolactin secretion of the control on day 8 of pp was significantly higher than previous day, BR alone was unable to bring down its level to the level of the previous day (Fig 1). The serum prolactin level measured in animals treated with BR both with OT or alone associated with the increased number and percentage of inactive corpora lutea, thus, may be demonstrated the termination of pp obtained in our study. Changes in progesterone serum level conform to prolactin changes discussed and may reflect the same type of changes. It is will known that the main secretory hormone of the corpora lutea in the rat is progesterone. (Plas-Roser et al; 1988). Administration of OT on day 6 caused a significant increase in progesterone serum level on day 7 and 8 respectively in comparison with the control (Fig. 3). This increase conforms to the prolactin increase due to OT treatment registered in the same groups. It may be possible to conclude that progesterone serum level

increase as consequent of OT administration is due to the intense stimulatory effect of prolactin on corpora luteal function. Several studies coincided with our results that prolactin also appeared to be capable of stimulating progesterone secretion by the corpora lutea (McLean et al; 1987). However, high prolactin caused a decrease in progesterone synthesis by the immature rat ovary in vitro (Kelly et al; 1987). Administration of BR alone or in association with OT caused a significant decrease in progesterone level. The result provides still further support for our assumption that changes obtained including progesterone increase are brought about indirectly through OT stimulation of prolactin release which in turn has provided additional support for corpora lutea function. Moreover, blood progesterone declined more rapidly after administration of BR (Taylor et al; 1986). However, progesterone levels were similar in animals treated with BR to those in the control group (Plas-Roser et al; 1988). Luteinizing hormone changes showed that OT or OT+BR had no cleat effect on its level after administration, although it increased significantly on day 11 & 12 after OT administration in comparison with control (Fig 4). This event occurs at a time when prolactin level was at its minimal values. In addition, Evans and Catt (1987) reported that OT was found to stimulate LH from cultured rat anterior pituitary cells. However, other authors claimed that OT has no significant effect on LH release in the rat (shani et al; 1976). Administration of BR alone induced a significant reduction in LH level in comparison with the control. Furthermore, BR treatment decreases the tonic LH secretion (Madhamurthy & Narayana, 1987). However, Boehm and his colleagues (1984) demonstrated that LH concentration increased when the female rats injected with BR on proestrus of the cycle. Moreover, LH injection caused to stimulate corpora lutea progesterone secretion, thus prolongation of the rat cycle by 24 h (Buffler and Roser, 1974). Our findings may indicate presence of interrelationship between these two hormones (Prl & LH), secreted by the anterior pituitary and have more or less similar effect on corpora lutea function (Boehm et al , 1984a; Plas-Roser et al, 1988). Moreover factors regulating prolactin secretion have a tendency for a similar effect on LH release (Medhamurthy and Narayana, 1987). This interrelationship may be supported by other results of our study namely administration of BR alone caused a significant reduction not only in prolactin level but also in LH. Moreover, the increasing in Prl level of OT treated animals was associated with a significant increase in LH level, especially during the day 11 & 12 of pp (Fig 3). In addition, Seki and Nagata (1990) found a significant rise in serum LH following administration of prolactin stimulator during the mid luteal phase in women. In conclusion, results of the present study showed clearly that oxytocin play an important luteotrophic role in the rat, and this role is mediated partly through stimulation of prolactin secretion which was associated with an increase in serum progesterone level, while the LH seem to play a role in this hormonal interaction since this levels increased significantly when pp was prolonged by oxytocin treatment.

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