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Reproductive efficiency of female albino rats after prolactin biosynthesis blockage

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Abstract

Prolactin is known to mediate many physiological functions including lactation, reproductive outcomes and sexual behaviors. Abnormally high levels, referred to as hyperprolactinemia has been associated with sexual disorders and infertility. However, little is known about the effect of blockade of prolactin biosynthesis (resulting in hypoprolactinaemia). This study aims to analyze this effect on the reproductive efficiency of female wistar rats. Prolactin biosynthesis blockade was induced using the dopamine agonist, bromocriptine at a dose of 2.5 mg/day. This was divided into two doses of 1.25 mg/day and was administered at 10.00 and 18.00 hours. The drug was administered for 10 days post acclimatization before mating and was continued until the end of gestation period. After acclimatization, the female rats were grouped into two groups (group 1; those without induction of blockade and group 2; those with blockade) prior to mating. After induction of prolactin biosynthesis blockade, the female rats numbering 32 females were grouped into 4 groups to allow for mating in ratio of 1:2 (1 male to 2 females). Group 1 served as the control group, group 2 (female rats with induction of prolactin biosynthesis blockade. Administration of bromocriptine stopped once pregnancy was confirmed.), group 3 (female rats with induction of prolactin biosynthesis blockade stopping at second week of gestation.) and group 4 (received bromocriptine till the end of gestation). It was noted that the live birth index for all the groups was 100%, mean litter size was noted as 5 pubs for all the groups except the control which was 6 pubs. There were also no still births, abortions or preterm deliveries. These findings may suggest that prolactin biosynthesis blockade did not negatively affect reproductive outcomes in the female rats.

Keywords: Prolactin; Biosynthesis; Blockade; Fertility; Mating; Bromocriptine.

1. Introduction

Prolactin is a polypeptide hormone that is synthesized in and secreted from specialized cells of the anterior pituitary gland, the lactotrophs (Riddle et. al., 1933). Prolactin is a pleiotropic hormone that is able to affect several physiologic functions including fertility as Prolactin receptors (PRLRs) are widely expressed in several tissues, including several brain regions and reproductive organs (Cabrera-Reyes et al., 2017). The effect of prolactin on reproduction however, is dependent on its serum level which is a function of its biosynthesis (Hollian et al., 2020. High level of prolactin in the serum results in hyperprolactinemia, a condition that can lead to menstrual disturbances, estrogen deficiency and testosterone deficiency (Hormone Health Network, 2019). Pituitary tumors, known as prolactinomas, and medications that reduce dopamine can also lead to increased prolactin levels (Majumdar and Mangal, 2015). Low serum level of prolactin results in a condition known as hypoprolactinaemia (Andrew, 2010). This is extremely rare but can take place when prolactin biosynthesis is blocked following administration of certain drugs such as bromocriptine, carbagoline and ergot alkaloid derivatives used in treatment of severe headaches (Bartke, 1986). The synthesis of prolactin is regulated by the hypothalamus via the release of dopamine which inhibits the synthesis of prolactin. Dopamine agonists such as Bromocriptine or cabergoline will inhibit prolactin secretion (Yoest et al., 2014). This results in hypoprolactinaemia, characterized by low serum prolactin levels. This commonly presents in women who after

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pregnancy are not able to produce sufficient milk (Jerome and Robert, 2013). No other proven health effects of low serum prolactin levels have been noted. This study seeks to evaluate the effects of induction of acute prolactin biosynthesis blockade (hypoprolactinaemia) on female reproductive outcomes. Reproductive outcomes are a measure of maternal and neonatal health and include live birth index, still births, abortions, miscarriages, etc. (Mmusi-Phetoe and Thupayagale-Tshweneagae, 2019).

2. Materials and methods

2.1. Experimental design

44 wistar rats of both male and female sex (32 females and 12 males) with reproductive ages between 3-4 months were obtained from the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. They were allowed to acclimatize for a week before the commencement of the study. They were housed in well aerated cages, under room temperature and 12 hours light and 12 hours dark cycles. They were fed with standard feed and distilled water and pellet diet ad libitum. Ethical approval was obtained from the Research and Ethical committee of Basic Medical Sciences, College of Health Sciences (NAU/CHS/NC/FMBS/405). Rats handling and treatments conform to the guidelines of the Nnamdi Azikiwe University Animal Research Ethics Committee (NAU-AREC) for laboratory animal care and use.

This study spanned 8 weeks. After acclimatization, the female rats were divided into two groups prior to mating, for the induction of prolactin biosynthesis blockade (administration of bromocriptine).

- Group 1: this group consisted of female wistar rats without induction of prolactin biosynthesis blockade.
- Group 2: this group consisted of female wistar rats with induction of prolactin biosynthesis blockade.

Following drug administration to group 2 of the female rats, prolactin assay was carried out on some randomly selected female rats from all the groups, to ascertain the prolactin levels of the groups.

The remaining animals were then grouped into 4 groups for mating to take place. These groups are:

- Group 1: 6 female rats without induction of prolactin biosynthesis blockade. This served as the control group.
- Group 2: 6 female rats with induction of prolactin biosynthesis blockade. Administration of bromocriptine stopped once pregnancy was confirmed.
- Group 3: 6 female rats with induction of prolactin biosynthesis blockade stopping at second week of gestation.
- Group 4: 6 female rats with induction of prolactin biosynthesis blockade continuing post mating until parturition.

2.2. Drug treatment (induction of prolactin biosynthesis blockade)

2.5 mg bromocriptine (Parlodel: Meda Pharmaceuticals ilaç Sanive Tic. Ltd. Sariyer/Istanbul) was used to induce blockade of prolactin biosynthesis (hypoprolactinaemia). This was administered to female rats that were induced at a dose of 2.5 mg/day (Faiza et al., 2022). The drug was dissolved in normal saline and administered via an oral gavage. The daily dose administration was divided into two doses of 1.25 mg and was administered at 10.00 and 18.00 hours. The treatment was administered for 10 days post acclimatization before mating was allowed.

2.3. Experimental animals

The animals were weighed at intervals throughout the study and their level of physical activity was observed. The female rats in each group were introduced to the male rats at a ratio of 2:1 and were allowed to remain together for additional 10 days to ensure the females passed through at least 2 estrus cycles. The vaginal opening of the female rats was observed every morning for signs of vaginal plug, which connotes that mating took place.

2.4. Radioimmunoassay of prolactin

After the administration of bromocriptine (parlodel) for 10 days in the test groups prior to mating, female rats were randomly selected from all the groups and ocular puncture was used to collect blood samples into plain bottles. Two samples were collected from each group and they were centrifuged and the serum was collected into plain bottles and stored at -20^{0}. Prolactin levels were determined by ELISA method (Beach et al., 1985) standard curve of 5,10,20,40 and 80 ng/ml PRL concentrations was prepared. Afterwards, 20 µl samples were added to the microplate, previously prepared with Anti-PRL antibody. Samples were analyzed with a microplate spectrophotometer (BIORAD 550) and

absorbance was read at 450 nm wave length, total prolactin concentrations were computed by linear regression fitting using Sigma Plot software (data were reported in ng/ml).

2.5. Determination of pregnancy

Pregnancy in the rats was determined through the following:

- Presence of vaginal plug: this indicates that mating was successful. A day after this was noted as day 1 of pregnancy.
- Body weight of the rat; a steady increase in the weight of the rat shows a progressing pregnancy (Paronis et al., 2015)

2.6. Determination of live births

Live births were calculated for each group using the formula below:

× 100

2.7. Determination of litter size

Litter size per group was determined using the formula below:

Litter size = Number of Pups / Numbers of pregnant female

2.8. Determination of still birth

Still birth is defined as fetal death before parturition. Therefore, any pub delivered without signs of life such as movements, squeaking would be termed as still birth (National Institute of Child Health and Development, 2014)

2.9. Determination of preterm birth

The day after observation of the presence of vaginal plug in the female rat is noted as day 1 of pregnancy. Gestational period in rats ranges from 21-23 days and so, any delivery before the 21st day of gestation was noted as preterm birth.

2.10. Determination of abortion in the female rats

Routine check was carried on the vagina of the female rats daily, to observe if there was discharge of blood prior to delivery. If the discharge was much, it was an indicator that the entire litter was lost, little discharge however showed that a pub or few pubs aborted.

2.11. Statistical analysis

Data collected were analyzed using SPGS version 25 and the results were expressed as mean ± SEM. The statistical significance between the means was analyzed using two-way Analysis of Variance (ANOVA) Turkey multiple comparison post hoc tests to determine the levels of significance between control and experimental groups.

3. Results

Table 1 Prolactin levels in the treated males and females and the control

SEX	GROUPS	PROLACTIN (ng/ml) MEAN ±SEM	t-Value	P-Value
FEMALE	1 (Control)	0.92±0.06	21.67	0.03*
	2 (Treated)	0.63±0.05		

*Indicates Significance at P<0.05

There was a significant reduction in prolactin level of the female rats treated with Parlodel when compared to the control group.

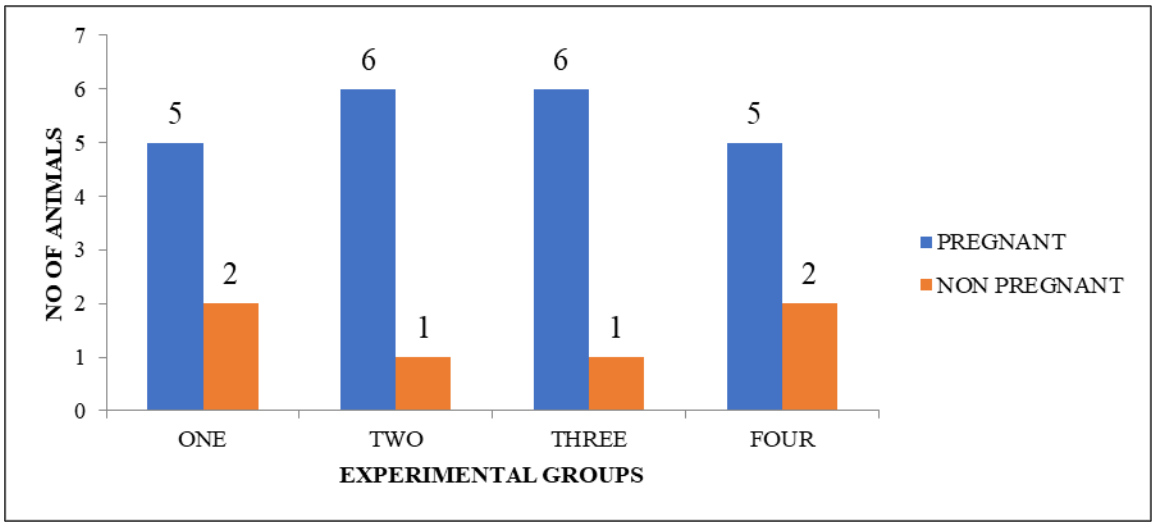

Figure 1 The numbers of the female rats that got pregnant and the ones that didn't get pregnant in the experimental groups

Table 2 Comparison of the number of pubs delivered between the control group and other test groups. P-value of <0.05 considered significant

Test Group	N	Mean± SEM	F-Value	P-VALUE
GROUP 1	6	5.80±0.58	0.53	
GROUP 2	6	4.83±0.83		0.35
GROUP 3	6	4.67±0.76		0.28
GR0UP 4	6	5.40±0.51		0.71

There was no significant difference in the number of pubs delivered, when that of the control group was compared to that of other test groups.

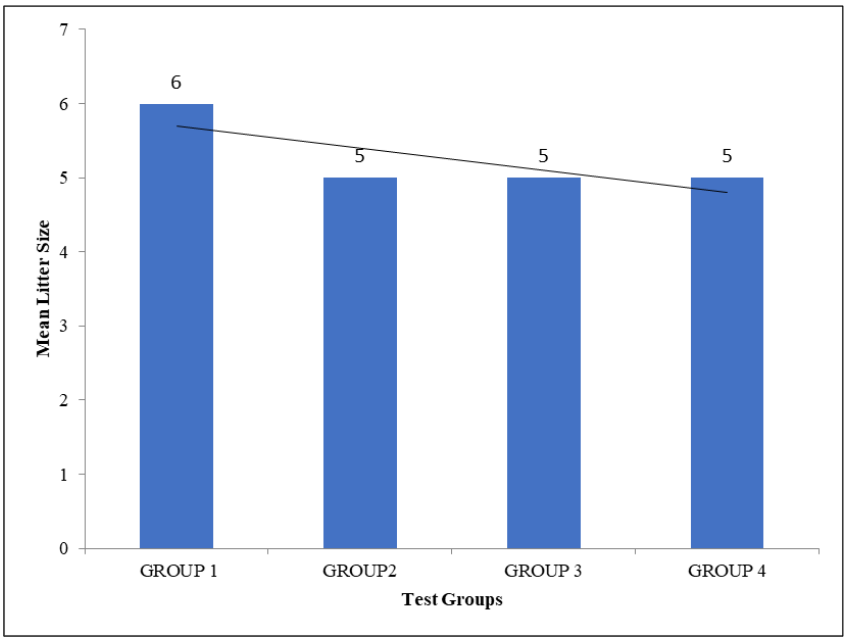

Figure 2 That the mean litter size of all the test groups was 5 except that of the control group which was 6

4. Discussion

An observation made in this study indicated significant reduction in prolactin level of the female rats treated with anti-prolactin drug bromocriptine when compared to the control group. This observation is a confirmation that bromocriptine used in this present study was able to induce prolactin biosynthesis blockage. This is in general agreement with an earlier study which stated that prolactin biosynthesis is blocked, following administration of certain drugs such as bromocriptine, carbagoline and ergot alkaloid derivatives used in treatment of severe headaches (Bartke 1986). In this study, it was also observed that the number of animals that got pregnant were higher than the ones that didn't get pregnant in various groups including the control. This is an indication that the rats used in this study were reproductively sound. This outcome also suggests that blockage of prolactin did not hinder the viability of the female rats. This study also showed that the live birth index of all the groups were 100 percent. This study also observed that the mean litter size of all the test groups was 5 except that of the control group which was 6 (Figure 2). The results still support that prolactin biosynthesis blockade did not possess anti-reproductive properties in the females rats at the drug dose used in this study. This however doesn't agree with a study by Anne and Nadine in 2007 which stated that PRL-/- female mice after mating with males of established fertility, failed to produce any litters despite several mating attempts.

No incidence of abortions and preterm deliveries were observed. This could be as a result of the conducive environment which the rats were kept in (no overcrowding, adequate nesting materials and little or no excessive noise). This agrees with earlier studies done by Govindaraj et al., 2017 and Carmichael et al., in 2007 which stated that maternal stress during gestation has been shown to cause birth defects.

5. Conclusion

Finally, the observations made in this study are interesting from a clinical view point, because the presented data demonstrated that prolactin biosynthesis blockage in female didn't affect reproductive output and this may help in ruling out hypoprolactinaemia as a risk factor for negative reproductive outcomes in sexually active women. However, further research is needed to ascertain the effect of chronic blockage of prolactin biosynthesis on female fecundity.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained from the Research and Ethical committee of Basic Medical Sciences, College of Health Sciences (NAU/CHS/NC/FMBS/405).

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