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Effect of Restraint Stress and Anxietyon Milk Production in Female Westar Rats

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ABSTRACT
There has been study on quality of breast-milk per feeding, exclusive breastfeeding but little or nothing on the effect of stress on milk production. This study was carried out to determine the effect of restraint stress and anxiety on milk production using animal model.20 female and 10 male Wistarrats were used for the study. The female rats were caged with mature males in the ratio 5 females to 2 males for two weeks. On delivery, the female rats were separated into their own cages. Each dam in a cage with adequate water and food. The experimental stressor was restraint stress (RSx1) for one hour on day 21. Rats were restrained in a customized Plexiglas Restrainer for restraint stress. After delivery and prior to exposure to physical restraint stressors, the pups of each dam and the dams were weighed using a digital weighing scale with 0.1g precision. These weights were recorded. The dams from each group were euthanized and their blood samples were collected in a plain test tube for hormonal assay using ELISA technique.The results showed that the rats which were subjected to restraint stress showed an increase in milk production compared to the control group ($P \leq 0.05$). In the control group the milk yield is 51.3ng/ml, which was relatively low compared to that of group four (the group that underwent restraint stress for 18 days) which is 291.7ng/ml. Hormonal assay showed elevations of prolactin and oxytocin level ($P \leq 0.05$).This study suggest the relationship between maternal stress and breastfeeding.

Keywords:
Stress,
Anxiety,
Milk,
Female,
Westar Rats.

INTRODUCTION

According to physiological theories, stress is defined by either a diminished (uncontrollable) neuroendocrine reactivity or an absence of an anticipatory response (unpredictable) (Koolhaas, 2011).Everyday stress triggers a range of physiological reactions in the central and peripheral systems, usually resulting in neuroendocrine reactions (glucocorticoid secretion) via the hypothalamic-pituitary-adrenal (HPA) axis. These physiological reactions are essential for maintaining brain homeostasis and adapting to stress; however, prolonged and exaggerated neuroendocrine reactions to chronic excessive stress are linked to the development of neuropsychiatric disorders, such as depression and posttraumatic stress disorder (Hirotaka, 2019).

Lactation describes as the secretion of milk from the mammary glands and the period that a mother lactates to feed her young, this process naturally occurs with all sexually mature females. Prolactin and oxytocin are the two hormones that regulate lactation, which is governed by the endocrine system (LibreText, 2023).

Prolactin also known as lactotropin or PRL is a hormone that is responsible for lactation, certain breast tissue development and contributes to hundreds of other bodily processes. Its levels are normally elevated in people that are pregnant or breastfeeding. Although prolactin is involved in hundreds of physiological processes, its two main roles are in the development of mammary glands in the breast tissues and the production of milk. Lactose, casein, and lipids are among the components of milk that are synthesized by the breast alveolar epithelial cells when prolactin is present. When progesterone levels are high, such as during pregnancy, the prolactin receptors on breast glandular tissue are down-regulated (Mustafa, 2023).

A peptide hormone called oxytocin is essential for controlling the female reproductive system, especially during labor and nursing. It is mostly made in the hypothalamus, and secreted by the posterior pituitary gland (Amy, 2024). Contracting the myoepithelial cells around the alveoli is caused by oxytocin. This causes the milk to flow through and fill the ducts after it has gathered in the alveoli. The "milk ejection reflex" or the "letdown reflex" are other terms for the oxytocin response.

Physical restraint, one of the most popular methods of

stress exposure in rodent models, involves putting the rats into various restraint apparatuses for a few minutes to several hours every day for a certain number of weeks. Both acute and chronic exposure to restraint stress has been shown to cause a variety of physiological and behavioral changes, including elevated levels of glucocorticoids, body weight loss, altered locomotor activity, heightened behaviors related to depression and anxiety, decreased learning, and memory impairments (Hirotaka, 2019).

The hypothalamic pituitary adrenal (HPA) coordinates and it is in charge of getting the body ready for the fight-or-flight response by coordinating the release of glucocorticoids into the blood from the adrenal cortex. The hyperprolactinemic state of lactation is characterized by a suppression of the HPA axis's reactivity to different stressors (Mustafa, 2023).The hyperprolactinemic state of lactation is characterized by a suppression of the HPA axis's reactivity to different stressors (Torner and Neumann, 2002). The kind and severity of stress have an impact on the prolactin hormone's release (Ranabir and Reetu, 2011), according to data from animal models. A reduced physiological reaction is the outcome of adaptation to the stimulus brought on by repeated exposure to the stressor. Therefore, an acute, unpredictable stress causes an increase in serum PRL levels (Agata, 2023).

When anxious or stressed, the body responds by releasing ACTH (adrenocorticotropin hormone) by the pituitary gland, this in turn will stimulate the adrenal glands to produce the hormone Cortisol. This cortisol provides negative feedback to the pituitary gland so that the prolactin levels in the body will be disturbed (Fatmawati, 2022).

There has been study on quality of breastmilk, quantity of breastmilk per feeding, exclusive breastfeeding but little or nothing on the effect of stress on milk production. This study was carried out to determine the effect of restraint stress and anxiety on milk production using animal model.

MATERIALS AND METHODS

Ethical Approval

The ethical approval was obtained from Bingham University Karu Nasarawa, Nigeria.Batch number BHU 203 was issued.

A total of twenty (20) female and ten (10) male Wistar rats were purchased and kept at the animal housing facility in Bingham university. Regular standard diet feedings were given to the animals. The rats were housed in typical lab settings with a light-dark cycle. The female rats were caged with mature males in the ratio 5 females to 2 males for two weeks. On delivery, the female rats were separated into their own cage. Each dam in a cage with adequate water and food.

The experimental stressor was restraint stress (RSx1) for one hour on day 21. Rats were restrained in a customized Plexiglas Restrainer for restraint stress (INCO, Ambala, India).

The dams from the treatment groups (group 2, 3 and 4) were exposed to the physical restraint stressors for a duration of one hour each for 7 days, 14 days and 18 days respectively. The first group served as a control group and were not exposed to this physical restraint stressors. The female rats were marked and weighed. After delivery and prior to exposure to physical restraint stressors, the pups of each dam and the dams were weighed using a digital weighing scale with 0.1g precision. These weights were recorded. The dams from each group were euthanized and their blood samples were collected in a plain test tube for hormonal assay using ELISA technique.

Data Analysis

Data analysis was performed using IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Oneway ANOVA, and Tukey's tests were used to compare the mean differences between the control group and the test groups. The results were reported as Mean ± SEM. To determine statistical significance, a probability level of 5% ($p<0.05$) was employed.

RESULTS AND DISCUSSION

Results

Table1: Effect of restraint stress on hormonal levels (oxytocin and prolactin)

Group	Prolactin{ng/ml)	Oxytocin (ng/ml)
1	0.82	14.75
2	0.79	17.06
3	0.98	18.37
4	1.02	19.01
P-Value	0.736	0.287

Table 2: Effect Of Restraint Stress On Milk Yield And Prolactin

Groups	Milk yield	Prolactin(ng/ml)
1	51.3	0.82
2	116.4	0.88
3	149.1	1.00
4	291.7	1.02

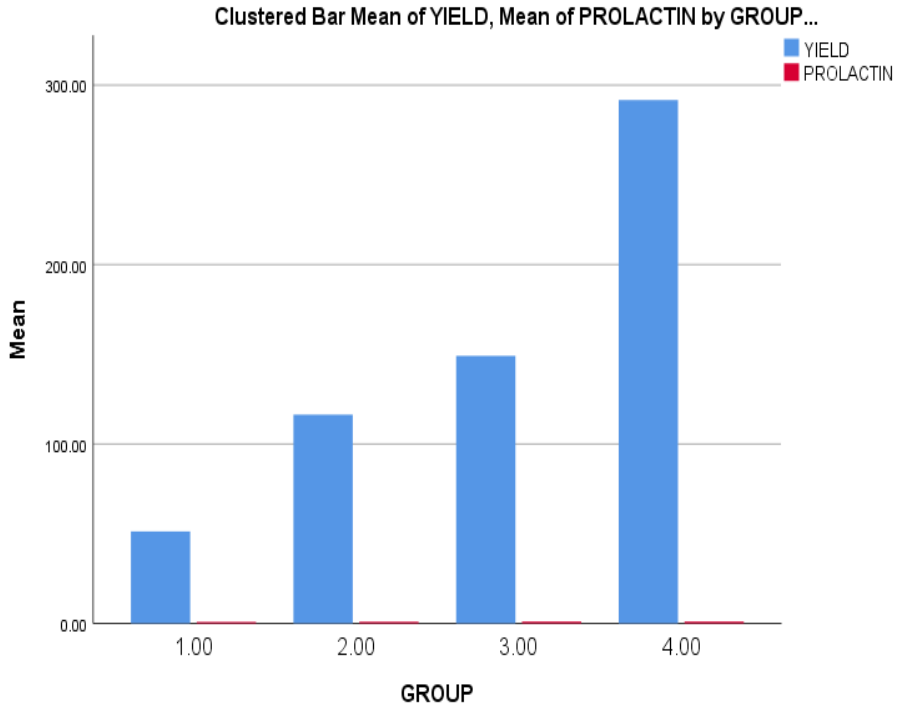

Figure 1: Graphical representation of mean milk yield and prolactin across the groups.

Table 3: Pearson Correlation of milk yield and prolactin across groups

	Milk yield	Prolactin(ng/ml)
	1	0.863
P-value	0.06	0.06

Table 2 above shows a +1 indicating a positive linear relationship between the milk yield and prolactin levels.

The primary aim of this study was to determine how restraint stress and anxiety affects milk production in female Wistar rats. The results showed that the rats which were subjected to restraint stress showed an increase in milk production compared to the control group (P ≤0.05.In the control group the milk yield is 51.3, which was relatively low compared to that of group four (the group that underwent restraint stress for 18 days)

which is 291.7(ng/ml) . Hormonal essay showed subsequent elevations of prolactin and oxytocin level further opposing the connection between chronic stress and milk production. P-value of milk yield is 0.160, for that of prolactin is 0.736(ng/ml) and for oxytocin which is 0.288(ng/ml) , these values are way greater than the p-value of this research which is $P \leq 0.05$.

There was also a +1 (positive correlation) between the milk yield and prolactin levels inferring that acute stress causes an increment in the release of prolactin thereby leading to the increase of milk production.

Discussion

This research revealed that female Wistar rats subjected to restraint stress exhibited a notable increase in prolactin levels and oxytocin levels (which are crucial and important markers of lactation) compared to the control group. This increment was consistent across multiple trials, indicating a robust response to stress. The milk yield also had a significant increase across the groups against the control (116.4 ng/ml, 149.1 ng/ml and 291.7 ng/ml) compared to 51.3 ng/ml). In a similar discovery by Jerome (2023), it was discovered that stress-induced rise in prolactin levels generally results in a doubling or tripling of prolactin levels. Acute exercise has also been regarded as a form of stress and results in an acute, transient increase in prolactin levels (Athanasiou *et al*., 2023).

Several physiological mechanisms may explain the increase in milk production, one of the possibilities is that the stress-induced release of certain hormones, such as oxytocin which might enhance lactation. Stress can trigger the release of oxytocin, particularly in response to the need to care for the offspring, creating a positive feedback loop where stress from the restraint stressor enhance milk let-down. A study conducted by Chantal (2005) observed that the initiation and maintenance of lactation require appropriate hormonal changes and maternal behavior, as such, the relationship with the infant, maternal interest in providing milk, and the demand and needs of the infant.

Chronic stress typically suppresses reproductive functions, but acute stress might have a different effect. In some cases, glucocorticoids can stimulate the synthesis of milk by influencing the metabolic processes that support lactation. Additionally, the activation of the hypothalamic pituitary adrenal (HPA) axis and following release of glucocorticoids could have paradoxical effect promoting milk synthesis and secretion of milk in some conditions (Perani, 2014). These paradoxical effects arise because while chronic stress generally inhibits reproductive processes as well as lactational processes, acute or mild stress may have a totally different effect. The body's response to the stress (acute stress) might prioritize immediate needs, such as breastfeeding an infant, leading to somewhat a temporary increase in lactation-related hormones. Moreover, chronic stress affects body composition by increasing fat accumulation (Stefanaki *et al*., 2018). This may result in a higher concentration of lipids and specific fatty acids in breast milk, because milk fat synthesis partly relies on maternal fat resource

This study tends to be in contrast to most existing literature, which generally reports on a negative impact of stress on lactation. However, little studies have shown that mild or acute stress can most times stimulate lactation in rats, potentially due to the adaptive physiological responses given and aimed at breastfeeding offspring to ensure adequate survival. In addition, the responses to stress varies widely among individuals, influenced by genetics, environmental and psychological factors. This variability means that in some women, stress might enhance lactation, while in others, it might have the opposite effect.

CONCLUSION

This research revealed that female wistar rats subjected to restraint stress exhibited a notable increase in prolactin levels and oxytocin levels (which are crucial and important markers of lactation) compared to the control group. This study results suggests the relationship between maternal stress and breastfeeding may be more complex than previously theorized, asthere was anincreased in milk production.

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