THE EUROPEAN SOCIETY OF MEDICINE
Medical Research Archives, Volume 12 Issue 9

RESEARCH ARTICLE

Breastfeeding's Impact on Postpartum Maternal Immune Homeostasis

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OPEN ACCESS

PUBLISHED
30 September 2024

CITATION
Tyldesley, MC and Bonney, EA., 2024. Breastfeeding's Impact on Postpartum Maternal Immune Homeostasis. Medical Research Archives, [online] 12(9). https://doi.org/10.18103/mra.v12i9.5691

DOI
https://doi.org/10.18103/mra.v12i9.5691

ISSN
2375-1924

ABSTRACT

There exists significant evidence of the beneficial effect of breastfeeding on the neonate, but there is comparatively little data on the effect on nursing mothers. It is said that the positive metabolic and vascular effects of breastfeeding are related to an extension or an amelioration of the adaptive mechanisms generated during pregnancy. However, many such vascular and metabolic effects are related to regulation or dysregulation of the immune system. Because of this, interest in some quarters has turned to the study of postpartum immunobiology. This review focuses on the association between breastfeeding and the postpartum immune system. It examines the role of the immune system in breast development and involution, and the molecular biology and potential role of sex and lactation-related hormones important to breastfeeding in immunoregulation. It further describes animal models that may be used to examine relevant underlying mechanisms. It then explores human observational studies that have examined both local and systemic outcomes of immune system related disease in breastfeeding and non-breast-feeding women. It is hoped that this review will further raise interest in the area and generate detailed examination in both animal models and humans.

Keywords: Breastfeeding, postpartum, immune system

Introduction

There is growing interest in the postpartum period. It is a time of healing, repair and asymptotic achievement of pre-pregnancy status in many organ systems. The postpartum is " the fourth trimester" when mechanisms of poor pregnancy outcomes can be revealed or examined[1], and factors engaged in the beginning of pregnancy may be observed as "the system" returns to homeostatic set points. It is also when future risk assessment can be initiated. In addition, it has been hypothesized that the postpartum period may be a unique period for intervention against ongoing and subsequent risk. In particular, behavioral interventions, e.g., diet, exercise, and counseling have been supported as means to ameliorate future risk[2]. One behavioral intervention which has long been supported is that of breastfeeding.

There is significant evidence that breastfeeding has many benefits to newborns, both nutritionally and immunologically[3-5]. However, evidence also suggests that breastfeeding exerts positive effects on the mother's overall emotional[6,7] and physiological health including those on lipid and glucose metabolism[8], and on tissue specific and systemic vascular biology[9-13]. Data on healthy mothers that thoroughly investigates the postpartum and breastfeeding period is evolving. Existing studies suggest that breastfeeding may contribute to the observed persistence of pregnancy-related adaptations which, in the case of the cardiovascular system, may persist up to one year after birth[14]. The idea that breastfeeding may prolong pregnancy-related immunological adaptation has been suggested, but not fully investigated[15]. A clear picture in this area is perhaps hampered by both a lack of experimental and observational data, but also a classically limited view of maternal immunity[16]. This review is intended as a preliminary exploration of the potential role and importance of breastfeeding in postpartum immune homeostasis.

Background

THE IMMUNE SYSTEM AND THE DEVELOPING BREAST

Some have wondered whether the proteins expressed in the developing breast during puberty or pregnancy might be new to the immune system and therefore a possible target of the immune response[17,18]. To some this might only be true if there was an inherent defect or dysregulation in the development processes of white adipose tissue expansion, neuronal network development, duct epithelial cell turnover and cyclic micro-alveolarization. Evidence suggests that there might be a unique population of immune cells that participate in this process, including eosinophils[19] and macrophages[20] which may participate in clearance of apoptotic debris, modulation of extracellular matrix components, angiogenesis or in important cell-cell signaling pathways. The high expression of TLRs on macrophages in the developing breast make them efficient at inciting an inflammatory response in the face of "opportunistic" infection with local microbes, but this inflammatory response can enhance any dysregulation in breast tissue components. T cells are also present within breast ducts, and data suggests that they may regulate epithelial cell proliferation and duct outgrowth and branching[21]. This regulatory function may depend on activation by local dendritic cells which presumably may present ductal peptide antigens[22]. This raises the interesting possibility of a unique, developmentally programed regulatory T cell pool, as has been suggested for other tissues[23]. One could also hypothesize that populations of regulatory B cells, for example capable of removing "self-antigens" generated by cellular homeostasis[24-28], may also participate in early breast development. These cell populations undergo their own fluctuations due to trafficking, death, proliferation and change of phenotype during pregnancy and lactation.

LOCAL IMMUNITY IN THE INVOLUTING BREAST.

Post weaning, breast alveolar epithelial cells undergo apoptosis and clearance, and adipocytes return to their pre-pregnancy state. Any residual milk products must also be removed. Dysregulation or incompleteness of this process has been associated with defects in the populations of tissue resident macrophages(reviewed in[20]). Macrophages in the involuting breast show an increasingly "alternatively

activated phenotype", including expression of arginase and the mannose receptor, and expression of molecules such as IL-10, which may be "suppressive" but may also contribute to regulation of angiogenesis[29] and wound healing[30]. A population of macrophages in the involuting breast express an immature phenotype, but it is not clear is these are new immigrants as opposed to tissue resident cells[20,31].

Other immune cell subsets experience regulated presence in involuting versus lactating mammary tissue. Dendritic cells in mice stay relatively constant through lactation, increasing at the time of involution and peaking after weaning[32]. They also experience phenotypic changes such as the reduction of molecules such as CD80 and CD86 until weaning. Evidence suggests however that these cells may have a lower capacity to activate naïve T cells. CD4 T cells also increase at involution and weaning. The CD4 T cell population expresses an activated and mixed phenotype of TH-17, TH2 and Treg like cells that evolved through involution. During involution, there may be an accumulation of memory CD4 T cells [32]. Emerging data also suggest the importance of B cells in the involution process[33]. The regulation of these cell types is very important in modulation of tumorigenesis (see below).

SEX HORMONES AS IMMUNOMODULATORY MOLECULES

Lactation produces lower circulating estrogen and progesterone in humans[34] and animals[35,36].

The exact underlying mechanisms are still under study, but likely relates to dysregulated production of luteinizing hormone or gonadotropin releasing hormone and lack of ovulation. Both estrogen and progesterone have immunomodulatory capacity, both in the development and function of immune cells (recently reviewed in[37]). Estrogen signaling has a complex trajectory (reviewed in[38] and elsewhere) with the possibility of binding to two receptors (ERa and ERb) in some species. This itself generates modulation of estrogen's function as the two can form a dimer. Binding of estrogen to its receptor(s) ultimately leads to trafficking to the nucleus where estrogen and its receptor can serve as transcription factors for several genes related to immune cell development and function. In addition, estrogen and its receptor can bind to the mitochondria and have effects related to not only gene expression but generation of ATP and decrease of ROS production. Finally, estrogen may bind to a membrane bound, G protein coupled receptor[39], which may mediate the estrogen signaling pathway by phosphorylating the estrogen bound ERa or ERb and modify the capacity for the estrogen receptor/ receptor complex to act as a transcription factor. Estrogen has also been shown to transcriptionally regulate gene expression not only in T and B cells, but also other immune cells such as dendritic cells[40] especially post activation. Expression of these receptors on natural killer cells[41], and other cells such as eosinophils [42] suggests broad-based regulation in the immune system. The resulting phenotype of estrogen responsiveness is likely complex. For example, estrogen may impair the negative selection of auto-reactive B cells, which could increase autoimmunity. In contrast, estrogen can also decrease B cell lymphopoiesis[43] and increase the expression of cytokines adaptive for the production of antibody responses. These responses can be highly protective of reproductive and gastrointestinal mucosal surfaces while inhibiting certain inflammatory responses which may be detrimental to developing fetal/placental tissues[44-46].

Progesterone signaling in cells may be equally complex as several isoforms of the full-length isoform PR-B (e.g., A, C, M, T,S) have been described (reviewed in[47,48]). PR-A and PR-B are the main isoforms, which bind progesterone, traffic to the nucleus and control gene transcription(reviewed in[49] and elsewhere). The A and B forms may regulate each other's action[50]. The C isoform may bind progesterone, but does not contain an DNA binding domain, which suggests its function is to regulate the effect of circulating progesterone. The M form may be localized to the mitochondria and support progesterone-enhanced function[51], such as

production of ATP. Low-affinity membrane-localized receptors for progesterone exist. These include a "family" of receptors,

(e.g., a, b, d, e,g) that may interact with molecules near the cell membrane which in turn activate signaling pathways and lead to molecular movement to the nucleus. Through these mechanisms, progesterone may indirectly mediate gene transcription at sites other than canonical progesterone response elements[49]. Another class of progesterone binding membrane receptor with similar activity include those receptors with progesterone receptor membrane components (reviewed in[48]). Furthering the complexity in progesterone receptor signaling pathways is the apparent cross talk between these and other steroid hormone signaling pathways[52].

While nuclear progesterone receptors are present in thymic epithelium[53] and their expression modulates the environment in which T cells develop during pregnancy, the expression of such receptors in T cells, is controversial[54,55]. Evidence in different species however suggests that the regulatory effect of progesterone in T cells (e.g., driving generation or regulatory T cells or decreasing activation) may occur via non-canonical receptors[54,56] or conversely via activation of glucocorticoid receptor pathways[57]. By flow cytometry, a small proportion of circulating B cells can express the "inhibitory" nuclear progesterone receptor A[58], perhaps as a method to downregulate inflammatory mechanisms in adverse pregnancies. However, evidence of membrane receptor expression in B cells is lacking[56]. In contrast most B cells express the glucocorticoid receptor[59], raising the hypothesis that crosstalk between this and the progesterone receptor may be a mechanism for progesterone responsiveness in these cells.

Within the immune system, progesterone is thought to inhibit inflammatory immune responsiveness by diminishing pro-inflammatory cytokine production in peripheral blood leukocytes[60]. The mechanism however may be via either direct or indirect transcriptional regulation of some cytokines, including IL-8, IL-6, and chemokines such as CXCL1, and CXCL1/2,[61]. This may occur not only through effects on T and B cells, but also by decreasing the population of innate immune cells, such as neutrophils and NK cells, or by decreasing the function of macrophages and dendritic cells (reviewed in[37]). Given the complexity of the timing and response to decreased progesterone and estrogen in lactating women, it is difficult to assert, based on these hormones, the likely inflammatory status of lactating versus non lactating postpartum women.

BRASTFEEDING HORMONES AS IMMUNOMODULATORY MOLECULES

While the underlying mechanisms are incompletely understood, it is thought that hormones instrumentally involved in the breastfeeding process may regulate the immune system locally (in the breast) and systemically. The two most compelling hormones in this respect are prolactin (reviewed in[62]) and oxytocin, which are in turn regulated by several other steroid hormones.

Prolactin is the hormone that induces synthesis of milk components like proteins and lipids. Prolactin receptors comprise many different membrane and soluble forms. The membrane-bound forms comprise homodimers of several different sizes (e.g., long, intermediate, short) depending on the size of the receptor extracellular or intracellular domain of the specific receptor (reviewed in[63]). Short/long heterodimer forms can be inhibitory. Soluble forms lack transmembrane and intracellular domains. They chiefly mediate the availability and effective local concentration of prolactin but may also form dimers with other receptor forms and regulate their activity[64]. Prolactin receptors are expressed on many cells throughout the immune system, including monocytes and macrophages[65], natural killer cells[66], and T cells[67]. The expression of differing isoforms may be regulated by infectious or inflammatory stimuli. Adding to the complexity of signaling through these receptors is their low-level affinity for other members of this receptor class (reviewed in[68]).

Prolactin's binding to its receptor activates several different signal transduction pathways (e.g., JAK/STAT

and MAPK), including those that induce gene expression and production of pro-inflammatory cytokines and chemokines in epithelial cells but also in macrophages[69]. It also increases expression of the IL-2 receptor, through which it can affect the function of NK cells[70] and the maturation of CD4 and CD8-thymocytes[45]. In addition, prolactin can increase both viability and function in dendritic cells, presumably through regulation of NFKB[71]. Another of the hypothesized effects of prolactin on the immune system is dose-dependent bimodal (e.g., low versus high dose) regulation of the transcription factor T-bet[72], a critical regulator of type 1 immune responses[45,73]. Additionally, a long form of the prolactin receptor has been identified in early bone marrow cells in animal models, suggesting a role in B cell development[45]. It can rescue immature B cells from apoptosis,[74] and inhibits B cell tolerance, while promoting B cell activation, proliferation and differentiation,[74-76]. Through this it increases the generation of autoantibodies and autoimmune disease[45,76-79]. Thus, prolactin is likely a broad-spectrum activator of postpartum immunity.

The neuropeptide oxytocin binds mainly to a G protein-coupled receptor that is found in several tissues of the central and peripheral nervous system. Interestingly, the spinal cord and brain - among other areas of the body - contain oxytocin neurons, which express receptors for immune cytokines including IL-6[80] and IL-1 [81] and respond to cytokine binding with increased release of oxytocin. However, oxytocin itself drives regulation of immunity.

The oxytocin receptor can aggregate to form homodimers or heterocomplexes of two or higher order with other elements (e.g., the dopamine 2 receptor) and this likely regulates its downstream signaling effects (reviewed in[82]). Oxytocin receptors are expressed on a wide variety of tissues and cells in the body, including those that participate in immunity[80]. In dendritic cells, oxytocin signaling may promote a "tolerogenic" phenotype by decreasing some functions while increasing others through activation of the PI3K/AKT pathway[83]. Similarly, macrophages express receptors for oxytocin[84]. Signaling by oxytocin in these cells results in a relative inhibition of NF-kB and decreased expression of molecules such as IL-1b and TNFa while increasing tendency to express indicators of "M-2" polarization such as arginase[84]. Inflammation increases expression of the oxytocin receptor, which then in the presence of the peptide decreases expression of inflammatory cytokines such as IL-6[85]. T cells expressing the oxytocin receptor can respond to oxytocin by increasing intracellular calcium[86]. This could have a wide-ranging effect on T cell activation (reviewed in [87]) or effector function[88]. However, enhanced premature activation within the thymus may support deletion of autoreactive T cells in favor of a mature response to environmental antigens. Though increased oxytocin expression by tumors increases B cell influx[89], evidence that oxytocin directly regulates B cell development of function is limited. That oxytocin release can be increased by inflammatory cytokines, that it can in turn down modulate specific immune cells along, and that its levels fluctuate with breastfeeding suggest it may play a major role in postpartum immunity.

Animal models to examine the role of breastfeeding in the mother

Animal models have been used to delineate causality and specific mechanisms underlying disease processes as well as healthy physiology, and studies of the immune system relative to pregnancy are numerous[90-92]. Studies specifically focused on breastfeeding are far fewer in number. Moreover, many animal studies of the immune system – including those which assess phenotype and activity of immune cells from peripheral blood relative to breastfeeding - compare animal mothers who are currently lactating to those who are not lactating, or to animals who have not been pregnant. Because pregnancy itself modifies the immune system and other elements of maternal physiology, this approach has made it somewhat difficult to specifically delineate the effects of breastfeeding. However, these studies are useful in the process of developing hypotheses

to test in humans and in increasingly refined animal models. What follows is a discussion of some of the approaches to using animal models to address this question.

USE OF BROMOCRIPTINE

One strategy that has been employed to approach this question is the use of bromocriptine, a complex dopamine receptor agonist/antagonist, to prevent lactation. Administration of bromocriptine to dams during the early stages of pregnancy was used to observe treatment-associated postpartum care of their pups; the delineator used was a home cage versus a novel cage along with differential bromocriptine administration, but immune parameters were not investigated[93]. The use of bromocriptine in animal models in studies concerning maternal immunity could enhance experimental design. Rather than using non-lactating, non-pregnant controls one could instead allow all animals to become pregnant and go through parturition and compare those treated with bromocriptine or vehicle control. Such an experiment might be a good parallel for bottle versus breast feeding after pregnancy, though the use of this drug to inhibit milk production is confounded by its regulation of prolactin[94](and reviewed in[95]) which also may influence immunity.

COMPARISON OF EARLY VERSUS LATE TIME POINTS IN LACTATION

One technique used to assess how lactation may affect the immune system of lactating animals is collecting samples from various points throughout lactation, as well as before parturition or after lactation ceases[96]. However, there appears to be no set standard for sample collection which allows for comparison across studies. One study done on dairy cows, for example, labeled animal subjects as being in lactation vs. dry periods to delineate subpopulations of T lymphocytes in mammary gland secretions[97], while another examined peripheral blood samples from postpartum and mid-late lactating animals to delineate CD8+ lymphocyte suppressor function[98]. Another study euthanized pregnant mice on days 8 and 15 of pregnancy and day 8 postpartum to assess the difference in β1,4-Galactosyltransferase expression, an enzyme that increases the number of N-terminal galactose molecules on IgG molecules during pregnancy, potentially influencing their effector function[99]. These techniques offer interesting insight into how the immune system of lactating animals changes postpartum, but do not specifically address the issue of breastfeeding/lactating versus non breastfeeding/nonlactating mothers who have recently given birth.

REMOVAL OF PUPS/FORCED WEANING.

While the literature includes studies in mice wherein the transfer of pups from one mother to another has been used to examine the effect on the pups' immune system, little attention has been paid to the examination of either mother's (biological or foster) immune system. A more informative approach might be to perform an experimental removal of the pups[32,36]. This would allow for the forced halting of milk production and prolactin release, albeit with a lag time of several days if not done immediately after parturition[36]. This method would allow a comparison of lactation to non-lactation but may generate a level of stress that could be a confounder in its own right. Forced weaning and mammary gland involution in mice leads to transient increase in mammary monocytes, macrophages, dendritic cells and T cells, along with complex phenotypic and functional changes suggestive of immune tolerance within the dendritic and T cell pools. Exactly what changes occur with forced weaning in distal sites, such as the spleen are unclear[32].

Removal of the nipples (thelectomy) could significantly inhibit lactation, (as does wearing a tight bra in humans[100]) but is also confounded by changes in behavior and possible other effects in rats[101] and mice[102]. An alternative in other species might be a temporary cessation of milking[103].

Observational studies in humans

Studies of specific phenotype and functional analysis of peripheral blood or local or systemic lymphoid tissue from healthy mothers who exclusively breastfed

their infants, when compared with mothers who exclusively formula-fed are relatively rare. Limited evidence suggests that exclusive breastfeeding is associated with a lower peripheral blood CD3+ cell percentage, but higher serum g-IFN, higher peripheral blood cell response to in vitro stimulation with PHA, and lower IL-10 production by PBMC in response to stimulation at 4-6 weeks postpartum, and lower symptoms of infection, suggesting that breastfeeding may play a positive role in maternal immunity[15]. However, some evidence suggests that in states of chronic infection, it is the postpartum state and not breastfeeding per se, that alters immune and inflammatory parameters[104]. Although studies directly observing breast versus bottle-feeding women is limited, some information may be gained by examining certain pathologic states.

BREASTFEEDING AND RISK OF BREAST CANCER

Breast cancer is the most diagnosed cancer in women in the U.S. and one of the most significant contributors to cancer deaths worldwide[105-107]. Immune system involvement may occur early in tumorigenesis, leading to removal of cancer cells, or late, perhaps decreasing metastasis. The role of the immune system in modifying breastfeeding and breast cancer is likely complex and confounded by diet, hormonal factors, toxin exposure, and other variables such as body weight and vitamin D status[107-109]. Studies suggest that breastfeeding may reduce the risk of breast cancer [106].

Particularly aggressive forms of breast cancer occur in the peripartum period, including cases that are triple-negative for estrogen, progesterone, and HER2 receptors[105,107]. These tumors have a high capacity for metastasis, since they may occupy or utilize the neo-lymphangiogenesis that occurs as the breast is involuting (reviewed in[110]). It is said that the involuting breast environment supports inflammation[111,112] and tumorigenesis,[113]. This environment may depend on mast cell presence[114], and B cell presence, phenotype and function,[33] and on infiltrating macrophage phenotypes, which have an immune "suppressive" phenotype[31], and which drive neo lymphangiogenesis[110]. T cells specific for breast cancer neoantigens have been described, and, moreover, pregnancy in a mouse model has been shown to generate antigen specific T cells that in turn decrease disease[115]. Consistent with this is the relative lack of both CD4 and CD8 T cells in the involuting breast tumor microenvironment[31], and the high ratio of macrophages to T cells. These data may support the thought that the immune system is a link between pregnancy and breast cancer and further supports examination of the role of the immune system and breastfeeding in breast cancer risk.

ALLERGIC DISORDERS

While a large body of literature has investigated the impact of maternal allergy, diet and breastfeeding on subsequent allergic disease in the offspring, little data exists on the effect of breastfeeding itself on maternal allergic disease. While breastfeeding can reveal or precipitate rare extremes of allergy[116], it is currently unclear if breastfeeding changes the course of chronic allergic disease in the mother[117].

AUTOIMMUNE DISORDERS

Autoimmune disorders result from a disruption, either acute or chronic, of tissue homeostasis that leads to an immune response. These disorders primarily affect women of reproductive age. Evidence from several studies is that autoimmune disorders relapse or abate during pregnancy and in the postpartum period recur, flare, or become clinically evident in previously non- symptomatic women[118-125]. This combined with the observations combined with the sexually dimorphic nature of the disorders[126], suggests regulation of the disorders by the sex chromosomes and their gene products.

One particularly devastating but rare pregnancy-related autoimmune disease is peripartum cardiomyopathy[127]. Though a mediator of this disease is thought to be a form of prolactin, there has been interest in the role of breastfeeding modification of the disease due to potential effects on the immune system. Interestingly, data suggests that in women with the disease, breastfeeding

increased prolactin, as expected, but in addition increased circulating CD8 T cells[128]. Circulating CD4 T cells were lower with breastfeeding. Breastfeeding did not significantly affect disease outcome as cardiac function was only slightly higher in breastfeeding versus non-breastfeeding patients[128]. Most data on breastfeeding and auto-immunity, however, comes from observation of only a handful of well-known autoimmune diseases.

Inflammatory Bowel disease is an example of a disease with increased risk for postpartum flares[123], although this may be related to therapy de-escalation[129]. Little data on the role played by breastfeeding in postpartum exists but suggest there may be a protective effect in some diagnoses[130].

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a variety of symptoms that has been shown to worsen during both pregnancy and the postpartum period particularly in patients with active disease prior to pregnancy (reviewed in[131]). There is particular interest in the field on how prolactin, which contributes to SLE pathogenesis and activity, may contribute to the worsening of disease activity[119]. Conflicting data exists. For example, one study of patients early postpartum (6 weeks) suggested that formula alone feeding was associated with higher disease activity as compared to breastfeeding (alone or with formula supplementation)[132], regardless of immune suppressive therapy. However, another study found no significant association[133], though there was observed a trend to increased disease severity in non-breastfeeding mothers at one-year post-partum. Small numbers and important confounders (e.g., smoking[134]) and the effect of time[134] of breastfeeding may blur the association. There moreover may be inherent selection bias in such data, as mothers may be hesitant to breastfeed due to fear of the safety profile of drugs used to treat the disease[135]. Larger studies of postpartum patients exist[136], but do not specifically address breastfeeding. While it is possible to assert that lack of breastfeeding may lead to increased disease activity, more research is needed in this area, including refinement of specific disease activity metrics delineated and perhaps a meta-analysis of existing data.

Rheumatoid arthritis (RA) is a complex disease in that many patients experience a decrease in disease severity while pregnant[119], while RA flares in[120] or may present with new onset disease[137] in the postpartum. The relationships between breastfeeding hormones are complex, e.g., prolactin and RA are complex, increasing the difficulty in understanding the association, if any, with breastfeeding. A 2015 meta-analysis found an inverse relationship between ever breastfeeding and subsequent RA development, with decreased subsequent risk in both women who breast fed for shorter (<12 months) or longer (>12 months) time frames[138], however this may be related to several factors not related to immune regulation. Studies specifically measuring the effect of breastfeeding on postpartum disease severity are rare, as many studies have focused on fear of medication and intent to breastfeed, and other sociodemographic factors supportive of breastfeeding (for example[139]). New tools to measure disease severity in the context of pregnancy may be better able to provide an assessment[140] of disease, and specific information on immune parameters.

Multiple sclerosis (MS) is another autoimmune disorder that more frequently affects women. Evidence suggests that while disease severity decreases during pregnancy, patients experience flares or worsening disease in the postpartum period[119], with 30% of women with MS experiencing a relapse in the first 3 months postpartum[122]. The relationship between breastfeeding hormones (e.g., prolactin) and RA are complex," increasing. Many relevant disease-modifying therapies are not recommended in pregnancy or lactation, so many women choose to return to these therapies and forego breastfeeding in order to prevent relapses, particularly if pre-pregnancy disease was severe. Conversely, those already with mild disease could choose to not restart medication and go on to breastfeed. An early large

prospective study of pregnant women with MS suggested that breastfeeding did not have a protective effect against postpartum disease severity (flares)[121]. Nearly two decades and several studies later,[141] a meta-analysis found a protective effect. Subsequently, a meta-analysis found that women who do choose to breastfeed have at least a 37% less chance of postpartum relapse compared with women who don't[122]. The benefit of breastfeeding was found to be stronger in the analysis of studies which required at least two months of exclusive breastfeeding than in the analysis including studies which allowed nonexclusive breastfeeding[122]. This may be because studies in the analysis differed with respect to the effect of nonexclusive breastfeeding with one study reporting that women who breastfed nonexclusively had comparable relapse risk to women who did not breastfeed at all. A second meta-analysis that same year found that population rates of MS relapse were not related to the proportion of women in the population who breastfed[142], and this finding may have been related to decreasing rates overall. Finally, in the era of more widespread use of pre-conceptional disease modifiers, a recent meta-analysis found that the use of such drugs pre-conceptionally was associated with increased and exclusive breastfeeding and also associated with decreased postpartum risk[143]. None of these analyses specifically looked at systemic immune parameters or metrics of systemic immunity (e.g., response to vaccines). Once again, more research is needed concerning progression and flares of this autoimmune disease and breastfeeding.

Though studies centering on these diseases could give us significant insight into the relationship between breastfeeding and the immune system, they are confounded by disease state and treatment using and are limited by the detail of the functionality and phenotype of the immune cells observed.

Conclusion

Breastfeeding is a critical driver of postpartum physiology in animal models[144] and in humans[6-8,10-13]. Breastfeeding is associated with hormones that may have a regulatory effect on immunity. The lack of breastfeeding may lead to a state of postpartum residual, low-level estrogen, which may be inflammatory, in the presence of falling or extremely low-level progesterone and lack of the immunoregulatory activity of breastfeeding hormones such as prolactin and oxytocin. It is hypothesized that this milieu may change immune cell development, homeostasis and effector function, but it may also lead to increased tissue (especially the breast) dysregulation which could in turn fuel inflammatory processes.

Studies examining the association between breastfeeding and postpartum flares of autoimmune disease are very complicated and confounded by such issues as baseline disease status, the availability of immunosuppressive drugs and biologics, and the changing overall presence of disease. They further comprise very little data on specific immune parameters. Further study of the development of breast cancer in young postpartum women is likely to be informative, but there still needs to be effort in the detailed examination of breast versus bottle feeding women after a normal pregnancy, as these may be less confounded by exogenous exposures, medications, and other interventions.

Finally, there is still a need for research using well-controlled experiments in animal models to delineate lactation-associated phenotypic and functional changes in immune cell populations as well as underlying mechanisms.

Conflict of Interest:

None

Funding:

MT is supported by the University of Vermont Summer Undergraduate Research Fellowship (SURF) program. EB is supported by NIH RO1HL141747 and NIH P30 GM118228 for the Vermont Center for Immunology and Infectious Disease.

Authors' contributions:

MT and EB both participated in both the literature search and review of data.

Acknowledgements:

This manuscript is dedicated to the memory or our colleague of Dr. Eric Testroet. We thank him and Martha Churchill for helpful reading and suggestions. We also thank Pauline DiGianivittorio for her assistance.

References:

1. Spelke B, Werner E. The Fourth Trimester of Pregnancy: Committing to Maternal Health and Well-Being Postpartum. *R I Med J* 2018;101(8):30-33.

2. Buchanan TA, Page KA. Approach to the patient with gestational diabetes after delivery. *J Clin Endocrinol Metab.* 2011;96(12):3592-3598.

3. Westerfield KL, Koenig K, Oh R. Breastfeeding: Common Questions and Answers. *Am Fam Physician.* 2018;98(6):368-373.

4. Gertosio C, Meazza C, Pagani S, Bozzola M. Breastfeeding and its gamut of benefits. *Minerva Pediatr.* 2016;68(3):201-212.

5. Vieira Borba V, Sharif K, Shoenfeld Y. Breastfeeding and autoimmunity: Programing health from the beginning. *Am J Reprod Immunol.* 2018; 79(1).

6. Krol KM, Grossmann T. Psychological effects of breastfeeding on children and mothers. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz.* 2018;61(8):977-985.

7. Groër MW. Differences between exclusive breastfeeders, formula-feeders, and controls: a study of stress, mood, and endocrine variables. *Biol Res Nurs.* 2005;7(2):106-117.

8. Stuebe AM, Rich-Edwards JW. The reset hypothesis: lactation and maternal metabolism. *Am J Perinatol.* 2009;26(1):81-88.

9. Adam I, Rayis DA, NA AL, Ahmed ABA, Sharif ME, Elbashir MI. Association between breastfeeding and preeclampsia in parous women: a case -control study. *Int Breastfeed J.* 2021;16 (1):48.

10. La Rosa M, Kechichian T, Olson G, Saade G, Bytautiene Prewit E. Lactation Leads to Modifications in Maternal Renin-Angiotensin System in Later Life. *Reprod Sci.* 2020;27(1):260-266.

11. Poole AT, Vincent KL, Olson GL, et al. Effect of lactation on maternal postpartum cardiac function and adiposity: a murine model. *Am J Obstet Gynecol.* 2014;211(4):424.e421-427.

12. Herrera SR, Vincent KL, Poole A, et al. Long-Term Effect of Lactation on Maternal Cardiovascular Function and Adiposity in a Murine Model. *Am J Perinatol.* 2019;36(5):490-497.

13. Stary CM, Xu L, Voloboueva LA, et al. Nursing Markedly Protects Postpartum Mice From Stroke: Associated Central and Peripheral Neuroimmune Changes and a Role for Oxytocin. *Frontiers in Neuroscience.* 2019;13.

14. Morris EA, Hale SA, Badger GJ, Magness RR, Bernstein IM. Pregnancy induces persistent changes in vascular compliance in primiparous women. *Am J Obstet Gynecol.* 2015;212(5):633.e 631-636.

15. Groer MW, Davis MW, Smith K, Casey K, Kramer V, Bukovsky E. Immunity, inflammation and infection in post-partum breast and formula feeders. *Am J Reprod Immunol.* 2005;54(4):222-231.

16. Bonney EA. A Framework for Understanding Maternal Immunity. *Immunol Allergy Clin North Am.* 2023;43(1s):e1-e20.

17. Matzinger P. Essay 1: the Danger model in its historical context. *Scand J Immunol.* 2001;54(1-2):4-9.

18. Kesaraju P, Jaini R, Johnson JM, et al. Experimental autoimmune breast failure: a model for lactation insufficiency, postnatal nutritional deprivation, and prophylactic breast cancer vaccination. *Am J Pathol.* 2012;181(3):775-784.

19. Gouon-Evans V, Lin EY, Pollard JW. Requirement of macrophages and eosinophils and their cytokines/chemokines for mammary gland development. *Breast Cancer Res.* 2002;4(4):155-164.

20. Wu Y, Teh YC, Chong SZ. Going Full TeRM: The Seminal Role of Tissue-Resident Macrophages in Organ Remodeling during Pregnancy and Lactation. *J Immunol.* 2024;212(4):513-521.

21. Nagy D, Gillis CMC, Davies K, et al. Developing ovine mammary terminal duct lobular units have a dynamic mucosal and stromal immune microenvironment. *Commun Biol.* 2021;4(1):993.

22. Plaks V, Boldajipour B, Linnemann JR, et al. Adaptive Immune Regulation of Mammary Postnatal Organogenesis. *Dev Cell.* 2015;34(5):493-504.

23. del Rio R, Sun Y, Alard P, Tung KS, Teuscher C. H2 control of natural T regulatory cell frequency in the lymph node correlates with susceptibility to day 3 thymectomy-induced autoimmune disease. *J Immunol.* 2011;186(1):382-389.

24. Ehrenstein MR, Notley CA. The importance of natural IgM: scavenger, protector and regulator. *Nat Rev Immunol.* 2010;10(11):778-786.

25. Vink A, Warnier G, Brombacher F, Renauld JC. Interleukin 9-induced in vivo expansion of the B-1 lymphocyte population. *J Exp Med.* 1999;189 (9):1413-1423.

26. Binder CJ, Chang M-K, Shaw PX, et al. Innate and acquired immunity in atherogenesis. *Nat Med.* 2002;8(11):1218-1226.

27. McKay JT, Haro MA, Daly CA, et al. PD-L2 Regulates B-1 Cell Antibody Production against Phosphorylcholine through an IL-5-Dependent Mechanism. *J Immunol.* 2017;199(6):2020-2029.

28. Moon BG, Takaki S, Miyake K, Takatsu K. The role of IL-5 for mature B-1 cells in homeostatic proliferation, cell survival, and Ig production. *J Immunol.* 2004;172(10):6020-6029.

29. Silvestre JS, Mallat Z, Duriez M, et al. Antiangiogenic effect of interleukin-10 in ischemia-induced angiogenesis in mice hindlimb. *Circ Res.* 2000;87(6):448-452.

30. Balaji S, Wang X, King A, et al. Interleukin-10-mediated regenerative postnatal tissue repair is dependent on regulation of hyaluronan metabolism via fibroblast-specific STAT3 signaling. *Faseb j.* 2017;31(3):868-881.

31. Martinson HA, Jindal S, Durand-Rougely C, Borges VF, Schedin P. Wound healing-like immune program facilitates postpartum mammary gland involution and tumor progression. *Int J Cancer.* 2015;136(8):1803-1813.

32. Betts CB, Pennock ND, Caruso BP, Ruffell B, Borges VF, Schedin P. Mucosal Immunity in the Female Murine Mammary Gland. *J Immunol.* 2018; 201(2):734-746.

33. Lefrère H, Moore K, Floris G, et al. Poor Outcome in Postpartum Breast Cancer Patients Is Associated with Distinct Molecular and Immunologic Features. *Clin Cancer Res.* 2023;29(18):3729-3743.

34. Jeppsson S, Rannevik G, Thorell JI, Wide L. Influence of LH/FSH releasing hormone (LRH) on the basal secretion of gonadotrophins in relation to plasma levels of oestradiol, progesterone and prolactin during the post-partum period in lactating and in non-lactating women. *Acta Endocrinol (Copenh).* 1977;84(4):713-728.

35. Chen WZ, Li Y, Yu HL, et al. Monitoring menstrual cycle, gestation and lactation by measuring urinary oestradiol and progesterone in the captive golden snub-nosed monkey (Rhinopithecus roxellanae). *Anim Reprod Sci.* 2017;181:79-85.

36. Hackwell ECR, Ladyman SR, Brown RSE, Grattan DR. Mechanisms of Lactation-induced Infertility in Female Mice. *Endocrinology.* 2023;164(5).

37. Hoffmann JP, Liu JA, Seddu K, Klein SL. Sex hormone signaling and regulation of immune function. *Immunity.* 2023;56(11):2472-2491.

38. Ramírez-de-Arellano A, Pereira-Suárez AL, Rico-Fuentes C, López-Pulido EI, Villegas-Pineda JC, Sierra-Diaz E. Distribution and Effects of Estrogen Receptors in Prostate Cancer: Associated Molecular Mechanisms. *Frontiers in Endocrinology.* 2022;12.

39. Notas G, Kampa M, Castanas E. G Protein-Coupled Estrogen Receptor in Immune Cells and Its Role in Immune-Related Diseases. *Front Endocrinol (Lausanne).* 2020;11:579420.

40. Carreras E, Turner S, Frank MB, et al. Estrogen receptor signaling promotes dendritic cell differentiation by increasing expression of the transcription factor IRF4. *Blood.* 2010;115(2):238-246.

41. Curran EM, Berghaus LJ, Vernetti NJ, Saporita AJ, Lubahn DB, Estes DM. Natural killer cells express estrogen receptor-alpha and estrogen receptor-beta and can respond to estrogen via a non-estrogen receptor-alpha-mediated pathway. *Cell Immunol.* 2001;214(1):12-20.

42. Tamaki M, Konno Y, Kobayashi Y, et al. Expression and functional roles of G-protein-coupled

estrogen receptor (GPER) in human eosinophils. *Immunol Lett.* 2014;160(1):72-78.

43. Pazos M, Sperling RS, Moran TM, Kraus TA. The influence of pregnancy on systemic immunity. *Immunol Res.* 2012;54(1-3):254-261.

44. Taneja V. Sex Hormones Determine Immune Response. *Front Immunol.* 2018;9:1931.

45. Borba VV, Zandman-Goddard G, Shoenfeld Y. Prolactin and Autoimmunity. *Front Immunol.* 2018;9:73.

46. Javadian A, Salehi E, Bidad K, Sahraian MA, Izad M. Effect of estrogen on Th1, Th2 and Th17 cytokines production by proteolipid protein and PHA activated peripheral blood mononuclear cells isolated from multiple sclerosis patients. *Arch Med Res.* 2014;45(2):177-182.

47. Pedroza DA, Subramani R, Lakshmanaswamy R. Classical and Non-Classical Progesterone Signaling in Breast Cancers. *Cancers (Basel).* 2020;12(9).

48. Medina-Laver Y, Rodríguez-Varela C, Salsano S, Labarta E, Domínguez F. What Do We Know about Classical and Non-Classical Progesterone Receptors in the Human Female Reproductive Tract? A Review. *Int J Mol Sci.* 2021;22(20).

49. Mittelman-Smith MA, Rudolph LM, Mohr MA, Micevych PE. Rodent Models of Non-classical Progesterone Action Regulating Ovulation. *Frontiers in Endocrinology.* 2017;8.

50. Tan H, Yi L, Rote NS, Hurd WW, Mesiano S. Progesterone receptor-A and -B have opposite effects on proinflammatory gene expression in human myometrial cells: implications for progesterone actions in human pregnancy and parturition. *J Clin Endocrinol Metab.* 2012;97(5):E719-730.

51. Dai Q, Provost MP, Raburn DJ, Price TM. Progesterone Increases Mitochondria Membrane Potential in Non-human Primate Oocytes and Embryos. *Reprod Sci.* 2020;27(5):1206-1214.

52. Leo JC, Guo C, Woon CT, Aw SE, Lin VC. Glucocorticoid and mineralocorticoid cross-talk with progesterone receptor to induce focal adhesion and growth inhibition in breast cancer cells. *Endocrinology.* 2004;145(3):1314-1321.

53. Ahn SH, Nguyen SL, Kim TH, et al. Nuclear Progesterone Receptor Expressed by the Cortical Thymic Epithelial Cells Dictates Thymus Involution in Murine Pregnancy. *Front Endocrinol (Lausanne).* 2022;13:846226.

54. Dosiou C, Hamilton AE, Pang Y, et al. Expression of membrane progesterone receptors on human T lymphocytes and Jurkat cells and activation of G-proteins by progesterone. *J Endocrinol.* 2008;196(1):67-77.

55. Chiu L, Nishimura M, Ishii Y, et al. Enhancement of the expression of progesterone receptor on progesterone-treated lymphocytes after immunotherapy in unexplained recurrent spontaneous abortion. *Am J Reprod Immunol.* 1996;35(6):552-557.

56. Ndiaye K, Poole DH, Walusimbi S, et al. Progesterone effects on lymphocytes may be mediated by membrane progesterone receptors. *J Reprod Immunol.* 2012;95(1-2):15-26.

57. Hierweger AM, Engler JB, Friese MA, et al. Progesterone modulates the T-cell response via glucocorticoid receptor-dependent pathways. *Am J Reprod Immunol.* 2019;81(2):e13084.

58. Campe KJ, Redlich A, Zenclussen AC, Busse M. An increased proportion of progesterone receptor A in peripheral B cells from women who ultimately underwent spontaneous preterm birth. *J Reprod Immunol.* 2022;154:103756.

59. Cain DW, Bortner CD, Diaz-Jimenez D, Petrillo MG, Gruver-Yates A, Cidlowski JA. Murine Glucocorticoid Receptors Orchestrate B Cell Migration Selectively between Bone Marrow and Blood. *J Immunol.* 2020;205(3):619-629.

60. Shah NM, Lai PF, Imami N, Johnson MR. Progesterone-Related Immune Modulation of Pregnancy and Labor. *Front Endocrinol (Lausanne).* 2019;10:198.

61. Goddard LM, Ton AN, Org T, Mikkola HK, Iruela-Arispe ML. Selective suppression of endothelial cytokine production by progesterone receptor. *Vascul Pharmacol.* 2013;59(1-2):36-43.

62. Legorreta-Haquet MV, Santana-Sánchez P, Chávez-Sánchez L, Chávez-Rueda AK. The effect of

prolactin on immune cell subsets involved in SLE pathogenesis. *Front Immunol.* 2022;13:1016427.

63. Kavarthapu R, Dufau ML. Prolactin receptor gene transcriptional control, regulatory modalities relevant to breast cancer resistance and invasiveness. *Front Endocrinol (Lausanne).* 2022;13:949396.

64. Abramicheva PA, Smirnova OV. Prolactin Receptor Isoforms as the Basis of Tissue-Specific Action of Prolactin in the Norm and Pathology. *Biochemistry (Mosc).* 2019;84(4):329-345.

65. López-Rincón G, Gutiérrez-Pabello J, Díaz-Otero F, Muñoz-Valle JF, Pereira-Suárez AL, Estrada-Chávez C. Mycobacterium bovis infection in cattle induces differential expression of prolactin receptor isoforms in macrophages. *Comp Immunol Microbiol Infect Dis.* 2013;36(6):619-629.

66. Chambers WH, Amoscato AA, Smith MS, Kenniston TW, Herberman RB, Appasamy PM. Prolactin receptor expression by rat NK cells. *Nat Immun.* 1995;14(3):145-156.

67. Gagnerault MC, Touraine P, Savino W, Kelly PA, Dardenne M. Expression of prolactin receptors in murine lymphoid cells in normal and autoimmune situations. *J Immunol.* 1993;150(12):5673-5681.

68. Szukiewicz D. Current Insights in Prolactin Signaling and Ovulatory Function. *Int J Mol Sci.* 2024;25(4).

69. Tang MW, Garcia S, Gerlag DM, Tak PP, Reedquist KA. Insight into the Endocrine System and the Immune System: A Review of the Inflammatory Role of Prolactin in Rheumatoid Arthritis and Psoriatic Arthritis. *Front Immunol.* 2017;8:720.

70. Sun R, Li AL, Wei HM, Tian ZG. Expression of prolactin receptor and response to prolactin stimulation of human NK cell lines. *Cell Res.* 2004; 14(1):67-73.

71. Yang L, Hu Y, Li X, Zhao J, Hou Y. Prolactin modulates the functions of murine spleen CD11c-positive dendritic cells. *Int Immunopharmacol.* 2006;6(9):1478-1486.

72. Tomio A, Schust DJ, Kawana K, et al. Prolactin can modulate CD4+ T-cell response through receptor-mediated alterations in the expression of T-bet. *Immunol Cell Biol.* 2008;86(7):616-621.

73. Lazarevic V, Glimcher LH, Lord GM. T-bet: a bridge between innate and adaptive immunity. *Nat Rev Immunol.* 2013;13(11):777-789.

74. Flores-Fernández R, Aponte-López A, Suárez-Arriaga MC, et al. Prolactin Rescues Immature B Cells from Apoptosis-Induced BCR-Aggregation through STAT3, Bcl2a1a, Bcl2l2, and Birc5 in Lupus-Prone MRL/lpr Mice. *Cells.* 2021;10(2).

75. Truchet S, Honvo-Houéto E. Physiology of milk secretion. *Best Pract Res Clin Endocrinol Metab.* 2017;31(4):367-384.

76. Carreón-Talavera R, Santana-Sánchez P, Fuentes-Pananá EM, et al. Prolactin promotes proliferation of germinal center B cells, formation of plasma cells, and elevated levels of IgG3 anti-dsDNA autoantibodies. *Front Immunol.* 2022;13: 1017115.

77. Jara LJ, Medina G, Saavedra MA, et al. Prolactin has a pathogenic role in systemic lupus erythematosus. *Immunol Res.* 2017;65(2):512-523.

78. Saha S, Gonzalez J, Rosenfeld G, Keiser H, Peeva E. Prolactin alters the mechanisms of B cell tolerance induction. *Arthritis Rheum.* 2009;60(6): 1743-1752.

79. Liu Y, Zhang Z, Jin Q, et al. Hyperprolactinemia is associated with a high prevalence of serum autoantibodies, high levels of inflammatory cytokines and an abnormal distribution of peripheral B-cell subsets. *Endocrine.* 2019;64(3):648-656.

80. Wang P, Yang HP, Tian S, et al. Oxytocin-secreting system: A major part of the neuroendocrine center regulating immunologic activity. *J Neuroimmunol.* 2015;289:152-161.

81. Landgraf R, Neumann I, Holsboer F, Pittman QJ. Interleukin-1 beta stimulates both central and peripheral release of vasopressin and oxytocin in the rat. *Eur J Neurosci.* 1995;7(4):592-598.

82. Borroto-Escuela DO, Cuesta-Marti C, Lopez-Salas A, et al. The oxytocin receptor represents a key hub in the GPCR heteroreceptor network: potential relevance for brain and behavior. *Front Mol Neurosci.* 2022;15:1055344.

83. Dou D, Liang J, Zhai X, et al. Oxytocin signalling in dendritic cells regulates immune tolerance in the intestine and alleviates DSS-induced colitis. *Clin Sci (Lond).* 2021;135(4):597-611.

84. Tang Y, Shi Y, Gao Y, et al. Oxytocin system alleviates intestinal inflammation by regulating macrophages polarization in experimental colitis. *Clin Sci (Lond).* 2019;133(18):1977-1992.

85. Szeto A, Sun-Suslow N, Mendez AJ, Hernandez RI, Wagner KV, McCabe PM. Regulation of the macrophage oxytocin receptor in response to inflammation. *Am J Physiol Endocrinol Metab.* 2017;312(3):E183-e189.

86. Ndiaye K, Poole DH, Pate JL. Expression and Regulation of Functional Oxytocin Receptors in Bovine T Lymphocytes1. *Biology of Reproduction.* 2008;78(4):786-793.

87. Diercks BP. The importance of Ca(2+) microdomains for the adaptive immune response. *Biochim Biophys Acta Mol Cell Res.* 2024;1871 (5):119710.

88. Nagaleekar VK, Diehl SA, Juncadella I, et al. IP3 receptor-mediated Ca2+ release in naive CD4 T cells dictates their cytokine program. *J Immunol.* 2008;181(12):8315-8322.

89. Sun J, Xu Z, Mao Y, Zhang T, Qin Y, Hua D. Prognostic role of oxytocin receptor in colon adenocarcinoma. *Open Med (Wars).* 2021;16(1): 1762-1776.

90. Oliveira LJ, Barreto RS, Perecin F, Mansouri-Attia N, Pereira FT, Meirelles FV. Modulation of maternal immune system during pregnancy in the cow. *Reprod Domest Anim.* 2012;47 Suppl 4:384-393.

91. Schäfer-Somi S, Gabriel C, Aslan S. Embryo-maternal communication in dogs: Immune system related factors. *Theriogenology.* 2020;150:382-387.

92. Ott TL. Immunological detection of pregnancy: Evidence for systemic immune modulation during early pregnancy in ruminants. *Theriogenology.* 2020;150:498-503.

93. Price AK, Bridges RS. The effects of bromocriptine treatment during early pregnancy on postpartum maternal behaviors in rats. *Dev Psychobiol.* 2014;56(6):1431-1437.

94. Murray IC, McComb DJ, Hellmann P, Kovacs K, Thorner MO. The effect of bromocriptine on the mammary glands of hyperprolactinemic rats. A histologic, ultrastructural and morphometric study. *Exp Pathol.* 1984;26(3):131-140.

95. Fitzgerald P, Dinan TG. Prolactin and dopamine: what is the connection? A review article. *J Psychopharmacol.* 2008;22(2 Suppl):12-19.

96. Beasley AM, Kahn LP, Windon RG. The periparturient relaxation of immunity in Merino ewes infected with Trichostrongylus colubriformis: parasitological and immunological responses. *Vet Parasitol.* 2010;168(1-2):60-70.

97. Asai K, Komine Y, Kozutsumi T, Yamaguchi T, Komine K, Kumagai K. Predominant subpopulations of T lymphocytes in the mammary gland secretions during lactation and intraepithelial T lymphocytes in the intestine of dairy cows. *Vet Immunol Immunopathol.* 2000;73(3-4):233-240.

98. Shafer-Weaver KA, Sordillo LM. Bovine CD8+ suppressor lymphocytes alter immune responsiveness during the postpartum period. *Vet Immunol Immunopathol.* 1997;56(1-2):53-64.

99. Jeddi PA, Keusch J, Lydyard PM, Delves PJ. The stability of lymphocytic beta 1,4-galactosyltransferase expression during pregnancy and lactation. *Scand J Immunol.* 1997;45(2):145-150.

100. Kochenour NK. Lactation suppression. *Clin Obstet Gynecol.* 1980;23(4):1045-1059.

101. Mayer AD, Carter L, Jorge WA, Mota MJ, Tannu S, Rosenblatt JS. Mammary stimulation and maternal aggression in rodents: thelectomy fails to reduce pre- or postpartum aggression in rats. *Horm Behav.* 1987;21(4):501-510.

102. Mann MA, Harvey PJ. Mice: the initiation and maintenance of pregnancy-induced aggression following thelectomy. *Behav Neural Biol.* 1988;49 (1):12-26.

103. Purba FY, Ishimoto Y, Nii T, Yoshimura Y, Isobe N. Effect of temporary cessation of milking

on the innate immune components in goat milk. *J Dairy Sci.* 2021;104(9):10374-10381.

104. Russell ES, Mohammed T, Smeaton L, et al. Immune activation markers in peripartum women in Botswana: association with feeding strategy and maternal morbidity. *PLoS One.* 2014;9(3):e89928.

105. Islami F, Liu Y, Jemal A, et al. Breastfeeding and breast cancer risk by receptor status--a systematic review and meta-analysis. *Ann Oncol.* 2015;26(12):2398-2407.

106. Zhou Y, Chen J, Li Q, Huang W, Lan H, Jiang H. Association between breastfeeding and breast cancer risk: evidence from a meta-analysis. *Breastfeed Med.* 2015;10(3):175-182.

107. Anstey EH, Shoemaker ML, Barrera CM, O'Neil ME, Verma AB, Holman DM. Breastfeeding and Breast Cancer Risk Reduction: Implications for Black Mothers. *Am J Prev Med.* 2017;53(3s1):S40-s46.

108. Kolak A, Kamińska M, Sygit K, et al. Primary and secondary prevention of breast cancer. *Ann Agric Environ Med.* 2017;24(4):549-553.

109. Coughlin SS. Epidemiology of Breast Cancer in Women. *Adv Exp Med Biol.* 2019;1152:9-29.

110. Elder AM, Stoller AR, Black SA, Lyons TR. Macphatics and PoEMs in Postpartum Mammary Development and Tumor Progression. *J Mammary Gland Biol Neoplasia.* 2020;25(2):103-113.

111. Basree MM, Shinde N, Koivisto C, et al. Abrupt involution induces inflammation, estrogenic signaling, and hyperplasia linking lack of breastfeeding with increased risk of breast cancer. *Breast Cancer Res.* 2019;21(1):80.

112. Pensa S, Watson CJ, Poli V. Stat3 and the inflammation/acute phase response in involution and breast cancer. *J Mammary Gland Biol Neoplasia.* 2009;14(2):121-129.

113. Froehlich K, Schmidt A, Heger JI, et al. Breast cancer, placenta and pregnancy. *Eur J Cancer.* 2019;115:68-78.

114. Ramirez RA, Lee A, Schedin P, Russell JS, Masso-Welch PA. Alterations in mast cell frequency and relationship to angiogenesis in the rat mammary gland during windows of physiologic tissue remodeling. *Dev Dyn.* 2012;241(5):890-900.

115. Jasti S, Farahbakhsh M, Nguyen S, Petroff BK, Petroff MG. Immune response to a model shared placenta/tumor-associated antigen reduces cancer risk in parous mice. *Biol Reprod.* 2017;96 (1):134-144.

116. MacDonell JW, Ito S. Breastfeeding anaphylaxis case study. *J Hum Lact.* 1998;14(3):243-244.

117. Ornek SA, Suroji Alkilinc A, Kızıltac U, Kızıltac K, Kocaturk E. Effect of Puberty, Menstruation, Pregnancy, Lactation, and Menopause on Chronic Urticaria Activity. *J Cutan Med Surg.* 2023;27(5): 466-471.

118. Lazarus JH, Parkes AB, Premawardhana LD. Postpartum thyroiditis. [Review] [55 refs]. *Autoimmunity.* 2002;35(3):169-173.

119. Borba VV, Zandman-Goddard G, Shoenfeld Y. Exacerbations of autoimmune diseases during pregnancy and postpartum. *Best Pract Res Clin Endocrinol Metab.* 2019;33(6):101321.

120. Vieira Borba V, Shoenfeld Y. Prolactin, autoimmunity, and motherhood: when should women avoid breastfeeding? *Clin Rheumatol.* 2019;38(5):1263-1270.

121. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T. Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in Multiple Sclerosis Group. *N Engl J Med.* 1998;339(5):285-291.

122. Krysko KM, Rutatangwa A, Graves J, Lazar A, Waubant E. Association Between Breastfeeding and Postpartum Multiple Sclerosis Relapses: A Systematic Review and Meta-analysis. *JAMA Neurol.* 2020;77(3):327-338.

123. Malhi G, Tandon P, Perlmutter JW, Nguyen G, Huang V. Risk Factors for Postpartum Disease Activity in Women With Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. *Inflamm Bowel Dis.* 2022;28(7):1090-1099.

124. Davies TF. The thyroid immunology of the postpartum period. *Thyroid.* 1999;9(7):675-684.

125. Chiba M, Tsuji T, Komatsu M, Watanabe H, Takahashi M. Ulcerative colitis in the postpartum period. *Autops Case Rep.* 2020;10(4):e2020187.

126. Christou EAA, Banos A, Kosmara D, Bertsias GK, Boumpas DT. Sexual dimorphism in SLE: above and beyond sex hormones. *Lupus.* 2019;28(1):3-10.

127. Ansari AA, Fett JD, Carraway RE, Mayne AE, Onlamoon N, Sundstrom JB. Autoimmune mechanisms as the basis for human peripartum cardiomyopathy. *Clin Rev Allergy Immunol.* 2002; 23(3):301-324.

128. Koczo A, Marino A, Jeyabalan A, et al. Breastfeeding, Cellular Immune Activation, and Myocardial Recovery in Peripartum Cardiomyopathy. *JACC Basic Transl Sci.* 2019;4(3):291-300.

129. Kane S, Lemieux N. The role of breastfeeding in postpartum disease activity in women with inflammatory bowel disease. *Am J Gastroenterol.* 2005;100(1):102-105.

130. Julsgaard M, Nørgaard M, Hvas CL, Grosen A, Hasseriis S, Christensen LA. Self-reported adherence to medical treatment, breastfeeding behaviour, and disease activity during the postpartum period in women with Crohn's disease. *Scand J Gastroenterol.* 2014;49(8):958-966.

131. Knight CL, Nelson-Piercy C. Management of systemic lupus erythematosus during pregnancy: challenges and solutions. *Open Access Rheumatol.* 2017;9:37-53.

132. Noviani M, Wasserman S, Clowse ME. Breastfeeding in mothers with systemic lupus erythematosus. *Lupus.* 2016;25(9):973-979.

133. Bjerkaas Hanssen M, Malm Gulati A, Koksvik H, Wallenius M. Breastfeeding in women with systemic lupus erythematosus: results from a Norwegian quality register. *Int Breastfeed J.* 2023; 18(1):37.

134. Orefice V, Ceccarelli F, Pirone C, et al. Breastfeeding in women affected by systemic lupus erythematosus: Rate, duration and associated factors. *Lupus.* 2021;30(6):913-920.

135. Li W, Wang T. Breastfeeding Initiation, Duration, and Associated Factors in Mothers with Systemic Lupus Erythematosus. *Breastfeed Med.* 2022;17(11):958-963.

136. Götestam Skorpen C, Lydersen S, Gilboe IM, et al. Disease Activity During Pregnancy and the First Year Postpartum in Women With Systemic Lupus Erythematosus. *Arthritis Care Res (Hoboken).* 2017;69(8):1201-1208.

137. Iijima T, Tada H, Hidaka Y, et al. Prediction of postpartum onset of rheumatoid arthritis. *Ann Rheum Dis.* 1998;57(8):460-463.

138. Chen H, Wang J, Zhou W, Yin H, Wang M. Breastfeeding and Risk of Rheumatoid Arthritis: A Systematic Review and Metaanalysis. *J Rheumatol.* 2015;42(9):1563-1569.

139. Carrazco-Chapa A, Perez-Barbosa L, Cardenas-de la Garza JA, et al. Sociodemographic and obstetric factors may affect breastfeeding attitudes, self-efficacy, and knowledge in women with rheumatic diseases: a retrospective analysis of self-reported surveys. *Rheumatol Int.* 2024.

140. Raine C, Ciurtin C, Jury EC, et al. DAS28(3) CRP is a reliable measure of disease activity in pregnant women with rheumatoid arthritis. *Clin Exp Rheumatol.* 2023;41(11):2224-2229.

141. Pakpoor J, Disanto G, Lacey MV, Hellwig K, Giovannoni G, Ramagopalan SV. Breastfeeding and multiple sclerosis relapses: a meta-analysis. *J Neurol.* 2012;259(10):2246-2248.

142. Dobson R, Jokubaitis VG, Giovannoni G. Change in pregnancy-associated multiple sclerosis relapse rates over time: a meta-analysis. *Mult Scler Relat Disord.* 2020;44:102241.

143. Schubert C, Steinberg L, Peper J, et al. Postpartum relapse risk in multiple sclerosis: a systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry.* 2023;94(9):718-725.

144. Gokina NI, Fairchild RI, Bishop NM, Dawson TE, Prakash K, Bonney EA. Kinetics of Postpartum Mesenteric Artery Structure and Function Relative to Pregnancy and Lactation in Mice. *Reprod Sci.* 2021;28(4):1200-1215.

