

# Beyond Milk and Nurture: Breastfeeding's Powerful Impact on Breast Cancer

## Jenseits von Milch und Umweltfaktoren: die starke Auswirkung von Stillen auf das Brustkrebsrisiko



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### Keywords

breast cancer, breastfeeding, breast cancer risk, HAMLET

### Schlüsselwörter

Brustkrebs, Stillen, Brustkrebsrisiko, HAMLET

received 29.1.2024

accepted after revision 21.4.2024

### Bibliography

Geburtsh Frauenheilk 2024; 84: 541–554

DOI 10.1055/a-2313-0637

ISSN 0016-5751

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### ABSTRACT

Breast cancer (BC) stands as a global concern, given its high incidence and impact on women's mortality. This complex disease has roots in various risk factors, some modifiable and others not. Understanding and identifying these factors can

be instrumental in both preventing BC and improving survival rates. Remarkably, women's reproductive behaviors have emerged as critical determinants of BC susceptibility. Numerous studies have shed light on how aspects including age of menarche, first pregnancy and menopause along with number of pregnancies, hormone replacement therapies, can influence one's risk of developing BC. Furthermore, the act of breastfeeding and its duration have shown an inverse relationship with BC risk. This review delves into the biological and molecular mechanisms associated with breastfeeding that contribute to BC protection. It highlights the role of endocrine processes triggered by suckling stimulation, the gradual onset of lactational amenorrhea, delayed weaning, reduced lifetime menstrual cycles, chromosomal repair mechanisms, and immunological events throughout the lactation cycle. These insights provide a potential explanation for the protective effects conferred by breastfeeding against breast carcinomas.

### ZUSAMMENFASSUNG

Angesichts der hohen Inzidenz von Brustkrebs und deren Auswirkung auf die Mortalität von Frauen bleibt Brustkrebs (BK) ein globales Problem. Diese komplexe Erkrankung hat ihren Ursprung in verschiedenen Risikofaktoren, von denen einige veränderbar sind und andere nicht. Das Verständnis und die Identifikation dieser Faktoren kann entscheidend sein, sowohl bei der Prävention von BK als auch bei der Verbesserung der Überlebensraten. Bemerkenswerterweise hat sich herausgestellt, dass das Fortpflanzungsverhalten von Frauen einen kritischen Faktor für die Anfälligkeit für BK darstellt. Zahlreiche Studien haben Aufschluss darüber gegeben, wie bestimmte Aspekte wie Alter beim Eintritt der ersten Menstruationsblutung, Alter bei der ersten Schwangerschaft und Alter beim Eintritt der Wechseljahre sowie Anzahl von Schwangerschaften und Hormonersatztherapien das Brustkrebsrisiko beeinflussen können. Es hat sich auch herausgestellt, dass das Stillen und die Stilldauer eine umgekehrte Relation zum Brustkrebsrisiko haben. Dieser Übersichtsartikel untersucht die mit Stillen assoziierten biologischen und molekularen Mechanis-

men, die helfen können, BK vorzubeugen. Die Rolle von durch Säugen stimulierten endokrinen Prozessen, z. B. das allmähliche Einsetzen der laktationsbedingten Amenorrhö, das verzögerte Abstillen, die verminderte Anzahl von Menstruationszyklen im Laufe des Lebens, die chromosomalen Reparatur-

mechanismen und immunologischen Ereignisse während des Laktationszyklus, werden beschrieben. Diese Einsichten bieten eine mögliche Erklärung für den durch das Stillen bedingten Schutz gegen Brustkrebs.

## Introduction

In the advancing and developed world breast cancer is a significant global challenge, impacting women [1, 2] “considerably”. Each year, around 2.3 million breast cancer cases are diagnosed worldwide, contributing to a 19.6 million Disability-Adjusted Life Years (DALYs) for women [3]. Each year, around 2.3 million breast cancer cases are diagnosed worldwide, contributing to a 19.6 million Disability-Adjusted Life Years (DALYs) for women [4]. Additionally, a rise from 140 to 170 thousand is expected to be observed in metastatic, violent breast cancer cases by the year 2025 [5]. Contrasting to these alarming figures, progress pace in reducing breast cancer mortalities has been slowed down over the past two decades. In the reduction of risk of breast cancer, breastfeeding comes in light as a key aspect [6]. A meta-analysis performed by Bernier et al., in 2000 including 40 studies showed that breastfeeding reduces breast cancer risk [7]. Another meta-analysis performed by Zhou et al. in 2015 which included 27 studies and involved 13907 breast cancer cases also came to the same deduction [8]. On the other hand, a systematic review, which included 31 studies between 1999 and 2007, also found breast cancer to be inversely proportional to breastfeeding [9]. Another systematic review including 65 studies between 2005 and 2015 also came to the same conclusion [10].

The total studies from the above-mentioned meta-analyses and systematic reviews, when pooled and organized based on distinct world regions, as shown in ► **Fig. 1**, provide insight into the heterogeneity among the varied populations studied alongside the global implications of the inverse correlation between breastfeeding and breast cancer.

Mothers and infants both benefit from breastfeeding. Immediate advantages which are experienced by breastfeeding mothers include reduced risk of postpartum depression, postpartum weight loss and lactational amenorrhea [6, 11]. A decreased risk of breast cancer, osteoporosis, type 2 diabetes, rheumatoid arthritis cardiovascular disease, and ovarian cancer are the benefits that mother receives in the long run [11, 12, 13].

Breastfeeding for over a year is shown by studies to decrease the risk of invasive breast cancer by approximately 4.3% [14]. All breastfeeding mothers have the risk of developing breast cancer lowered to 11%, while mothers nursing their babies for over a year experience a 26% reduction in breast carcinoma development [11]. Despite the well-known advantages of breastfeeding, breastfeeding rates around the globe remain below recommended levels [13, 15]. Various biological, psychosocial, and social factors contribute to the lack of change in breastfeeding status. Common reasons for discontinuing breastfeeding prematurely include insufficient milk supply, maternal fatigue, and returning to work or

school. Socioeconomic factors like a mother’s education, income, and lifestyle also influence breastfeeding [13, 15]. To enhance breastfeeding rates worldwide, organizations like United Nations International Children’s Emergency Fund (UNICEF) and World Health Organization (WHO) are making strides towards it. This review seeks to explore how disruptions or discontinuation of breastfeeding can increase the risk of developing breast cancer. Understanding these mechanisms, we aim to promote breastfeeding in both low and high-income as a natural protective measure against breast cancer.

## Breast Morphology

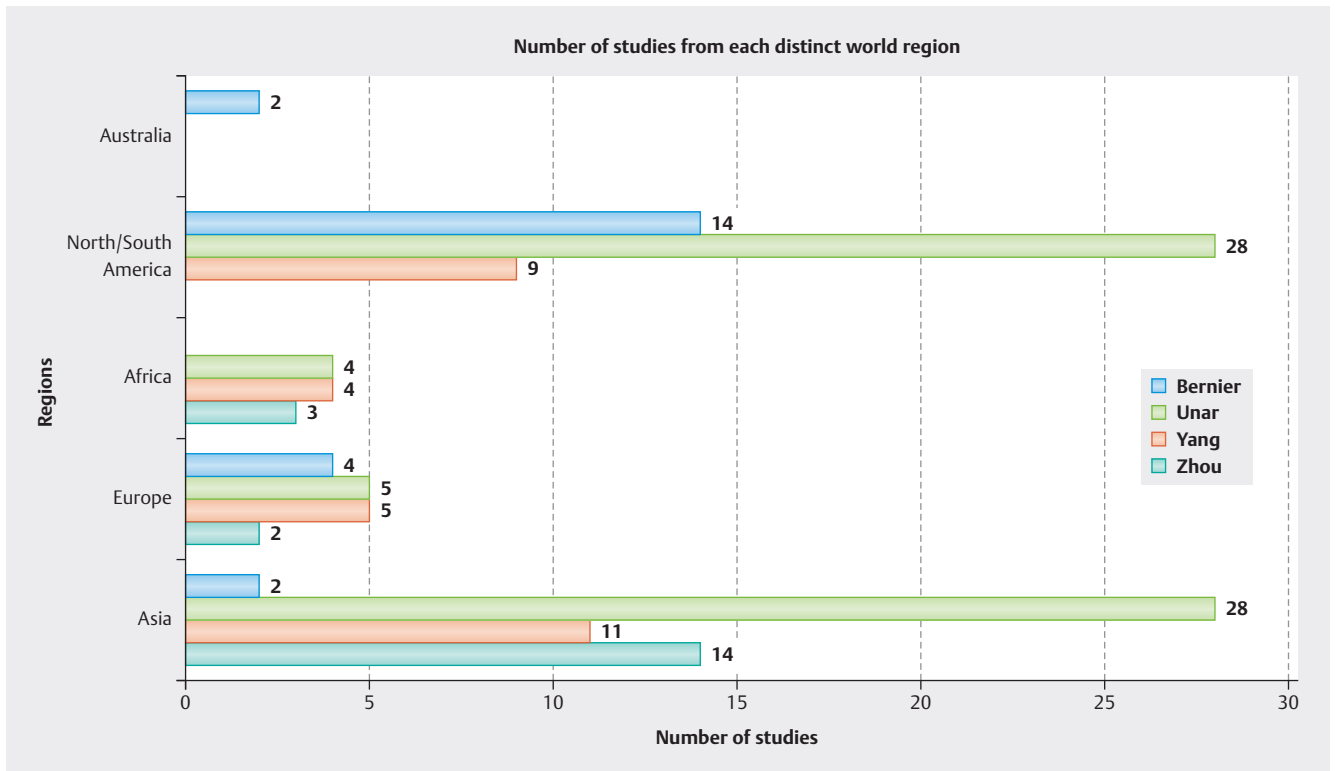
Breast development in females bodies the mammary glands essential for milk production, which is crucial for the nourishment of newborns [16]. A comprehensive understanding of breast anatomy is vital for grasping its intricate links with breast cancer. The surface anatomy of the mammary gland encompasses the areola and nipple, collectively forming the Nipple-Areola Complex (NAC). The nipple, highly sensitive due to its rich nerve supply, is penetrated by 15 to 20 lactiferous ducts on average, with some nipples having more ductal orifices [17]. Surrounding the nipple is the areola, a dark-hued area replete with sweat and sebaceous glands, producing oils that help prevent nipple cracking. The NAC has a key role regarding milk ejection during breastfeeding and it can be influenced by hormonal changes [18, 19, 20]. ► **Fig. 2** shows mammogenesis from embryo to lactation, followed by involution. Subsequent observations indicating an elevated risk of developing cancer in the breast not suckled during lactation in women who nursed from only one breast seemed to further endorse a potential link [21].

In their case-control study involving 528 breast cancer cases, Freudenheim et al. discovered that being breastfed was linked to a decreased risk of breast cancer (RR = 0.74, 95% CI 0.56–0.99). This reduction in risk in observed for both pre- and postmenopausal breast cancer amongst women who had breastfed [22].

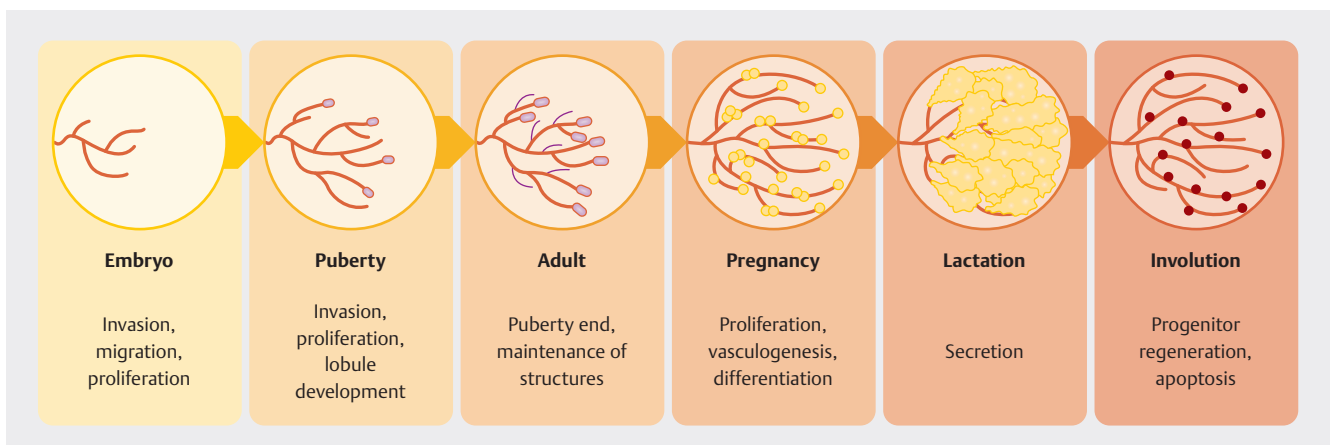
## Review

### Lactation induces resistance to carcinogens

Studies suggest that using medication to suppress lactation did not show any association with the risk of postmenopausal breast cancer, irrespective of the age at first use. Moreover, women who mentioned insufficient milk supply as their reason for not breastfeeding did not exhibit an elevated risk of breast cancer when compared to women with a sufficient milk supply, after accounting for the total duration of lactation [23].



► **Fig. 1** Organized studies from the above-mentioned meta-analyses and systematic reviews based on distinct world regions (Fig. based on data from [7, 8, 9, 10]).



► **Fig. 2** Mammogenesis from embryo to lactation, followed by involution.

Studies, coupled with findings from various reports, cast doubt on the idea that the apparent protective impact of lactation is linked to a heightened risk among women who cannot lactate. Furthermore, there is no indication from this study or recent studies to support an increased risk of breast cancer among those who use lactation suppressants [24, 25, 26, 27, 28, 29].

Research involving lactating rats and mice suggests that they demonstrate a certain resistance to the effects of chemical carcinogens when compared to their non-lactating counterparts [30, 31]. This resistance is thought to be a result of lower rates of DNA synthesis during lactation or an increased elimination of carcinogens by the mammary glands during secretion [30, 31, 32, 33, 34].

## The protective role of suppressing ovulation and prolonged breastfeeding against breast cancer

Among the various mechanisms suggested to account for the beneficial effects of lactation on breast cancer risk, it is still uncertain which ones precisely align with epidemiologic observations. The suppression of ovulation that comes with prolonged breastfeeding might play a role in diminishing exposure to the cyclic hormones associated with reproductive life. Notably, in studies that have identified an impact of lactation, a prolonged duration of breastfeeding emerges as the most influential predictor of breast cancer risk. This highlights the significance of exploring the ovulatory suppression mechanism in order to understand the potential protective effects of lactation against breast cancer [35].

The initial suggestion of an association between lactation and breast cancer dates back to Lane-Clayton, who noted that women with breast cancer frequently reported difficulties in breastfeeding [20]. Subsequent observations indicating an elevated risk of developing cancer in the breast not suckled during lactation in women who nursed from only one breast seemed to further endorse a potential link [21]. In their case-control study involving 528 breast cancer cases, Freudenheim et al. discovered that being breastfed was linked to a decreased risk of breast cancer (RR = 0.74, 95% CI 0.56–0.99). This reduction in risk was observed for both pre- and postmenopausal breast cancer amongst women who had breastfed [22].

## Breastfeeding and the risks of breast cancer in different subtypes

During breast cancer, certain protein hormone receptors serve as indicators of breast cancer due to an overexpression leading to speedy proliferation, mainly being the Estrogen (ER), Progesterone (PR) and the Human Epidermal growth factor Receptor 2 (HER2) [36]. According to a research study, increased parity and breastfeeding history emerged as the strongest protective factors among luminal A BC. Conversely, earlier menarche and later FFTP elevated the risk. For TN tumors, both later menarche and prior breastfeeding exhibited protective effects [37]. Scientists looked at 38 studies on breast cancer risk factors in women before and after menopause, including Caucasians and Asians. They found that known risk factors mostly apply to a specific type of breast cancer called luminal A (ER+ and/or PR+, HER2-), while distinct risk factors may be associated with other subtypes [38]. A recent study has shown that ever breastfeeding or longer breastfeeding was associated with low risk and protection for HER2+, TN, luminal A and luminal B type breast cancers [39]. A meta-analysis of 15 studies of varied origin suggested higher parity and younger age at FFTP to be associated with a reduced risk of luminal BC [40], while breastfeeding exhibited a protective effect for both luminal and TN BC [41]. In a study based on 890 breast cancer participants, IHC staining revealed that a little or no breastfeeding along with a high waist to hip ratio was related to most of the TNBCs [42]. The results observed for younger women were of varied nature as some showed relation of age of menarche with protection against TNBC, while others did not. However, reproductive

risk factors for hormone-dependent tumors (ER+) align with previous findings showing significant protection against ER+ and ER- tumors with breastfeeding durations lasting up to 6 months and 12 months respectively [37]. When the effect of breastfeeding was studied together with the number of parities, a strong protective effect was shown for women who breastfed two or more children. In addition old age of menarche was protective for both ER- and TNBC [43, 44]. However, conception or higher number of parities did not associate with any risk of ER- and TNBC [37]. Similar results were observed in a later study, indicating strong protection against ER- and TNBC with longer breastfeeding durations (12 months) and even a shorter duration of 6 months to exhibit protection against ER+ breast cancers [45].

## Epidemiology of breastfeeding and breast cancer

Research has uncovered a link between breastfeeding, one of the modifiable risk factors, and the susceptibility to breast cancer [46]. These investigations shed light on the intricate interplay between the breastfeeding duration, the number of pregnancies (parity), and the age of menarche (first menstrual cycle), all of which bear significance in the context of breast cancer etiology and its prognostic implications [46]. Epidemiological investigations commonly consider two key factors, alongside the duration of breastfeeding, when exploring the association between breastfeeding practices and the risk of breast cancer: the age of females and their pregnancy (parity) status [47]. This hormonal exposure is intricately linked to the number of times the cells of a female breast undergo proliferation during each menstrual cycle, thereby influencing the potential for breast cancer development. Moreover, the number of pregnancies a woman experiences is related to the frequency of her breast preparation for lactation, exposing her to the concomitant hormonal and physiological changes. Notably, when investigating the breast cancer risk associated with women's reproductive behaviors, a study among the Chinese population revealed a higher prevalence of Luminal A type breast cancer compared to the Triple-Negative Breast Cancer (TNBC). Additionally, the more aggressive forms of breast cancer, such as Luminal B type and HER2-enriched BC, were found to be less prevalent among women who adhered to regular breastfeeding practices. This study also underscored the significant protective role of early age menarche and the increasing number of pregnancies towards mitigating the risk of breast cancer [48]. Women, who are reluctant or unable to breastfeed, experience the more aggressive 'Parity Associated Breast Cancer' (PABC), such as TNBC. Importantly, a significant 50% reduction in PABC risk has been noted in young women who engaged in longer durations of breastfeeding, reaching up to 12 months during their lifetime [49]

## Pregnancies, breastfeeding, and breast cancer risk

Breastfeeding was found to be a risk-reducing factor for breast cancer, however, certain variations exist across different cancer subtypes. A negative correlation was found to exist between the duration of breastfeeding and the breast cancer risk. A comprehensive meta-analysis, which integrated findings from six signifi-

cant studies and applied the Preferred reporting items for systematic review and meta-analysis, (PRISMA) flowchart methodology, confirmed strong connections between extended periods of breastfeeding and a reduced risk of breast cancer [50].

Estrogen and progesterone, recognized as key factors driving breast cancer, promote the proliferation of cells in breast tissue. The levels of these hormones undergo substantial changes during pregnancy and lactation, which have a significant impact on the development of the mammary glands [51]. Throughout this process, two essential hormone groups play a critical role: reproductive hormones (estrogen, progesterone, prolactin, placental lactogen, oxytocin) and metabolic hormones (growth hormones, glucocorticoids, thyroid hormones, insulin). Estrogen and growth hormone influence ductal morphogenesis, whereas progesterone, placental lactogen, and prolactin initiate alveolar development. Lactation involves lactogenesis 1 and 2, where progesterone inhibits active milk secretion in the former, and prolactin becomes prominent in the latter. Prolactin regulates milk secretion, and oxytocin controls milk ejection [52].

Prolactin, a hormone primarily synthesized by the pituitary gland and various other tissues, is linked to an increased risk of breast cancer, particularly in postmenopausal women. While it does not directly impact cancer cells, it encourages undifferentiated breast cells, making them more vulnerable to becoming cancerous [52]. Additionally, prolactin fosters a pro-cancer environment by inciting inflammation, thickening breast tissue, and elevating cancer risk, particularly in estrogen receptor-positive (ER+) breast cancers where prolactin receptors (PRLRs) are abundant. Efforts to block PRL receptors or reduce PRL levels for breast cancer treatment have yielded mixed results, and prolactin aids breast cancer cells in resisting chemotherapy drugs, activating pro-cancer pathways like “phosphoinositide-3-kinase” PI3/Akt, and bolstering cancer cell survival. Oxytocin, known for its role in childbirth and milk secretion, has been linked to cancer development, with elevated levels observed in breast cancer patients. Oxytocin has the potential to boost the growth of breast cancer cells while amplifying the effectiveness of tamoxifen, a drug used in breast cancer treatment. Progesterone, intricately involved in breast cell division, exhibits both proliferative and inhibitory effects on breast cancer cells, initially promoting growth but later potentially slowing it down [53]. It can render breast cancer cells more responsive to growth signals, contributing to tumor progression and increasing resistance to treatment. Progesterone receptors (PR) serve as valuable biomarkers for studying estrogen receptor-alpha (ER $\alpha$ ) function and predicting breast cancer prognosis [54]. Finally, estrogen, crucial in estrogen receptor-positive breast cancer, constitutes 70% of cases, and imbalanced estrogen metabolism can produce harmful molecules, known as estrogen quinones, which can damage DNA and heighten breast cancer risk [55].

## Varied factors favoring breast cancer protection through hormonal events

Various breastfeeding-related factors contribute significantly to breast cancer prevention by influencing endocrine and hormonal regulation. These factors encompass:

### Suckling stimulus

Suckling intensity, influenced by factors like frequency, bout duration, and daily duration, correlates with the duration of postpartum amenorrhea [56]. During infant suckling, sensory receptors in the nipple send signals to the anterior pituitary gland, triggering the release of prolactin and oxytocin. This process stimulates the release of milk through a positive feedback loop. Prolactin, previously believed to directly maintain amenorrhea, is currently regarded as an indicator of how often the infant is nursing [57].

### Role of growth factor $\beta$

If we consider the direct correlation between the cumulative number of ovulatory cycles and the risk of breast cancer, the ovulatory suppression associated with prolonged breastfeeding should contribute to a reduction in both factors [58]. Notably, an extended duration of breastfeeding consistently emerges as a robust predictor of breast cancer risk across studies. Beyond temporary or long-term changes in pituitary and ovarian hormones [59, 60, 61, 62]. Noteworthy among these factors is transforming growth factor-beta, which exhibits hormonally regulated negative effects on breast cancer cells [63, 64]. The impact on the expression of these factors and their receptors assumes importance due to their intricate connections with oncogenes, proto-oncogenes, and the expression of tumor suppressors [65]. Specifically, there is compelling evidence suggesting that transforming growth factor-beta (TGF- $\beta$ ), expressed during lactation, serves as a hormonally regulated negative growth factor in human breast cancer cells. Behavioral and environmental factors influencing the expression of these growth factors assume significance due to their intricate connections with oncogenes, proto-oncogenes, and the expression of tumor suppressors. Understanding the interplay between hormonal regulation, environmental influences, and the expression of growth factors like TGF- $\beta$  is essential for unraveling the complex dynamics influencing breast cancer development [65].

## Hormonal changes and their impact

The hormonal shifts associated with lactation, characterized by heightened prolactin levels, and reduced estrogen production, may potentially hinder the initiation or growth of breast tumors. Lactation is often accompanied by a reduction or cessation of ovulation, which could contribute to additional protection against breast cancer. The interplay between these hormonal changes during lactation appears to create an environment that is less conducive to the development and progression of breast tumors [60].

## Longer lactational amenorrhea

Lactational amenorrhea, a natural contraceptive method, relies on breastfeeding patterns. It postpones the resumption of regular ovarian cycles by interfering with the release pattern of gonadotropin-releasing hormone (GnRH) from the hypothalamus and, consequently, luteinizing hormone (LH) from the pituitary gland [11]. Although the follicle-stimulating hormone (FSH) levels remain sufficient for follicle growth during lactation, the disrupted LH signaling diminishes estradiol production by these follicles. This altered hormonal environment impedes the typical preovulatory LH surge, leading to follicles that either fail to rupture or become atretic or cystic [57]. In women, hyperprolactinemia often leads to amenorrhea, resembling the lactational amenorrhea situation [66, 67]. Suckling-induced prolactin release can directly suppress the menstrual cycle, affect the ovary, or decrease GnRH release [57].

## Age at menarche and menopause

The impact of reproductive factors contributing to breast cancer risk is crucial for comprehensive risk assessment and preventive strategies. Although early onset of menstruation and late cessation of menstruation are associated with modest increases in breast cancer risk, the most consistently observed risk factor across diverse populations is the age at which a woman undergoes her first full-term pregnancy. This pivotal reproductive milestone emerges as a common denominator in breast cancer risk assessment, emphasizing its significance in shaping preventive strategies across various demographic contexts [68].

## Estrogen window hypothesis

Proposed in 1980 by Korenman, the “estrogen window hypothesis” suggests that the most favorable conditions for breast cancer induction result from unopposed estrogen stimulation, while normal post ovulation progesterone secretion reduces susceptibility. The hypothesis posits that the interplay of normal estrogen stimulation and luteal inadequacy, marked by diminished progesterone secretion, can explain the main epidemiological features of breast cancer. It underscores the notion that unopposed estrogen stimulation is particularly conducive to tumor induction. Despite the initial assumption that progesterone acts as an antiestrogen on breast epithelium akin to its impact on endometrial epithelium, a comprehensive evaluation of epidemiologic and experimental evidence challenges this premise. Contrary to expectations, the accumulated data indicates that a higher frequency of ovulatory cycles, not a reduction, constitutes the primary determinant of breast cancer risk. This reframing emphasizes the complexity of hormonal dynamics influencing breast cancer etiology and encourages ongoing exploration in this field [58].

## Direct physical changes

The act of lactation has beneficial impact on the interaction between the mammary epithelium and the stroma. Breast tissue could be particularly responsive to these lactational changes during early reproductive life [69, 70].

## Cumulative lactation

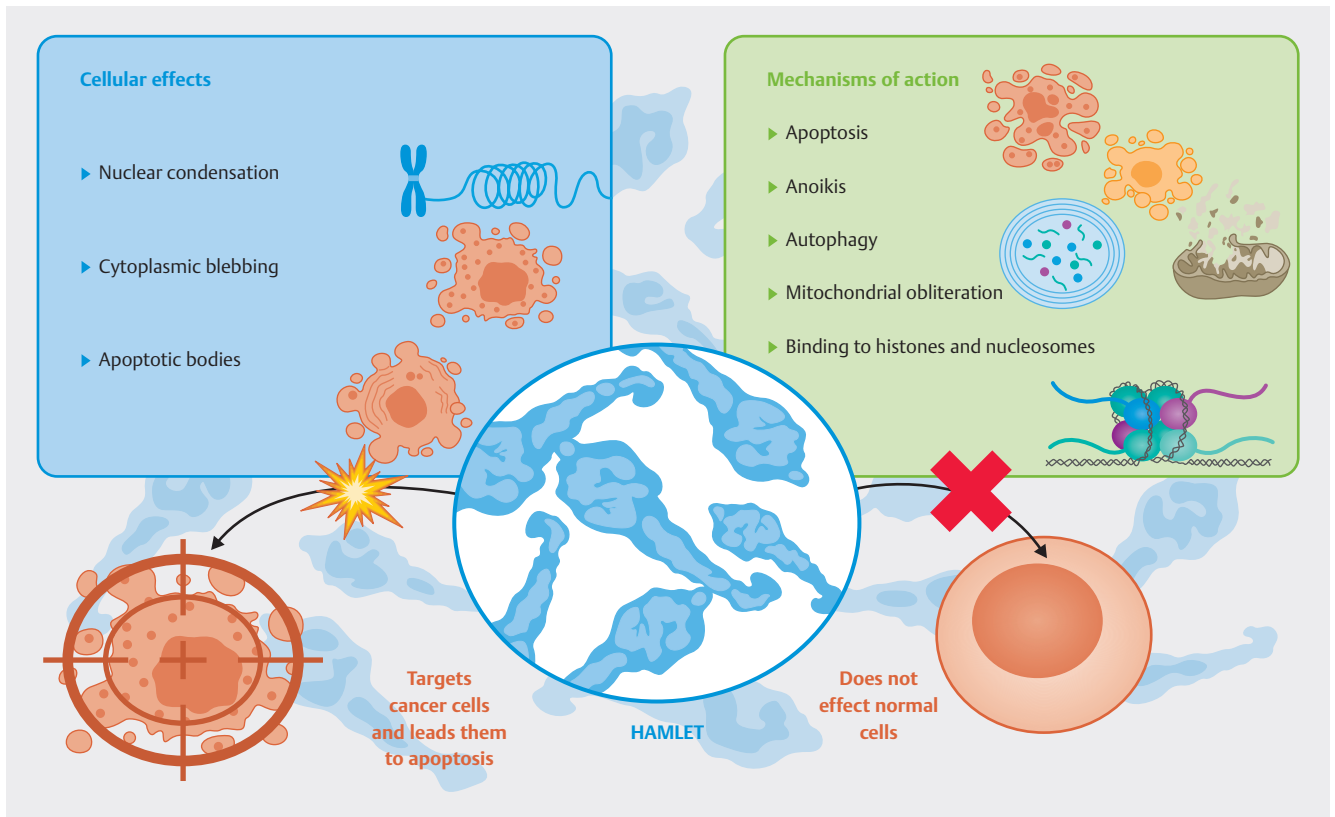
The prolonged duration of breastfeeding stands out as the most robust predictor of breast cancer risk. This is underscored by the fact that concentrations of toxic organochlorines in human breast milk decrease as the cumulative duration of lactation increases [71]. The suppression of ovulation associated with prolonged breastfeeding should effectively reduce both factors if connected to breast cancer risk [58]. This dual impact highlights the potential protective role of extended breastfeeding, addressing not only the presence of harmful substances but also contributing to a decreased risk of breast cancer. Consistent with reported duration effects, the elimination of carcinogens through breast milk secretion aligns with observed trends. In humans, there is an observable decrease in breast milk concentrations of toxic organochlorines with increasing cumulative lactation. This correlation sheds light on the potential protective mechanisms associated with extended breastfeeding, as it not only nourishes infants but also serves as a means of reducing the presence of harmful substances [72].

## Molecular Mechanisms Underlying Protection

### Cellular differentiation and maturation

Acknowledging the epidemiological, clinical, and experimental evidence that highlights the crucial connection between ovarian function and the risk of breast cancer, it becomes clear that factors such as early onset of menstruation, delayed menopause, and parity play a significant role in influencing susceptibility [73, 74]. ▶ **Fig. 3** shows molecular mechanisms of breastfeeding aiding in protection against breast cancer.

In the context of breast development, different stages of breast lobules become evident, starting with the simple, undifferentiated lobules type 1 (Lob 1), and progressing to the more complex lobules type 2 and lobules type 3 [75, 76]. Pregnancy, along with lactation, emerge as hallmark episodes, driving the breast's most development marked by lobules type 4 [76]. However, postmenopause both nulliparous and parous women find common ground, characterized by Lob 1 predominance. It is worth noting that the increased risk of breast cancer in women who have never given birth (nulliparous) compared to those who have (parous), even when their postmenopausal lobular compositions appear to be similar, suggests the presence of subtle inherent differences or varying susceptibility to carcinogenic factors within lobules type 1 [77]. A conjecture emerges positing that Lob 1 in nulliparous and certain parous women remains in an undifferentiated state, housing epithelial cells predisposed to carcinogenic triggers and consequent neoplastic transformation, denoted as Stem cells one. In contrast, Lob 1 formations within early postmenopausal parous women, untouched by mammary pathology, host transformation-resistant stem cells 2 [76]. Central to this notion is the hypothesis that early pregnancy-triggered differentiation confers a distinct genomic signature upon stem cells two, diverging them onto a course resilient against carcinogenic activation. While in-depth exploration is requisite to decipher the intricate mechanisms and gene interplay shaping stem cells 2 exclusive genomic landscape, the cumulative available data indicate that the differentiation induced by pregnancy guides stem cells 1's transition into the stead-



▶ **Fig. 3** Molecular mechanisms of breastfeeding aiding in protection against breast cancer.

fast stem cells 2, collectively endowing the mammary gland with an inherent armor against malignant progression [76].

Throughout pregnancy and lactation, dynamic transformations transpire within the breast tissue in anticipation of milk synthesis and nursing.

### Apoptosis (programmed cell death)

Apoptosis, a fundamental process of controlled cell death, plays a crucial role in various aspects of mammary development, ranging from the initial construction of the mammary gland during early embryonic stages to the regression phase after the lactation cycle. The highest frequency of apoptotic events occurs during mammary involution, marked by a significant wave of programmed cell death [78]. During this period, most of the secretory epithelium in the lactating breast undergoes apoptosis, as the mammary gland regresses and undergoes restructuring in anticipation of the next lactation cycle [79]. An examination of morphological constituents and gene expressions intimates a two-phased portrayal of involution-driven apoptosis: a preliminary controlled apoptosis prompted by hormonal withdrawal, succeeded by a subsequent wide-ranging apoptosis modulated by proteases. This later stage is triggered by changes in cell-matrix interactions and detachment from anchoring. Intriguingly, breastfeeding emerges as an activator of elevated apoptosis rates within breast tissue. This discernment implies that cells marred by DNA damage or mutations are nudged towards apoptosis, curtailing their malignant evolution.

The regulation of apoptosis is a complex interplay orchestrated by intricate signaling pathways intrinsically embedded within cells. The transformative impact of breastfeeding encompasses hormonal and molecular signals, potentially amplifying the activation of pro-apoptotic pathways. This mechanism, in turn, furnishes a strategic avenue for the eradication of cells bearing incipient cancerous modifications.

### Reduced ovulation frequency

Extensive investigations have substantiated a correlation between a woman's susceptibility to breast cancer and her interaction with hormones produced by her ovaries, specifically the endogenous estrogen and progesterone. Reproductive factors that prolong the duration and intensity of exposure to ovarian hormones, which in turn stimulate cellular proliferation, have demonstrated associations with an increased risk of breast cancer [80, 81]. Some scientific inferences suggest that these differentiated cells exhibit heightened resilience against malignant transformation compared to their undifferentiated counterparts, underpinning the notion that differentiation fosters resistance to cancer cell metamorphosis [76]. Breastfeeding, particularly exclusive breastfeeding, can initiate a process called lactational amenorrhea, where the cessation of ovulation and menstruation results from hormonal shifts tied to lactation. This occurrence is partially regulated by the hormone prolactin, which inhibits the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus. Consequently, this leads to

a reduction in the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland [11]. These hormones are integral to fostering ovarian follicle maturation and triggering ovulation. Reduced frequency of ovulation translates to a decrease in menstrual cycles and diminished exposure to estrogen and progesterone—hormones that wield influence over the progression of hormone-responsive breast cancers [82, 83]. It is important to highlight that estrogen can stimulate the proliferation of breast cells, which contributes to the development of breast cancer [84]. Through this transient attenuation of ovulation and curtailed hormonal exposure, breastfeeding holds the promise of mitigating the risk linked to breast cancer engendered by estrogen influence.

## HAMLET

HAMLET, short for “Human  $\alpha$ -lactalbumin made lethal to tumor cells”, represents an exceptional protein-lipid complex distinguished by its remarkable capacity to selectively target and eliminate tumor and immature cells, all the while preserving the healthy and differentiated ones [85, 86]. The complex structure results from the combination of partially unfolded  $\alpha$ -lactalbumin and oleic acid, both of which are common components found in human milk. This configuration shift transpires upon calcium ion ( $\text{Ca}^{2+}$ ) removal from native  $\alpha$ -lactalbumin, leading to protein unfolding that unveils fresh fatty acid binding domains, securely accommodating oleic acid [87, 88]. Critical to its tumor-targeting potency, oleic acid is indispensable, as solely the unfolded protein lacks the capacity to induce tumor cell death [89, 90].

### HAMLET a shield against tumorigenesis

HAMLET (Human  $\alpha$ -lactalbumin made lethal to tumor cells), a unique complex formed from  $\alpha$ -lactalbumin and oleic acid present in human milk, has been shown to induce cell death in both tumor and bacterial cells [91]. Tumor cells experience apoptosis triggered by HAMLET, while well-differentiated normal cells exhibit resilience against its impact [92]. The widespread effectiveness of HAMLET in combating tumors is emphasized by its ability to act against a wide range of tumor cells, including more than 40 different types of lymphoma and *in vitro* carcinoma cell lines [90]. This breadth suggests the initiation of fundamental cell death pathways within tumor cells [91]. Although the mechanism leading to cell death is complex, thorough examination has outlined multiple pathways through which HAMLET induces cell death in tumor cells, including apoptosis, anoikis, and autophagy [91, 93]. Following cellular internalization, HAMLET instigates swift mitochondrial obliteration [93]. Nucleic acid translocation exerts its impact by intricately binding to histones and nucleosomes. It disrupts the functionality of transcription machinery. The discovery of HAMLET's activity was an unexpected outcome during research involving human milk fractions aimed at studying bacterial adhesion to lung carcinoma cell lines. In addition to its role in blocking adhesion, a specific milk fraction surprisingly revealed its ability to trigger cell death, leading to apoptosis [93]. HAMLET possesses unique biological properties, selectively eliminating cancerous

cells through a mechanism resembling apoptosis, while leaving normal cells unharmed [88]. This indicates that HAMLET manages to bypass the diverse mechanisms of resistance to apoptosis that are often exhibited by tumor cells. Instead, it initiates alternative cell death pathways that remain functional in these tumor cells. HAMLET highlights the therapeutic possibilities within human milk, where a wealth of molecules may have beneficial implications for various human health conditions. The conditions necessary for HAMLET formation occur in the stomachs of breast-fed infants. The lower pH environment in the stomach may induce protein unfolding through calcium release, while acid-sensitive lipases catalyze the breakdown of milk triglycerides to release oleic acid [94]. The potential consequences extend to lymphoid cells within the gut-associated lymphoid tissue, as breast-fed infants demonstrate significantly lower rates of lymphoma compared to bottle-fed infants. HAMLET's broad anti-tumor effectiveness is evident in laboratory studies, and its therapeutic capabilities are confirmed through *in vivo* experiments using a rat model of human glioblastoma, as well as in patients with skin cancer (papillomas) and bladder cancer [94, 95].

In a previous investigation, the influence of HAMLET on mammary cells was examined closely. HAMLET-embedded plastic pellets were administered to lactating mice and a three-day exposure resulted in morphological changes characteristic of apoptosis. There was also an increase in caspase-3 activity observed in alveolar epithelial cells close to the HAMLET pellets, with no discernible effect in distant areas or in contralateral glands. This effect was unique to HAMLET, as native  $\alpha$ -lactalbumin or isolated fatty acids showed no impact on mammary glands. Additionally, HAMLET induced cell death in a mouse mammary epithelial cell line [96]. HAMLET is capable to induce apoptotic cell death in mammary gland tissue. Prototypic strategies for prophylactic cancer vaccination have been established using various mouse breast cancer models.  $\alpha$ -lactalbumin, a differentiation protein specific to the breast and prominently expressed in most human breast carcinomas and solely in lactating mammary epithelial cells, were chosen as the autoantigen for vaccination. Developing immunity against  $\alpha$ -lactalbumin provides significant protection against spontaneous tumor growth in transgenic breast cancer mouse models and transplanted 4 T1 breast tumors in BALB/c mice [96]. Since  $\alpha$ -lactalbumin is expressed conditionally during lactation, vaccination-induced protection occurs without noticeable inflammation in non-lactating breast tissue [96]. This highlights the potential safety and effectiveness of  $\alpha$ -lactalbumin vaccination in protecting against breast cancer development in premenopausal women during their post-childbearing years, a period when lactation can be avoided, and the risk of breast cancer is higher. An overview of breastfeeding associated molecular mechanisms aiding in protection against breast cancer is given in ► **Table 1**.

### Link between duration of breastfeeding and breast cancer

Long noncoding RNAs have regulatory roles in multiple processes, including cell differentiation, proliferation, migration, and the cell cycle. A recent study identified upstream Eleanor (u-Eleanor), a



► **Table 1** Overview of breastfeeding associated molecular mechanisms aiding in protection against breast cancer.

Breastfeeding associated molecular mechanism	Role in preventing breast cancer risk	Sources
Differentiation and maturation	Throughout pregnancy, labor, and lactation, the mammary gland undergoes a dynamic remodeling process orchestrated by the complex interplay of lactogenic hormones. This intricate sequence involves the activation of mammary stem and progenitor cells, ultimately leading to the differentiation and maturation of cells responsible for milk production. Importantly, this maturation process during breastfeeding contributes to a reduction in the likelihood of tumor development or the onset of breast cancer.	Ambrosone et al. 2020 [97] Witkowska-Zimny et al. 2017 [98]
Apoptosis	The mammary gland microenvironment undergoes significant remodeling during lactation, potentially influencing breast cancer susceptibility. Elevated calcium concentrations in breast milk actively suppress cellular apoptosis and necrosis through disruption of intercellular connections. Additionally, Secretory IgA (SIgA) and Lactalbumin Alpha (LALBA) exhibit anti-tumorigenic properties by suppressing breast cancer cell growth and promoting apoptosis. Collectively, these findings suggest that breastfeeding may contribute to the elimination of premalignant and malignant cells, providing a possible mechanism for its observed association with reduced breast cancer risk.	Karbasi et al. 2022 [99] Honorio-França et al. 2016 [100]
Immune System	Prolonged breastfeeding (> 12 months) reduces IRIS (BRCA1 splice variant) expression through VD/VDR/STAT3 signaling, promoting terminal differentiation and immune clearance of these cells upon involution [101]. Extended lactation promotes terminal differentiation of mammary epithelial cells, promoting their post-involution clearance. Conversely, insufficient breastfeeding may leave IRIS-overexpressing progenitors susceptible to immune evasion and potential tumorigenesis during involution. These findings suggest a potential link between breastfeeding duration, IRIS regulation, and breast cancer risk.	Castillo et al. 2022 [102] ElShamy et al. 2016 [49]
Reduced Ovulation	Breastfeeding extends the postpartum period of amenorrhea, primarily through prolactin-mediated inhibition of GnRH. This delay in ovulation translates to reduced lifetime estrogen exposure, a known risk factor for breast cancer [103].	Beaber et al. 2008 [104] Chen et al. 2023 [105]
HAMLET	Alpha-lactalbumin, a prominent protein in human milk, undergoes a remarkable transformation when encountering oleic acid. This interaction forms HAMLET (Human Alpha-lactalbumin Made Lethal to Tumors), a complex with potent cytotoxic activity towards tumor cells. The most intriguing aspect of HAMLET lies in its selectivity. Unlike conventional chemotherapeutic agents, HAMLET preferentially induces apoptosis (programmed cell death) in tumor cells while sparing normal, differentiated cells.	do Carmo França-Botelho et al. 2012 [106] Abraham et al. 2023 [107]

novel lncRNA with key functions in breast cancer [101]. The connection between hormone-dependent reproductive risk factors in breast neoplasms and lncRNAs, u-Eleanor, and HOTAIR was discovered. Studies showed that women who had not lactated in the past had a higher level of u-Eleanor expression compared to those who had breastfed [108]. Furthermore, there was an observed increase in u-Eleanor expression as the duration of lactation decreased. Similarly, another study revealed a higher level of u-Eleanor in women who breastfed for a shorter duration (one to six months) compared to those who breastfed for a longer period (greater than 24 months) [109, 110]. Both exclusive breastfeeding for up to six months and continued breastfeeding accompanied with solid meals, for up to two years, according to the WHO standards, confer great short-term and long-term health benefits for both the child and mother alike [103, 111]. It not only provides cognitive and immune development for the infant, but also prevents the threat of childhood cancers, obesity, sudden infant death syndrome (SID) and respiratory diseases [103, 112, 113,

114, 115]. The immediate, as well as long-term impacts of breastfeeding have significant importance for women health, wellbeing, and survival [14, 116]. Instant effects of breastfeeding show up soon after childbirth, in the form of visceral fat loss and improved metabolic functions [117, 118]. It also serves as a natural contraceptive through inhibited ovulations and ensures uterine health through suckling stimulus derived uterine contractions during breastfeeding, aiding in the removal of fetal components which may otherwise lead to postpartum hemorrhage [119, 120]. Lactation speeds up the reversal of pregnancy-induced metabolic changes and fat accumulation, which would otherwise pose a risk of metabolic diseases, insulin resistance, gestational diabetes, and hypertension [121, 122].

On the other hand, long-term impact of breastfeeding includes prevention of hypertension, osteoporosis, cerebrovascular incidence and cancers, particularly the BRCA1 mutation-associated breast cancer [117, 123]. Numerous studies have confirmed opposing effects of extended breastfeeding practices upon the risk

of endometrial, ovarian and breast carcinomas in women [112, 124]. It has been shown that breastfeeding not only prevents the risk of breast cancer development, but also its recurrence in breast cancer treated cases [125].

### Lactation inhibition and hormone use

Hormones like estrogens have been commonly employed to hinder lactation. For instance, in the study by Newcomb et al., 43% of women aged 20–74 years in the control group reported using hormones to inhibit milk flow [126]. Given that diethylstilbestrol has been linked to a slight increase in breast cancer risk among older women, various studies have explored whether the apparent protective link between lactation and breast cancer risk might be influenced by an elevated risk among users of lactation suppressants [70]. The suppressants utilized encompass a diverse group, including prolactin inhibitors, androgens, vitamins, and estrogen. Consequently, it is likely that their effects, if any, are also diverse. In general, no heightened risk of breast cancer has been observed among women who reported using lactation suppressants [35]. In early reproductive life, direct physical changes accompanying milk production in the breast may favorably influence breast tissue [60, 61]. Lactation is believed to reduce the risk of breast cancer, potentially by interrupting ovulation or modifying pituitary and ovarian hormone secretion [60, 61, 127, 128]. The use of hormones for lactation suppression, including pills, injections, and unknown forms, was associated with an exceedingly small and nonsignificant increase in risk among premenopausal women. Postmenopausal women, however, exhibited a slight increase in risk with no discernible gradient of effect. Additionally, diethylstilbestrol use during pregnancy has been linked to a modest increase in breast cancer risk among older women [129, 130]. This underscores the potential protective role of extended breastfeeding in mitigating the impact of ovulation on breast cancer risk [58].

### Future directions and multidisciplinary approaches

The future implications stemming from breast cancer and breastfeeding research are both promising and diverse. They offer the potential for personalized risk evaluation, precisely targeted therapies, and the identification of early detection markers grounded in cellular differentiation and maturation mechanisms. A comprehensive understanding of the pathways that lead to apoptosis induced by breastfeeding opens innovative avenues for cancer treatment and the prevention of resistance mechanisms. Finally, the clinical applications of HAMLET, including its role in cancer treatment and prevention, along with the development of breast cancer vaccines that target specific proteins, provide exciting prospects for improving breast cancer outcomes and advancing personalized healthcare.

### Conclusion

In this extensive examination of the intricate interplay between breast cancer and breastfeeding, we have ventured into the complex molecular mechanisms that underlie this vital connection. Breast cancer remains a significant global health challenge, im-

pacting women across diverse regions and communities. Despite advances in medical practices, its incidence continues to climb, posing both public health dilemmas and economic burdens. Reproductive factors, including breastfeeding practices, have emerged as modifiable risk factors capable of exerting a profound influence on breast cancer susceptibility. Beyond its primary role in infant nutrition, breastfeeding bestows a plethora of benefits upon both mothers and infants. Notably, breastfeeding has demonstrated to be effective in risk reduction of breast cancer, with each additional 12 months of breastfeeding correlating with a substantial reduction in this risk. Our investigation into the molecular aspects of this relationship has unveiled critical pathways that breastfeeding confers to prevent breast cancer: cellular differentiation and maturation, apoptosis (programmed cell death), reduced ovulation frequency, and the intriguing Human  $\alpha$ -lactalbumin made lethal to tumor cells (HAMLET). The intricate molecular details emphasize the multifaceted aspect of breast cancer, especially in its association with breast feeding. While breastfeeding undeniably provides concrete protective advantages, the Health Organization (WHO) and UNICEF aim to heighten awareness and bolster breastfeeding practices on a global scale. By shedding light on the intricate molecular mechanisms through which breastfeeding mitigates breast cancer risk, this review underscores the imperative of fostering increased breastfeeding rates, both in low- and high-income countries. Such efforts promote breastfeeding, which significantly reduces the risk of breast cancer. It is noteworthy that global breastfeeding rates still fall below recommended levels.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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