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Abstract:

Background: Breastfeeding has many short-term and long-term health benefits for infants. Short-term benefits include protection against childhood infections and mortality in low income countries. The adult long-term effects usually emphasized are a reduction of excess weight and type 2 diabetes. However, there is a lack of available data on the impact of **having been** breastfed on adult fertility. Indeed, infertility **probably has** a multifactorial origin, including an environmental origin. The aim of this study was to investigate whether having been breastfed could be associated **with** unexplained infertility.

Materials and Methods: This research is an ancillary study of the case-control study ALIFERT, for which both fertile and infertile couples were recruited. Breastfeeding statuses, collected f**rom** childhood health records, were compared among fertile and infertile individuals. Anthropometrics parameters were also used for analysis.

Results: 65.6% of infertile women and 63.3% of fertile women were breastfed, and 69% of infertile men and 67.4% of fertile men were breastfed. There was no statistically significant difference between fertile and infertile groups. Nevertheless, infertile women who were not breastfed had a significantly higher BMI than those who were breastfed (25.8 kg/m2 vs 23.2 kg/m²).

Conclusion: In our study, we did not observe an**y association between having been breastfed** and fertility in adulthood. However, we observed that, in infertile women, **having not been** breastfed may **influence** weight in adulthood.

Trial registration: NCT01093378 ALIFERT. Registered: March 25, 2010.

Keywords: breastfeeding; BMI, fertility

Introduction

Several studies have reported that breastfeeding has many **short and long term** health benefits to infants. In 2001, World Health Organization (WHO) gave some worldwide recommendations on breastfeeding. Specifically, it encouraged exclusive breastfeeding until the age of 6 months[1]. More evidence based on a systematic **literature** review **published i**n 2007 confirmed these recommendations[2]. These observations published by the WHO were recently confirmed in a meta-analysis published in the Lancet[3]. The short-term benefits highlighted were a protection against childhood infection such as diarrhoea and respiratory infections. Forty-six studies conducted in low income countries showed that breastfeeding is associated with a 68% reduction in malocclusions[4]. A reduction of the risk of death in high income countries was also highlighted[5].

Long-term effects of breastfeeding were associated with a 13% reduction in adults becoming overweight or obese and a 35% reduction in the incidence of type 2 diabetes in adulthood[3]. **Having been breastfed** was also associated with increase**d** performance in intelligence tests **during** childhood and adolescence, with a 3- to 4-point increase in intelligence quotient (IQ) points[6].

Given the manifest health benefits of breastfeeding, we wondered about the possibility of an association between **having been breastfed** and fertility in adulthood. To our knowledge, there is no published research on this particular aspect of breastfeeding.

Few animal studies have been published **about** the influence of newborns overfeeding or underfeeding on their reproductive functions. Castellano demonstrated the influence of changes in early postnatal feeding on the timing of puberty and development of the hypothalamic kisspeptin system involved in the reproductive function[7]. These results have been confirmed by Caron's study[8]. They showed that neonatally undernourished and overnourished females

display perturbed development of neural projections from the arcuate nucleus to the preoptic region with adverse consequences on neural projection of kisspeptin and puberty onset.

These experiments underline the importance of early nutrition in the development of the reproductive system. Similarly, **having been** breastfed has largely been described as protective against several illnesses; thus, in this paper, we intended to evaluate if it may impact fertility in adulthood. In order to answer to this question, we compared the breastfeeding **status of** both fertile and infertile couples.

Materials and Methods

Couple recruitment

Data from patients recruited for the ALIFERT case-control study were analysed[9]. The purpose **of** the ALIFERT study was to **evaluate** the link between unexplained infertility and the patient's nutritional behaviour. Unexplained infertility is defined by a lack of diagnosis for couples that have failed to conceive after one years of unprotected sexual intercourse. Standard investigation protocol of unexplained infertility is somewhat limited and mainly involves **normal** ovulation and tubal **assessment** for women, **as well as normal** semen analysis for men.

Couples were recruited from September 2009 to December 2013 from 4 **fertility** centres in France (Jean Verdier Hospital in Bondy, Cochin Hospital in Paris, North Hospital in Saint Etienne, and Polyclinic Navarre in Pau).

The inclusion criteria for the infertile groups were: individuals who had experienced more than 12 months of unexplained infertility; **female or male ages** between 18 **and** 38**, or** 18 **and** 45 **years old, respectively;** and individuals being in possession of childhood health records.

The fertile couples were healthy volunteers recruited nearby these hospitals. The inclusion criteria for the fertile group were: **female or male age** between 18 **and** 38**, or** 18 to 45 **years old, respectively**; individuals who were the biological parent of a child under 2 years of age, spontaneous**ly** conceived in less than 12 months, and in possession of their childhood health records.

Data collection

To avoid reporting bias, only childhood health records completed by doctors were accepted as viable records of a participant's breastfeeding status. Each participant had his or her weight and height measured by the same trained investigator, using the same calibrated devices. The body mass index (BMI) of each participant was calculated as the weight in kilograms divided by the square of height in metres.

All participants gave their written informed consent. The ALIFERT study was approved by an ethics committee. (National biomedical research ID no. P071224; ethics committee approval ('Comité de Protection des Personnes') ID no. AOM 2009-A00256–51; NEudra CT ID no. 08180; clinicaltrials.gov ID no. NCT01093378).

Statistical analysis

Data was summarized using means and standard deviations. Statistical differences were analysed using unpaired Student's *t* test for the quantitative data; and the Chi 2 test for the qualitative data.

$P < 0.05$ was considered significant.

Results

Ninety-three infertile women, 98 fertile women, 87 infertile men and 95 fertile men were included in this ancillary study. All participants were born at term (gestational age was between 37 and 41 weeks of amenorrhea).

Age, BMI and breastfeeding status of the participants are presented in Table 1. Fertile and infertile men had comparable ages whereas fertile women were slightly older than the infertile women. The BMI of infertile men and women was significantly higher compared to fertile participants.

65.6% of infertile women and 63.3% of fertile women had been breastfed and 69% of infertile men and 67.4% of fertile men had been breastfed. The difference was not statistically significant between groups (respectively: p= 0.764 and p= 0.874).

We examined more specifically the BMI according to the different groups. We observed that infertile women who had not been breastfed had a significantly higher BMI than those who had been breastfed (25.8 kg/m2 +/-5.55 vs 23.2 kg/m2+/-4.13, p=0.018). We did not observe such differences in the other groups (Table 2).

Discussion

We did not observe any association between unexplained infertility in adulthood and **having been breastfed** in the neonate period, neither for women or men. Nevertheless, interestingly, among the infertile group, we noted that the non-breastfed women had significantly higher BMI than those who had been breastfed, with **a shift toward** the overweight BMI category.

Breastfeeding has many beneficial and protective effects on the short- and long-term health of individuals, as evidenced by the recommendations of the WHO on breastfeeding [3,6,10]. Several studies have shown that long-term health programming mechanisms are established during the prenatal and first years of life [11]. This concept is well-known as the ”first 1000 days” of life

(including gestation and the first two years of life), a period of vulnerability in human development [12,13].

In the ALIFERT cohort, we had previously reported that an increased birth weight was a risk factor for unexplained infertility **both** in men [14] and women [15]**, suggesting a** link between prenatal period and fertility in adulthood. In the present study, we aimed to examine the potential impact of early postnatal period on fertility **at** adulthood. A**n association** between **having been** breastfed and fertility was not highlighted; but we observed a link between **having been** breastfed and **female** weight **in the infertile subgroup.**

Studies have indicated that nutrient imbalance in early life influences the risk of obesity later in life [16,17], suggesting that obesity may result from "developmental programming". Breastfed newborns have a better regulation of the amount of milk ingested [18] and they are significantly lighter at 9 months of age[19]. Some reports highlighted an association between **having been** breastfed and **a relative** protection against obesity later in life. Higher plasma-insulin concentrations in bottle-fed compared to breast-fed infants could stimulate fat deposition and lead to **an** early development of adipocytes[20]. Although the origin of obesity is complex and multifactorial, rapid weight gain in early childhood has been clearly identified as a risk factor for the development of subsequent obesity and metabolic dysfunction [21]. Thus, breastfeeding is known to have a protective effect on the early rebound of adiposity in children, which is known to have deleterious effects on the onset of puberty and increases the risk of long-term obesity[22,23]. Pubertal timing is an indicative marker for the neuroendocrine system, which regulates the development of reproductive system. A recent large-scale study showed that early pubertal timing was associated with a lower sperm concentration and negatively associated with estrogen levels[24]. Both testicular somatic cells and germ cells are sources of estrogen in mammals. Exposure of testis to extra-estrogen contributes to lower sperm concentration[25].

178 Pubertal timing can therefore **be** used as an indicative marker for hormone levels in adult men,
179 and consequently for their fertility.

180 **O**verweight **and** obesity are **known** risk factors of infertility in both men [26] and women [27]. We
181 assume that the lack of protective breastfeeding in early life, combined with an unhealthy
182 lifestyle in adulthood, could lead to obesity and therefore, by extension, **could** contribute to
183 infertility. An accumulation of risk factors could be envisaged, reinforcing our hypothesis that
184 unexplained infertility origin is multifactorial and may have a developmental origin (pre- and
185 post-natal). Studies have shown that infertile individuals are in poorer health than fertile
186 individuals [28,29], and would **have been** more susceptible to unfavourable foetal or neonatal
187 programming. These hypotheses underline a possible indirect impact of **having been** breastfed
188 on the reproductive functions in adulthood

189 A direct effect of **having been** breastfed on fertility in adulthood may also be considered. Thus,
190 leptin is present in breast milk[30] and plays a critical role in the long-term protection against
191 obesity and metabolic disorders[31]. Leptin is also an essential factor for brain development and
192 neural projection[32]. A lack of leptin intake during the neonatal period could have consequences
193 on the development of the reproductive axis[7,8,33] and, therefore, have consequences on fertility
194 in adulthood. Another theory is the potential role of epigenetics mechanisms through early
195 postnatal nutrition in the developmental programming. **Leptin may play a critical role** in the
196 DNA methylation patterns **establishment** and the response to dietary conditions in later life[34].
197 Furthermore, miRNA**s are** present in high concentration in breast milk [35] and **could** influence
198 individual development.

199 The strengths of the study are the recruitment of two comparable groups of fertile and infertile
200 couples. The assessments on breastfeeding **status** were **registered** from health book completed
201 by a medical staff, **in order to** limit bias due to declarative information. However, our study

had some limitations, such as the lack of information concerning the duration of breastfeeding and the type of breastfeeding (exclusive or not). In some meta-analyses, the duration of exclusive breastfeeding is a protective factor for obesity in adulthood [36,37]. The duration of breastfeeding may also have an impact on the age at which adiposity rebound occurs[38,39]. We recognise that the limited sample size of the groups may decrease the **accuracy** of our study. Consequently, further studies are needed for meaningful conclusions; in particular, studies which take into account breastfeeding characteristics such as its duration and the type of breastfeeding (exclusive or not). It should be noted that animal studies could be useful in obtaining quick answers.

In conclusion, in our study, we did not observe **any association between having been breastfed** and fertility in adulthood. **However,** an association **was observed** between **having** not been breastfed and a high BMI **in the subgroup of infertile women, suggesting that n**ot being breastfed **could constitute** a factor contributing to the onset of infertility. Nevertheless, infertility may be multifactorial. **Although f**urther studies are needed to fully understand this phenomenon, breastfeeding should continue to be encouraged.

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Conflicts of Interest

There are no potential conflicts of interest relevant to this article.

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Availability of data and materials

Data are the property of the Public Assistance – Paris Hospitals [Assistance Publique – Hôpitaux de Paris (AP-HP)] that does not authorize as a promoter the sharing of data without a contract. Consultation by the editorial board or interested researchers may nevertheless be considered.

Authors' contributions

CD, CF participated in the study conception and design, in patient's recruitment, data acquisition, interpretation and analysis, and drafting of the manuscript. PT participated in study design, performed statistical analysis and participated in drafting of the manuscript. SC and NS participated in study conception and design, in patient recruitment and critical revisions of the manuscript for intellectual content. RL participated in study conception and design, interpretation of data, critical revision of the manuscript for intellectual content and supervised

the study. The collaborators of the ALIFERT collaborative group participated in study design and were involved in patients' recruitment. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The ethics committee ("Comité de Protection des Personnes") approved the study. ALIFERT study (national biomedical research P071224/AOM 08180:NEudra CT 2009-A00256–51/clinical trials NCT01093378). All the participants signed a written informed consent.

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	Infertile Women	Fertile Women	Infertile Men	Fertile Men
Total	93	98	87	95
Age	***30.9+/-4.25****	***32.1+/-3.18****	33.1+/-5.19	34.3+/-3.85
BMI (kg/m^2)	***24.1+/-4.77****	***21.9+/-3.02****	***26.3+/-4.17****	***23.9+/-2.64****
Breastfeeding (%)	61 (65.6%)	62 (63.3%)	60 (68.9%)	64(67.3%)

373 *p=0.02, **p<0.001

374 Table1: Age, BMI and breastfeeding status of the fertile and infertile women and men. Data are means ± standard deviations. Significant
375 differences are written in bold italic.

376

	Infertile Women		Fertile Women		Infertile Men		Fertile Men	
	Breastfed	**Not breastfed**	**Breastfed**	**Not breastfed**	**Breastfed**	**Not breastfed**	**Breastfed**	**Not breastfed**
Total	61	32	62	36	60	27	64	31
Weight (kg)	***63.4 +/- 11.8****	***71 +/- 16****	60.2 +/- 7.8	58.8 +/- 6.8	83.5 +/- 15.4	83.9 +/- 17.2	75.8 +/-9.3	77.1 +/- 11.5
Height (m)	1.65 +/- 0.06	1.66 +/- 0.05	1.66 +/- 0.04	1.65 +/- 0.05	1.78 +/-0.07	1.79 +/- 0.06	1.79 +/- 0.06	1.77 +/- 0.07
BMI (kg/m2)	***23.2 +/- 4.1****	***25.8 +/- 5.5****	21.8 +/- 2.7	22 +/- 3.6	26.4 +/- 3.9	26.2 +/- 4.7	23.6+/- 2.4	24.6 +/- 2.9

*p=0.012, **p=0.018

Table 2: BMI of infertile and fertile women and men. Data are means ± standard deviations. Significant differences are written in bold italic.