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Abstract

Background:
Non-celiac wheat sensitivity (NCWS) most frequently presents clinically with irritable bowel syndrome (IBS)-like symptoms, although many extra-intestinal manifestations have also been attributed to it. No studies to date have evaluated the presence and frequency of gynecological symptoms in NCWS.
Aim:
To evaluate the frequency of gynecological disorders in patients with NCWS.
Patients and methods:
Sixty-eight women with NCWS were included in the study. A questionnaire investigating gynecological symptoms and recurrent cystitis was administered, and patients reporting symptoms were then examined

by specialists. Three control groups were selected: 52 patients with IBS not related to NCWS, 56 patients with celiac disease (CD), and 71 healthy controls.
Results:
59% of the patients with NCWS showed gynecological symptoms, a higher frequency than in healthy controls ($P = 0.04$), IBS controls ($P = 0.01$) and CD controls ($P = 0.02$). Menstrual cycle alterations were more frequent in patients with NCWS than in healthy controls (26.5% vs 11.3%; $P = 0.03$); the patients with NCWS suffered from recurrent vaginitis (16%) and dyspareunia (6%) significantly more frequently than healthy controls. Twenty-nine percent of patients with NCWS reported recurrent cystitis, a finding higher than in the control groups (vs healthy $P = 0.0001$, vs IBS $P = 0.001$, vs CD controls $P = 0.04$). Microbiological examinations were negative in most of the patients with NCWS and recurrent vaginitis or cystitis. During the 1-year follow-up, 46% of patients with menstrual disorders and 36% with recurrent vaginitis reported resolution of symptoms on a wheat-free diet.
Conclusions:
Patients with NCWS showed a significantly higher frequency of gynecological symptoms and recurrent cystitis than patients with IBS.

Keywords (separated by '-') Non-celiac wheat sensitivity - Vaginitis - Cystitis - Menstrual cycle abnormalities - Obstetric diseases - Irritable bowel syndrome

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ORIGINAL ARTICLE

Gynecological Disorders in Patients with Non-Celiac Wheat Sensitivity

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Background Non-celiac wheat sensitivity (NCWS) most frequently presents clinically with irritable bowel syndrome (IBS)-like symptoms, although many extra-intestinal manifestations have also been attributed to it. No studies to date have evaluated the presence and frequency of gynecological symptoms in NCWS.

Aim To evaluate the frequency of gynecological disorders in patients with NCWS.

Patients and methods Sixty-eight women with NCWS were included in the study. A questionnaire investigating gynecological symptoms and recurrent cystitis was administered, and patients reporting symptoms were then examined by specialists. Three control groups were selected: 52 patients with IBS not related to NCWS, 56 patients with celiac disease (CD), and 71 healthy controls.

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Conclusions Patients with NCWS showed a significantly higher frequency of gynecological symptoms and recurrent cystitis than patients with IBS.

Keywords Non-celiac wheat sensitivity · Vaginitis · Cystitis · Menstrual cycle abnormalities · Obstetric diseases · Irritable bowel syndrome

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Abbreviations

NCWS	Non-celiac wheat sensitivity
CD	Celiac disease
IBS	Irritable bowel syndrome
DBPC	Double-blind placebo controlled
IEL	Intra-epithelial lymphocyte(s)

Introduction

Non-celiac wheat sensitivity (NCWS) is a relatively recent definition for a condition characterized by intestinal and extra-intestinal clinical manifestations, triggered by wheat ingestion [1–3]. Patients with NCWS report symptoms after wheat ingestion and the disappearance of symptoms on a wheat-free diet, although they are not suffering from celiac

disease (CD) or wheat allergy (WA), the two well-known diseases associated with wheat (or gluten) ingestion.

The most frequent clinical presentations of NCWS include irritable bowel syndrome (IBS)-like manifestations [4], but many extra-intestinal manifestations, such as neurological dysfunction, fibromyalgia, and skin rashes have been attributed to NCWS [5, 6]. In our experience, gynecological symptoms can also be included among the possible manifestations of NCWS [7], but no studies to date have been designed to evaluate their presence and frequency in NCWS.

The aims of the present study were: (1) to evaluate the frequency of gynecological and obstetric disorders and symptoms in prospectively recruited patients with NCWS and (2) investigate possible correlations/associations between the presence of gynecological and obstetric symptoms and other clinical characteristics present in patients with NCWS.

Patients and Methods

Patients Enrolled

We prospectively enrolled female adult patients with a definitive diagnosis of NCWS based on a DBPC wheat challenge, most of them presenting with IBS-like clinical symptoms, according to the Rome IV criteria [8, 9]. 68 study patients (mean age 39 ± 11 years; range 18-67 years) were consecutively recruited between January 2017 and July 2018 at the outpatient clinic of the Department of Internal Medicine of the Hospital of Sciacca.

Control Groups

Three control groups were selected, age- and sex-matched (± 2 years) with the patients suffering from NCWS, and recruited at random during the study period at the outpatient clinics of the Department of Internal Medicine of the Hospital of Sciacca and at the Department of Internal Medicine of the University Hospital of Palermo. The first included 52 females (mean age 38 ± 10 years; range 18–65 years) with a diagnosis of IBS unrelated to NCWS or other types of food "intolerance/allergy." These patients underwent a 4-week period of elimination diet without wheat, but their IBS symptoms did not improve.

The second control group included 56 CD female patients at CD diagnosis (mean age 39.5 ± 9.5 years; range 19–66 years), and the third control group included 71 healthy and asymptomatic women (mean age 39 ± 12 years; range 18–64 years), who were undergoing a gynecological examination for a routine PAP test at the outpatient clinic of one of the authors (S.I.).

In this last group (healthy controls) and in the control group composed of patients with IBS not related to wheat ingestion, CD diagnosis was excluded on the basis of negative serum assays for CD, anti-tissue transglutaminase (anti-tTG) IgA and anti-deamidated gliadin peptide (anti-DGP) IgG antibodies.

Non-Celiac Wheat Sensitivity Diagnosis

As the first step of evaluation, CD and WA diagnoses were ruled out in all the 68 study patients according to the following criteria: (1) negative serum assays for CD, anti-tissue transglutaminase (anti-tTG) IgA and anti-deamidated gliadin peptide (anti-DGP) IgG antibodies; (2) the absence of intestinal villous atrophy, documented in all the patients carrying the DQ2 and/or DQ8 HLA haplotypes; and (3) negative IgE-mediated immune-allergy tests to wheat (skin prick tests and/or specific serum IgE testing). Inflammatory bowel diseases and other diagnoses were also excluded in accordance with current recommendations, as previously described [10, 11].

The NCWS diagnostic criteria adopted were: (a) resolution of symptoms on a standard elimination diet without wheat, cow's milk, yeast and other food(s) causing self-reported symptoms; (b) reappearance of symptoms on a DBPC wheat challenge, performed as described previously [10, 11]. For details see Supplemental File 1.

Additional inclusion criteria were: age over 18 years, follow-up period of more than 12 months after the initial NCWS diagnosis, and at least two outpatient visits during follow-up to confirm the diagnosis [10, 12].

Exclusion criteria were: (a) age < 18 years; (b) self-exclusion of wheat from the diet and refusal to reintroduce it before entering the study; (c) presence of other "organic" gastrointestinal diseases; (e) pregnancy; (f) immune deficiency disorders.

Clinical Characteristics of the Patients with NCWS

The presence/absence of the following were recorded: (a) multiple food sensitivity, evaluated according to the reaction to the DBPC challenge with cow's milk proteins and to open challenges with other foods; (b) coexistent atopic diseases (asthma, rhinitis, dermatitis); (c) autoimmune diseases; (d) anemia (hemoglobin value < 12 g/dl); (e) IBS-like symptoms (diarrhea, constipation, alternating bowel habits); (f) dyspepsia; (g) intra-epithelial lymphocyte (IEL) infiltration in the duodenal mucosa (measurements were categorized as < 25 IEL/100 enterocytes (Marsh 0), or > 25 IEL/100 enterocytes (Marsh 1); (h) eosinophil infiltration in the lamina propria of the duodenal mucosa (results were categorized as: < 40 lamina propria eosinophils per 10 high-power fields = "no infiltration," or > 40 lamina propria eosinophils per 10 high-power fields = "presence of infiltration"); (i)

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serum anti-nuclear antibodies (ANA); (l) the heterodimer codifying for the HLA DQ2 and/or the DQ8 alleles.

Questionnaire for Gynecologic and Obstetric Diseases

A structured questionnaire was administered to all study patients and the controls with IBS at entry to the study, and thus before a definitive diagnosis of NCWS or IBS unrelated to NCWS had been made. Both the healthy controls and the controls suffering from CD also received the same questionnaire. The following items were included: (A) menstrual cycle alterations: oligomenorrhea, hypermenorrhea, polymenorrhea, hypomenorrhea, metrorrhagia, amenorrhea, dysmenorrhea; (B) gynecological symptoms/disorders: spotting, vulvar pruritus, recurrent vaginitis, vulvodynia, recurrent cystitis, vaginism, dyspareunia, chronic pelvic pain, infertility; (C) obstetric diseases and conditions: pre-eclampsia, eclampsia, recurrent miscarriage, preterm delivery, placental abruption. Recurrent cystitis was also recorded. The questionnaires were administered, explained and filled in by two of the authors (S.I. and A.C.) who were blinded to the final diagnosis of the patients. All the relevant definitions are included in Supplemental File 2.

Microbiologic Examinations

All patients included in the study (with NCWS, CD, or IBS not related to wheat ingestion) who reported gynecological symptoms were examined by a gynecologist (G.I.). A number of laboratory investigations were also performed to investigate possible infectious diseases causing the symptoms: stained bacterial smears; cultures for common vulvovaginal pathogens (i.e., *Candida albicans, Gardnerella vaginalis*, *Mycoplasma* spp., Human papilloma virus, etc.). Dermatological causes of vulvovaginitis, such as psoriasis, pemphigus, lichen, etc., were also investigated.

Patients reporting cystitis underwent urine culture tests for both bacteria and yeast, using standard commercial microbiological culture systems.

Statistical Analysis

Data were expressed as mean ± SD when distribution was Gaussian, and differences were calculated using Student's *t* test. Otherwise, data were expressed as median and range and analyzed with the Mann–Whitney *U* test. Fisher's exact test or the Chi-square test were used where appropriate.

Results

Clinical and Laboratory Characteristics of the Patients with NCWS

Table 1 shows the demographic, clinical and laboratory characteristics of the 68 women with NCWS included in the study. Most of them (81%) reported IBS-like clinical symptoms. Two patients referred recurrent vaginitis and recurrent cystitis, respectively, as the main reasons for consulting a specialist, and were self-reporting a relationship between wheat consumption and the onset or worsening of these symptoms.

Thirty-five patients showed multiple food sensitivity (14 to yeast, 15 to cow's milk, 8 to tomato, 5 to soy, 3 to egg, 4 to peach, 2 to chocolate). Notably, 10 patients with NCWS tolerated other gluten-containing cereals (rye or barley).

Twenty-four patients had an autoimmune disease (autoimmune thyroiditis in almost all cases) and 37 were positive for serum ANAs. None, as per protocol, presented

Table 1 Demographic, clinical and laboratory characteristics in 68 women suffering from NCWS, included in the present study

Age (mean ± standard deviation)	39 ± 11
Presence of multiple food sensitivity	35 (51%)
Presence of atopic diseases (rhinitis, asthma, dermatitis)	32 (47%)
Presence of autoimmune diseases	24 (35%)
Presence of anemia	29 (43%)
IBS-like presentation	55 (81%)
Diarrhea	28 (51%)
Constipation	12 (22%)
Alternating bowel habits	15 (27%)
Functional dyspepsia	30 (44%)
Intra-epithelial lymphocyte infiltration in the duodenal mucosa (Marsh 1 lesion)	32 (48%)
Eosinophil infiltration in the lamina propria of the duodenal mucosa	34 (50%)
Serum ANA positivity	37 (54%)
DQ2 and/or DQ8 HLA haplotype positivity	40 (59%)

Values are given as an absolute number and as a percentage (in brackets)

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duodenal mucosa villi atrophy, but 32 of them (48%) showed > 25 IEL/100 enterocytes (see Table 1).

Gynecological Symptoms in Patients with NCWS

Figure 1 shows the frequency of obstetric and gynecological disorders in the groups studied. When untreated on a wheat-containing diet, the patients with NCWS showed a higher frequency of disorders/symptoms than healthy controls ($P = 0.04$), controls with IBS ($P = 0.01$), and controls with CD ($P = 0.02$), whereas no significant difference was observed between patients with CD or IBS and Healthy controls.

Menstrual cycle alterations were more frequent in the patients with NCWS than in healthy controls (26.5% vs 11.3%; $P < 0.03$), including both oligomenorrhea and polymenorrhea (7.5% and 11.8%, respectively). However, there were no significant differences in the single specific alterations between the groups (see Table 2).

Six patients with NCWS were in menopause, but there was no significant difference in frequency or in age at menopause onset between them and the subjects of the three control groups.

Other gynecological disorders and symptoms were more frequent in women with NCWS than in the controls with IBS and those with CD ($P = 0.04$ for both). As regards the specific symptoms (see Table 3), a significantly higher frequency was found in NCWS for recurrent vaginitis (16%) and dyspareunia (6%) than in healthy controls. A detailed analysis of clinical history showed that all patients with recurrent vaginitis had previously tried various empirical treatments, including oral and topical preparations of antibiotics and anti-fungal drugs, but without solving the problem completely or preventing its recurrence.

Frequency of Urinary Symptoms

Recurrent cystitis was significantly more frequent in patients with NCWS (29%) than in all the three control groups. Patients with CD showed a higher frequency of recurrent cystitis than healthy controls (see Fig. 2).

Microbiology Results

The eleven patients with NCWS who reported recurrent vaginitis underwent vaginal swab tests for both bacteria

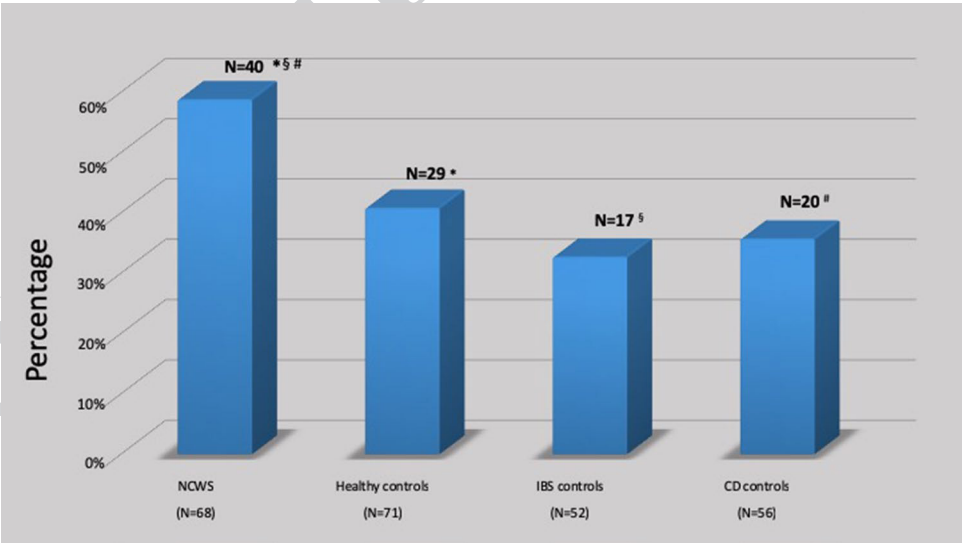

Fig. 1 Frequency of gynecological symptoms, considered as the presence of at least one symptom, in patients with non-celiac wheat sensitivity (NCWS, $N = 68$), and in healthy controls ($N = 71$), in irritable bowel disease (IBS) controls whose symptoms did not improve on elimination diet ($N = 52$), and in celiac disease (CD) controls ($N = 56$). *NCWS versus healthy controls $P = 0.04$; §NCWS versus IBS controls $P = 0.01$; #NCWS versus CD controls $P = 0.02$

Table 2 Frequency (number of cases and percentage) of menstrual disorders in 68 women with non-celiac wheat sensitivity (NCWS), in 71 healthy controls, in 52 subjects with irritable bowel syndrome (IBS) and symptoms not related to wheat or other food sensitivity, and in 56 patients with celiac disease (CD)

	NCWS ($n = 68$)	Healthy controls ($n = 71$)	IBS controls ($n = 52$)	CD controls ($n = 56$)
Oligomenorrhea	5 (7.5%)	3 (4.2%)	4 (7.7%)	3 (5.4%)
Polymenorrhea	8 (11.8%)	1 (1.4%)	5 (9.6%)	5 (8.9%)
Hypermenorrhea	0	0	0	0
Hypomenorrhea	1 (1.5%)	1 (1.4%)	0	1 (1.8%)
Menorrhagia	1 (1.5%)	2 (2.8%)	0	1 (1.8%)
Amenorrhea	0	0	1 (1.9%)	0
Dysmenorrhea	3 (4.4%)	2 (2.8%)	0	0

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Table 3 Frequency (number of cases and percentage) of gynecological disorders and/or symptoms in 68 women with NCWS, in 71 healthy controls, in 52 subjects with irritable bowel syndrome (IBS) and symptoms not related to wheat or other food sensitivity, and in 56 patients with celiac disease (CD)

	NCWS ($n=68$)	Healthy controls ($n=71$)	IBS controls ($n=52$)	CD controls ($n=56$)
Spotting	2 (2.9%)	2 (2.8%)	0	1 (1.8%)
Vulvar itching	0	2 (2.8%)	0	0
Recurrent vaginitis	11 (16.2%)*	2 (2.8%)*	6 (11.5%)	5 (8.9%)
Vulvodynia	1 (1.5%)	0	0	0
Vaginism	1 (1.5%)	0	0	0
Dyspareunia	4 (5.9%)§	0§	1 (1.9%)	0
Infertility	1 (1.5%)	5 (7%)	1 (1.9%)	1 (1.8%)
Chronic pelvic pain	0	1 (1.4%)	0	0
Endometriosis	1 (1.5%)	2 (2.8%)	1 (1.9%)	0

*$P=0.01$; $^{\S}P=0.05$

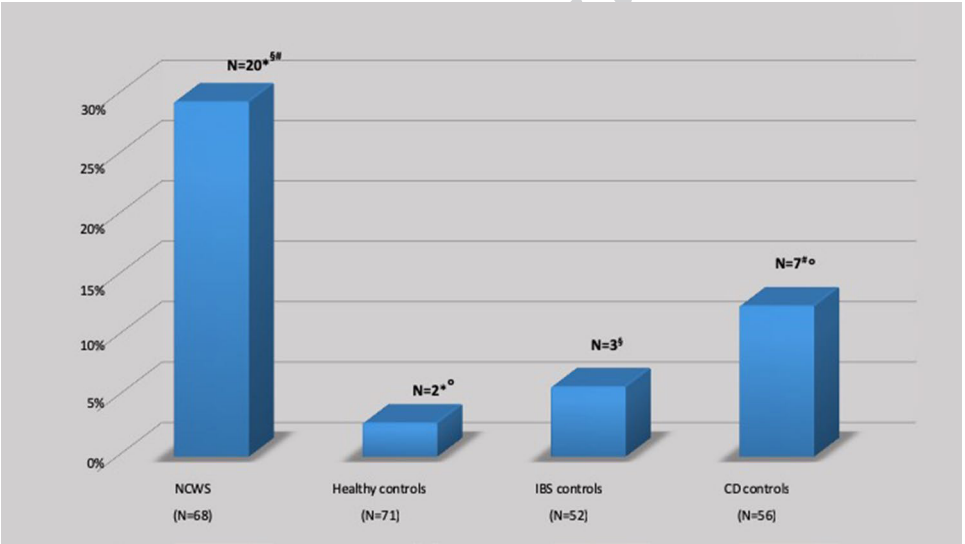

Fig. 2 Frequency of recurrent cystitis in patients with non-celiac wheat sensitivity (NCWS, $N=68$), and in healthy controls ($N=71$), in irritable bowel disease (IBS) controls whose symptoms did not improve on elimination diet ($N=52$), and in celiac disease (CD) controls ($N=56$). *NCWS versus healthy controls $P=0.0001$; §NCWS versus IBS controls $P=0.001$; $^{\#}$NCWS versus CD controls $P=0.04$; °CD controls versus healthy controls $P=0.04$

and yeast when they were symptomatic for itching, burning, and abnormal discharge. Culture was positive in only one patient (presence of *Escherichia coli*), whereas all the others tested negative for *Candida* and bacteria.

Fifteen of the twenty patients with NCWS and recurrent cystitis underwent a urine culture test when they were symptomatic. Only five of these patients (33%) tested positive for bacterial infection (all for *Escherichia coli* spp.).

Obstetric Disorders

No significant differences were observed between the four groups studied as regards obstetric disorders. Recurrent miscarriage was recorded in two subjects with NCWS and one CD control, but in none of the IBS or healthy controls (differences not significant).

Correlation/Association Between the Presence of Gynecologic Symptoms and Other Clinical Characteristics in Patients with NCWS

Analysis of the characteristics of the patients with NCWS presenting gynecological disorders or symptoms showed that they were associated with the presence of IBS-like manifestations. Indeed, these were present in 38 of the 40 (95%) who presented gynecological symptoms, but in only 17 of the 28 (59%) who did not ($P=0.001$). Furthermore, there was a higher frequency of multiple food allergy (14/18 patients) among the patients with menstrual cycle disorders than in those without (21/50) ($P=0.02$), and also of atopic disease (14/18 vs 18/48, respectively; $P=0.005$).

No other associations between NCWS clinical characteristics and specific gynecological disorders or symptoms were observed.

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Follow-up Data in the Patients with NCWS

During the follow-up, although gastrointestinal condition was re-evaluated in all patients with NCWS, no specific charts were filled in for the gynecological diseases. However, six of the thirteen (46%) patients with menstrual disorders (oligo- or polymenorrhea) reported that periods regularized during the second or third month after wheat was eliminated from the diet. Four out of eleven (36%) patients with recurrent vaginitis reported no more than one episode during the one-year follow-up, but in the others there was no improvement. Symptoms persisted in the dyspareunia patients on elimination diet (wheat-free). Finally, only two of the twenty patients with recurrent cystitis reported a lower frequency or a resolution of this disorder during follow-up.

Discussion

NCWS is characterized by a series of intestinal and extra-intestinal manifestations; the best studied of these are probably neurological disorders, which have been identified as "wheat or gluten-dependent" [13, 14], but many other symptoms have been reported to be part of the NCWS spectrum, despite the lack of specifically designed studies.

In our study, we prospectively included patients with NCWS diagnosed using the current clinical gold standard, the DBPC wheat challenge, and, for the first time, evaluated the frequency of gynecological and obstetric disorders associated with the NCWS condition. Only one case study to date has reported a relationship between NCWS and infertility [15].

We found that patients with NCWS had a significantly higher frequency of gynecological diseases and recurrent cystitis than healthy controls, IBS controls, and CD controls. In fact, 40 of the 68 (59%) study patients with NCWS had a gynecological condition; in two cases the gynecological symptoms were the reason behind the request for consultation with a specialist, as these patients self-reported that wheat ingestion caused their symptoms.

The specific conditions significantly associated with NCWS, which were more frequent in this group than in the healthy controls were: altered menstrual cycle duration (both oligomenorrhea and polymenorrhea), recurrent vaginitis, dyspareunia, and recurrent cystitis. This last symptom was significantly more frequent in NCWS, not only than in healthy controls, but also than in the controls with IBS or CD.

The relationship between gynecological disorders and IBS is well known and has been extensively studied in the literature [16–20], and unjustified pelvic surgery has even been reported in patients with IBS [21]. A recent primary care registry study on over 13,000 subjects with IBS confirmed a clear association with urogenital symptoms and infections [22].

The results of the present study, however, seem to indicate that our patients with NCWS constituted a subgroup of IBS showing a stronger association with uro-gynecological diseases than the subjects with IBS whose condition was not related to wheat ingestion (who served as controls in this study).

Of particular interest is the high frequency of recurrent vaginitis and cystitis observed in the patients with NCWS and the findings that all those with vaginitis, except for one, tested negative for vaginal swab culture test, and that two-thirds of those with cystitis had a negative urine culture test. These inflammations and their related symptoms were therefore not linked to infections. A pathogenetic hypothesis could be that the inflammation is due to an allergic mechanism, based on a non-IgE-mediated wheat allergy. Previous studies by our group have suggested this hypothesis for intestinal inflammation in NCWS [7, 23, 24].

Furthermore, the possibility of recurrent vaginitis linked to IgE-mediated allergic mechanisms has been suggested [25], and allergic vulvovaginitis is generally included as an important cause of vulvovaginitis [26]. Interestingly, the vaginal mucosa has been demonstrated to contain several immune-competent cells, such as mast cells, eosinophils, plasma cells and macrophages, and that allergens can reach the mucosa not only directly, but also as inhaled or digested allergens, thus activating the release of histamine and other inflammatory mediators [27].

Moreover, our recent data showed an abundant eosinophil infiltration in the rectal mucosa of most patients with NCWS, revealing that rectal inflammation is also a characteristic of NCWS [11]. Consequently, the anatomic proximity between an "inflamed" rectum and other pelvic organs could be the pathogenetic explanation for the high frequency of urogenital disturbances in NCWS, this "specific subgroup" of IBS patients.

Despite the strength of this study—the first prospective study specifically designed to investigate gynecological diseases in NCWS and based on the current diagnostic gold standard (the DBPC wheat challenge)—its limitations must be underlined.

First, the questionnaire used to record the gynecological conditions had not been previously validated.

Second, the pathogenetic hypothesis that rectal inflammation could possibly explain the pelvic involvement was not supported by histology; our patients did not undergo rectal biopsy, nor bladder mucosa biopsy in the case of those with recurrent cystitis. It would have been of great interest to investigate possible mast cell infiltration and degranulation, a mechanism which has been reported, especially in interstitial cystitis [27], and has been supported in a sensitized

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rat model, used to study the role of colon irritation in pelvic organ cross-sensitization [28].

Third, our NCWS population was characterized by the clinical stigmata of immunologic reaction: There was a high frequency of multiple food sensitivity, associated autoimmune diseases, and serum anti-nuclear antibodies. Although these are regular characteristics, which we have frequently seen in patients diagnosed with NCWS, our findings must be viewed with caution and not be extended to all "people who avoid gluten."

Finally, the 1-year follow-up was not specifically designed for the study of the gynecological disturbances and, although our data seem to indicate an improvement in both menstrual irregularities and recurrent vaginitis on a wheat-free diet, we cannot affirm that the gynecological disorders were indeed due to wheat ingestion and resolved with the elimination of wheat from the diet.

In conclusion, this study demonstrated that the patients with NCWS had a very high frequency of gynecological symptoms and recurrent cystitis. This frequency seemed to distinguish these patients from "simple" patients with IBS who did not benefit from the elimination diet. Thus, when subjects with suspected NCWS present coexistent gynecological and intestinal symptoms the clinician could consider prescribing an elimination diet.

The eventual benefit of a wheat-free diet in treating the gynecological disorders seems to have been demonstrated in a percentage of cases, but it must be further evaluated.

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Author's contribution AC, SI, and MS had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. AC contributed to study concept and design. AC, SI, PM, FLB, AD, and GI collected the data. FF contributed to serum marking. Histology study was done by AMF. Microbiological study was done by GF. MS carried out the statistical analysis. AC, SI, and PM carried out the analysis and interpretation of data. Drafting of the manuscript was done by AC and MS. Critical revision of the manuscript for important intellectual content was performed by AC, PM, and MS. All authors had access to the study data and reviewed and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of interest None of the authors have conflict of interest to declare.

Ethical approval The study protocol conformed to the ethical guidelines of the Declaration of Helsinki. It was approved by the Human Research Committee of the University of Palermo and registered at clinicaltrials.gov (Registration Number: NCT03027492, "Gynecological Disorders in Not-celiac Wheat Sensitivity").

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