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María Gónzalez , Miriam Al-Adib , Ana Beatriz Rodríguez , Cristina Carrasco *

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Menstrual-Related Disturbances Following SARS-CoV-2 Vaccination: A Spanish Retrospective Observational Study in Formerly Menstruating Women

María Gónzalez [1,†], Miriam Al-Adib [2,†], Ana B. Rodríguez [1] and Cristina Carrasco [1,*]

[1] Neuroimmunophysiology and Chrononutrition Research Group. Department of Physiology, University of Extremadura (Avenida de Elvas s/n, CP 06006, Badajoz, Spain); mgonzalewt@alumnos.unex.es / moratino@unex.es / ccarom@unex.es

[2] Obstetrics & Gynecology clinics "Miriam Gine" (C/ Guadalupe 9, CP 06200, Almendralejo, Badajoz); consulta@miriamginecologia.com

* Correspondence: ccarom@unex.es; Tel.: (+34) 924 28 69 47

† These authors contributed equally to this work.

Abstract: To date, the impact of COVID-19 vaccination on formerly menstruating women remains unknown. For this reason, a retrospective observational cross-sectional study was conducted (N= 548) using an online survey. General characteristics, medical history, and adverse events following COVID-19 vaccination were recorded. In comparison with the first dose, significantly higher percentages of respondents experienced menstrual-related disturbances (dose 1: 38.5% vs. dose 2: 44.8%; McNemar=9.15; mid P-value=0.002), as well as the simultaneous occurrence of two or more of these symptoms (dose 1: 11.2% vs. dose 2: 15.3%; McNemar=13.53; mid P-value=0.044) after receiving the second one. Among them, those related with the length and flow stand out, being of long-term nature in about 17-20% of cases. Interindividual factors influencing this unexpected event after receiving the dose 1 may include weight (AOR 1.02, CI 95% 1.01–1.03, P<.001), perimenopause (AOR 2.28, CI 95% 1.37–3.77, P=.001), pre-existing diagnoses of non-autoimmune rheumatic/articular conditions (AOR 0.31, CI 95% 0.10–1.00, P=0.05), hormonal contraceptive use (AOR 0.25, CI 95% 0.07-0.82, P=0.02), suffering from other vaccine side effects – such as arm pain (AOR 0.61, CI 95% 0.39–0.95, P=0.03), headache (AOR 0.53, CI 95% 0.35 – 0.80, P=.003), swollen glands (AOR 0.29, CI 95% 0.15 – 0.60, P=.001) and nauseas (AOR 0.35, CI 95% 0.14 – 0.86, P=0.02) – and the number of previous pregnancies (AOR 2.70, CI 95% 1.54 – 4.76, P=.001). Formerly menstruating women may experience long-term menstrual-related disturbances following COVID-19 vaccination.

Keywords: women's health; COVID-19 vaccine; menstrual-related disturbances; formerly menstruating women; secondary amenorrhea

1. Introduction

The development of SARS-CoV-2 vaccines has been one of the main preventive strategies to halt the advance of the COVID-19 pandemic. To this end, the drug regulatory agencies accelerated the established procedures, which allow to perform mass vaccination campaigns all over the world in record time [1–6]. Short-term side effects are similar to those observed for other vaccines and medicines, the expected benefits have been considered to outweigh any currently known adverse events [7–9]. However, its safety must be under continuous study. Despite the logical doubts in a context of global health crisis, mass immunization campaigns have been a success in nations such as Spain, one of the most affected European countries since the pandemic began.

However, as with any vaccine or drug, its safety must be under continuous study. Since vaccination campaigns started, individuals and health professionals worldwide have shared their testimonies about the occurrence of menstrual changes (MC) after COVID-19 vaccination on social media, an unexpected event that has not been monitored in clinical trials [10–13]. Furthermore, a

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considerable number of reports has been also officially documented by national bodies responsible for public health surveillance [7,8,14,15]. In the light of a growing concern about this health issue, several studies were launched. It should be remembered that the menstrual cycle is a sign of female health and fertility. Driving by a complex hormonal process, it may be affected by several external factors [16,17]. Nevertheless, any abnormal changes hereof should not be ignored [18,19], even in a context of public health emergency.

To date, there are few available data about the link between SARS-CoV-2 vaccine and MC in menstruating people [14,16,17,20–28]. Furthermore, the prevalence of this event in formerly menstruating people remains unknown, because different causes of secondary amenorrhea − pregnancy, breastfeeding, menopause, contraceptives methods, and gynecological conditions [29,30] – has been considered as exclusion criteria in these studies. With the purpose to provide additional insights to the limited extant literature about this subpopulation [26], this study was aimed to analyze not only the occurrence of menstrual-related disturbances (MRD) but also the influencing factors.

2. Materials and Methods

2.1. Experimental design

A retrospective observational cross-sectional study was conducted on Spanish adult women using an online survey (Microsoft Forms®, Microsoft Corporation, Washington, USA).

2.2. Recruitment, data collection and participants

The online survey was released in December 2021 via social networks, applying the snowball method. An informed consent was only obtained from those who agree to be contacted via email by the research group for additional data collection. Thus, 17,512 were recruited within 15 days; from this sample, the data pertaining to a subpopulation of formerly menstruating women (FMW) was selected for the present analysis (N=548, Figure 1). The inclusion criteria were women: (i) over 18 years of age, (ii) having secondary amenorrhea for different causes and (iii) having received at least one dose of the COVID-19 vaccine.

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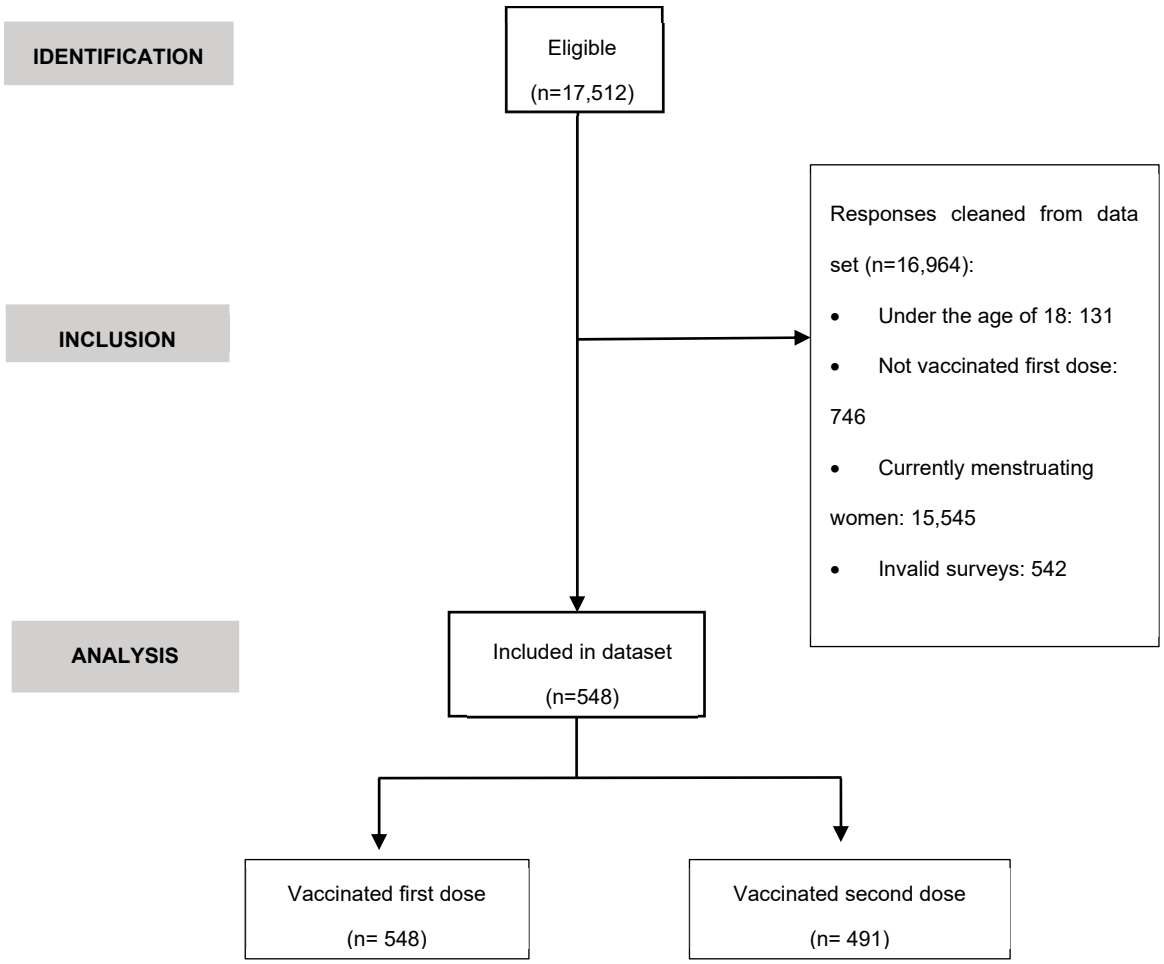

Figure 1. STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) flow diagram.

2.3. Survey information

We designed a customized questionnaire based on the survey launched on April 2021 by Lee et al. [26]. It was composed of 56 multiple-choice and text entry questions divided into 6 sections. Participants were asked about 1) the general characteristics of their menstrual cycles –, 2) COVID-19 disease, 3) COVID-19 vaccine, 4) menstrual experiences both after the infection and after receiving each dose of the vaccine in comparison with the expected period symptoms, 5) other MRD, 6) time between infection/vaccine and MRD, 7) duration of MRD, 8) side effects from each dose of the vaccine, 9) reproductive history, 10) medical history, and 11) demographics. The survey took approximately 10-15 min to complete.

2.4. Statistical analysis

Participants were categorized according to the occurrence (MRD subgroup) or not (n-MRD subgroup) of MRD after vaccination against the SARS-CoV-2 virus. Values were expressed as mean±standard deviation for quantitative variables and number of participants and frequency (%) for qualitative ones. Chi-square and Mann-Whitney U tests were performed for qualitative and quantitative variables, respectively. McNemar mid-P test was applied to detect differences between doses of the COVID-19 vaccine in the occurrence of MRD. Subsequently, a bivariate logistic regression analysis was performed to investigate possible associations between the occurrence of MRD following vaccination (dependent variable) and the independent variables that were significant ($P<0.05$) in the previous analyses. Thus, the results were presented as adjusted odds ratios (ORAs) with 95% confidence intervals (CI). The aforementioned analyses were performed using the Statistical

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Package for Social Sciences (SPSS v.25, IBM, New York, USA) for Windows. Statistical significance was set at P≤0.05.

3. Results

3.1. Anthropometric characteristics and medical history

53.8% of the study participants (N=548, mean age 40.4±10.7 years) had normal weight (mean BMI value=25.0±5.5), 12.4% had autoimmune diseases, and 25.0% had other clinical conditions. The secondary amenorrhea at the time of vaccination was caused by the contraceptive use (29.6%), post-menopause (17.3%), perimenopause (16.8%), and breastfeeding (14.8%). 37.8% of women used contraceptives for less than 10 years (79.3%), 60.2% had been pregnant, and 54.4% reported having been diagnosed with a gynecological disease (Table 1).

Table 1. Anthropometric characteristics and medical history of the study population (formerly menstruating women, N=548).

Variable		Category	Total (N=548)
Age (years)[a]		-	40.4±10.7
Weight (kg) [a]		-	67.3±15.3
Height (cm)[a]		-	164.2±6.3
BMI [a,b]		-	25.0±5.5
		Underweight	32 (5.8)
		Normal weight	295 (53.8)
		Pre-obesity/overweight	118 (21.5)
		Obesity I	67 (12.2)
		Obesity II	21 (3.8)
		Obesity III	9 (1.6)
Medical history [a,b]			
Autoimmune diseases	Diagnosis	Yes	68 (12.4)
		No	480 (87.6)
	Comorbidity	Yes	5 (7.4)
		No	63 (92.6)
	Types	Thyroid	31 (5.4)
		Gastrointestinal	14 (2.4)
		Dermatological	11 (1.9)
		Rheumatic/articular	7 (1.2)
		None of the above	7 (1.2)
Other clinical conditions	Diagnosis	Yes	136 (25.0)
		No	407 (75.0)
	Comorbidity	Yes	39 (27.7)
		No	102 (72.3)
	Types	Gynecological	30 (22.1)
		Cancer	19 (14.0)
		Neurological/mental	17 (12.5)
		Rheumatic/articular	16 (11.8)
		Gastrointestinal	14 (10.3)
		Respiratory	14 (10.3)
		Thyroid	13 (9.6)
		Dermatological	6 (4.4)
		HPV	8 (5.9)
		Cardiovascular	7 (5.1)
		None of the above	37 (27.2)
Allergies	Diagnosis	Yes	190 (34.7)

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		No	358 (63.3)
COVID-19	Diagnosis	Yes	72 (13.1)
		No	476 (86.9)
Gynecological history [a,b]			
Menarcheal age			12.6±1.5
Amenorrhea	Causes	Contraceptives	162 (29.6)
		Postmenopause	95 (17.3)
		Perimenopause	92 (17.3)
		Breastfeeding	81 (14.8)
		Hysterectomy	7 (1.3)
		None of the above	111 (1.3)
Current/past use of contraceptives	Time of use	< 10 years	161 (79.3)
		> 10 years	42 (20.7)
	Types	None	341 (62.2)
		Hormonal	150 (27.4)
		IUD (nonhormonal)	57 (10.4)
Reproduction	Have you been pregnant?	Yes	330 (60.2)
		No	218 (39.8)
	Nº pregnancies	0-2	470 (86.1)
		>2	76 (13.1)
	Nº children	0-2	523 (95.4)
		>2	25 (4.6)
Diseases	Diagnosis	Yes	298 (54.4)
		No	250 (45.6)
	Comorbidity	Yes	70 (23.4)
		No	229 (76.6)
	Types	PCOS	110 (36.8)
		Heavy menstrual bleeding	105 (35.1)
		Endometriosis	57 (19.1)
		Fibroids	30 (10.1)
		Menorrhagia	21 (7.0)
		Adenomyosis	10 (3.3)
		Uterine bleeding	9 (3.0)
		None of the above	53 (17.7)

Values are expressed as: [a] mean ± standard deviation; [b] n (%). Abbreviations: IUD, Intrauterine Device; BMI, Body Mass Index; PCOS, Polycystic Ovary Syndrome; HPV, Human Papillomavirus.

3.2. Impact of vaccination on the menstrual health of formerly menstruating women

Pfizer-BioNTech Comirnaty® vaccine was received in most cases, followed by Moderna Spikevax® and Oxford-AstraZeneca® ones (Table 2).

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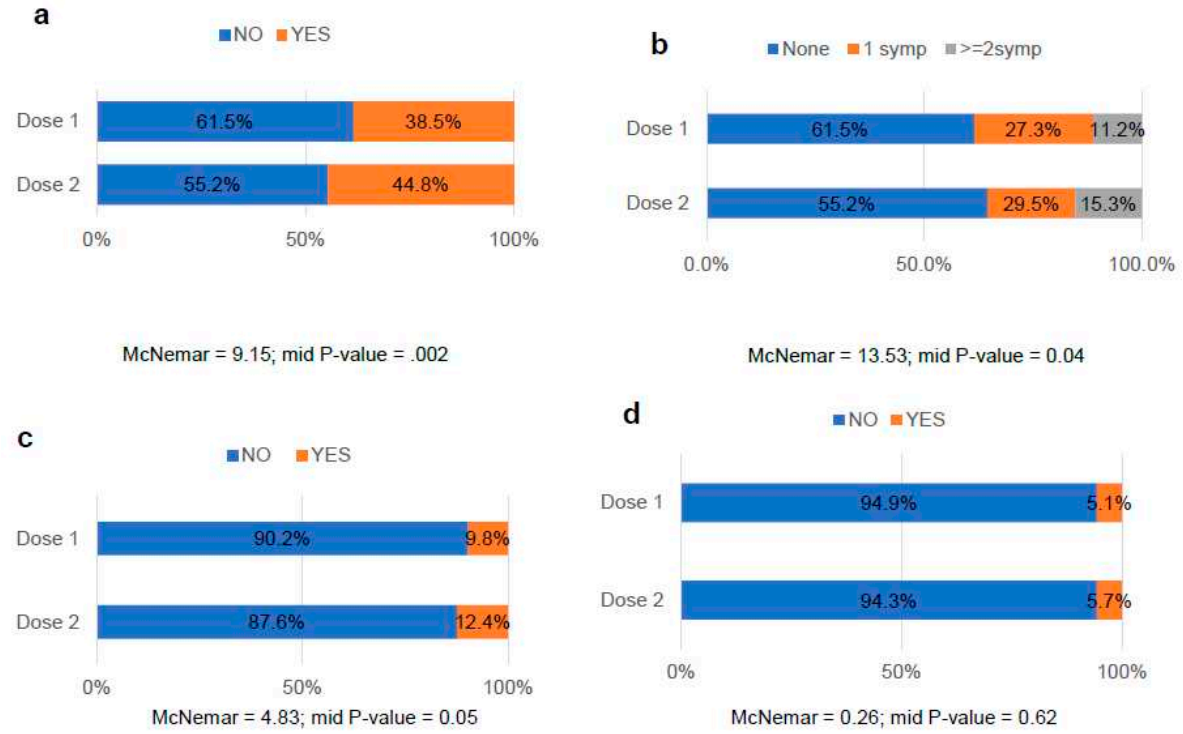
Table 2. COVID-19 vaccination of the study population (formerly menstruating women, N=548), according to the dose received.

Variable	Category	Dose 1 (n=548)	Dose 2 (n=491)
Vaccination	Yes	517 (94.6)	491 (89.6)
	No	31 (5.4)	57 (10.4)
Brand	Pfizer-BioNTech	373 (68.1)	352 (71.7)
	Moderna	95 (17.3)	89 (18.1)
	Oxford-AstraZeneca	54 (9.9)	48 (9.8)
	None of the above	26 (4.7)	2 (0.4)

3.3. Comparative analysis of the occurrence of MRD after COVID-19 vaccination

A significantly higher percentage of FMW experienced MRD after the second dose (Figure 2a), as is the case with the simultaneous occurrence of two or more symptoms (Figure 2b). Among the main disturbances, significant differences between doses were detected for the variables "spotting" (Figure 2c), "breast pain" (Figure 2e), and "abnormal bleeding (Figure 2h). Hot flashes were more frequent after the first dose (Figure 2f)



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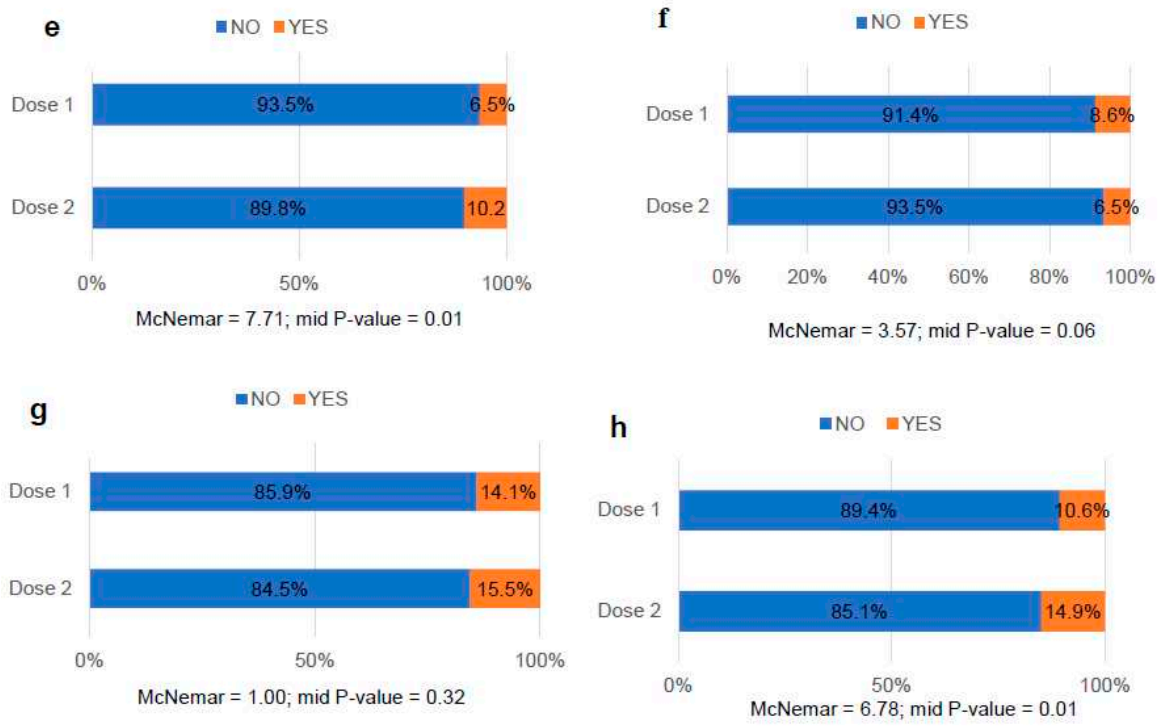

Figure 2. Comparison between the frequencies (%) obtained for the variables linked to MRD after the administration of doses 1 and 2 of the SARS-CoV-2 vaccine (formerly menstruating women, N=548).

3.4. Comparative analysis of menstrual bleeding changes after COVID-19 vaccination

Significant differences between doses were observed for the variables "length" (Figure 3a) and "flow" (Figure 3b). Most surveyed did not experience abnormal bleeding, and those who reported it pointed that this change mainly occurred after more than 14 days after the vaccination (Figure 3c). It is noteworthy that, after receiving the second dose, 20.4% of women reported that these alterations last ''until today'' compared to 17.1% who reported this situation after receiving dose 1, as well as for other durations (Figure 3d).

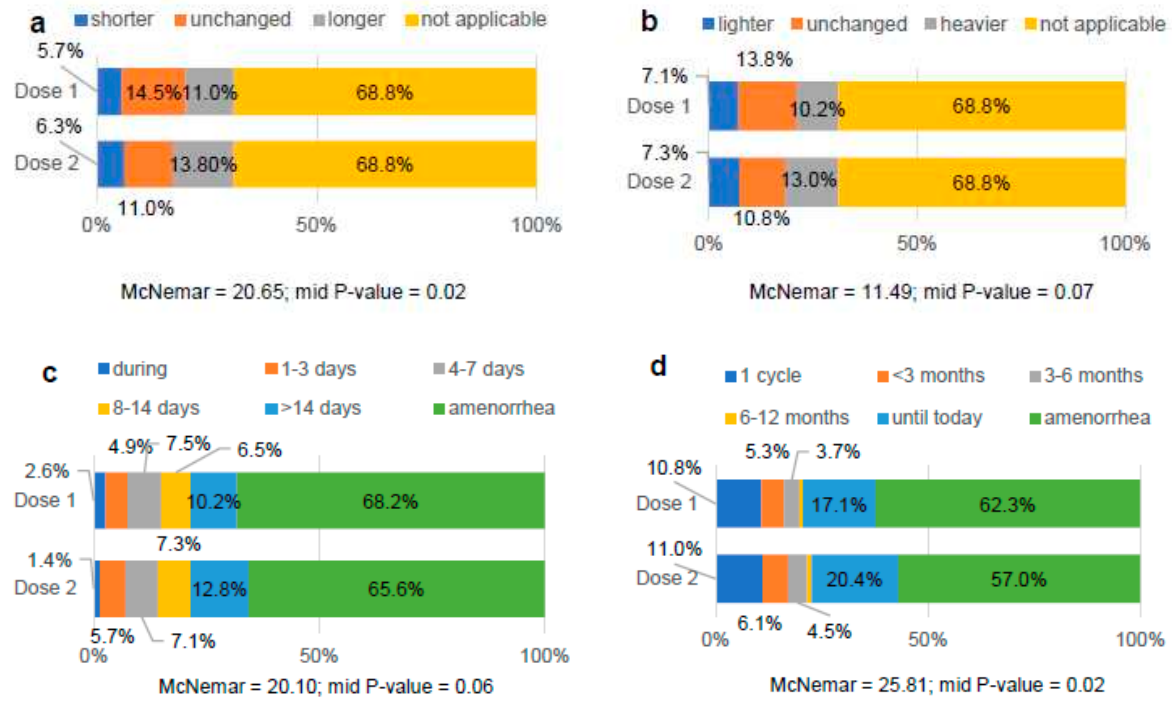

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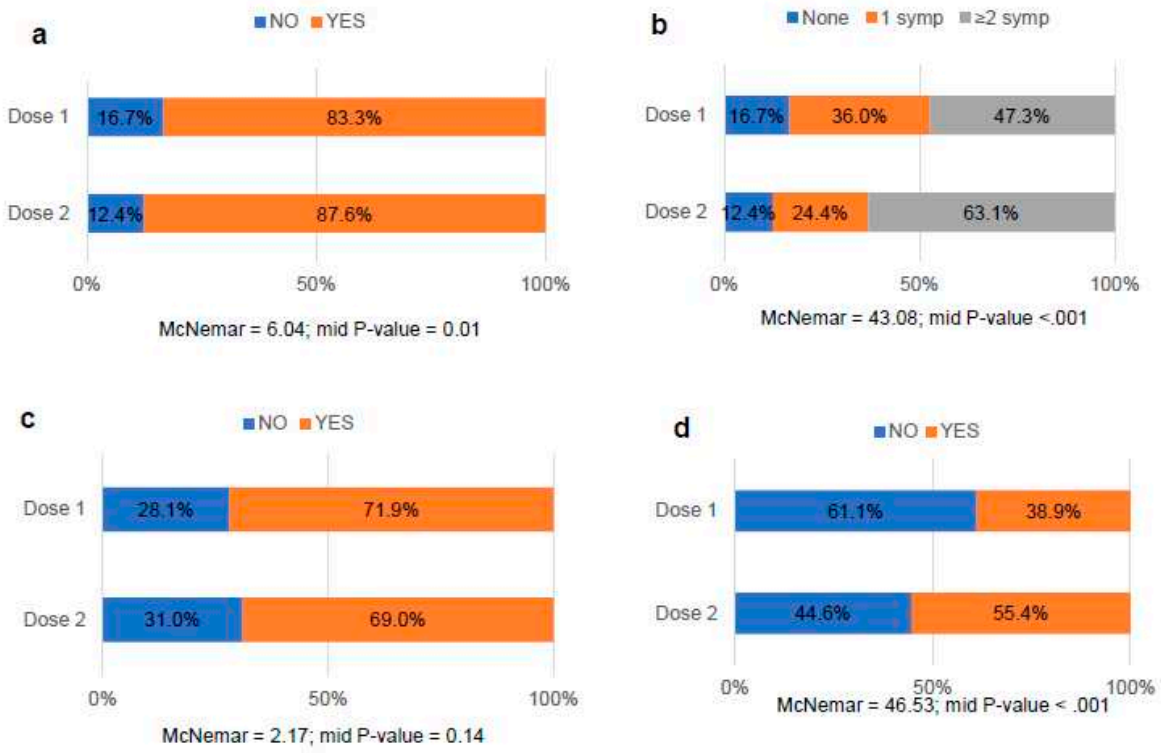
Figure 3. Comparison between the frequencies (%) obtained for the variables linked to menstrual bleeding changes after the administration of doses 1 and 2 of the SARS-CoV-2 vaccine (formerly menstruating women, N= 548). a. Length; b. Flow; c. Abnormal bleeding; d. Duration of menstrual bleeding changes.

3.5. Comparative analysis of the side effects after COVID-19 vaccination

After the second dose, there was a higher occurrence of side effects compared to the first one (Figure 4a), as well as the simultaneous occurrence of two or more symptoms (Figure 4b). Fatigue (Figure 4d), fever (Figure 4e), headache (Figure 4f), and nauseas (Figure 4g) appeared more frequently after dose 2.



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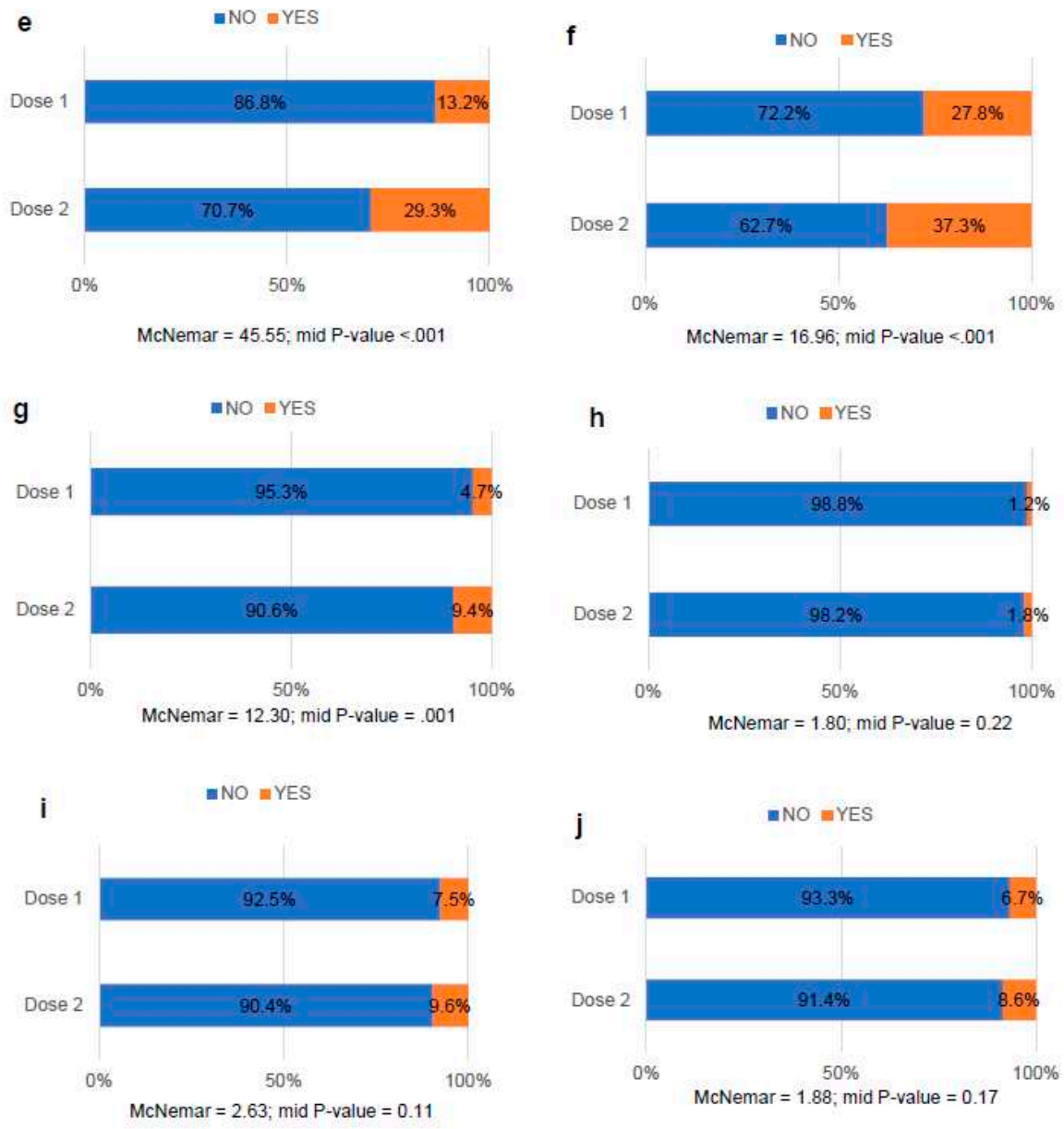

Figure 4. Comparison between the frequencies (%) obtained for the variables related to the side effects after the administration of doses 1 and 2 of the SARS-CoV-2 vaccine (formerly menstruating women, N= 548). a. Occurrence of side effects; b. Number of side effects; c. Arm pain; d. Fatigue; e. Fever; f. Headache; g. Nauseas; h. Breast lumps; i. Swollen glands; j. Other side effects.

3.6. Factors associated with the occurrence or not of MRD after COVID-19 vaccination

The mean values of weight and body mass index (BMI) were significantly different between the two subgroups (n-MRD and MRD) and for the two doses.

Based on the gynaecological history data (Table 3), a higher number of women who have used contraceptives for less than 10 years were recorded in the MRD subgroup for dose 2; moreover, hormonal contraceptives were significantly more used by these FMW. The number of children was significantly different between subgroups for the first dose. On the other hand, the percentage of perimenopausal women was higher in the subgroup experiencing MRD after receiving both doses. Significant differences were also detected in relation to suffering from gynaecological diseases such as endometriosis or heavy menstrual bleeding, in both cases being higher in the MRD than n-MRD for the second dose. Other noteworthy results are those related to side effects. Thus, after receiving the first dose, the occurrence of breast lumps, fatigue, arm pain, headache, swollen glands, and

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nauseas were higher in the subgroup experiencing MRD; for the second dose, the different side effects between subgroups included breast lumps, fatigue, headache, and swollen glands. It should be noted that the analysis of each subgroup of amenorrhea separately (contraceptives, menopause and other causes) for the first (Appendix 1) and second (Appendix 2) doses of the vaccine did not altered the above findings.

Table 3. Differences in the study variables according to the dose received (1 and 2) and the occurrence or not of MRD after COVID-19 vaccination (formerly menstruating women, N=548).

			Dose1				Dose2			
Variable		**Category**	**n-MRD (n=335)**	**MRD (n=213)**	**X²**	**P-value**	**n-MRD (n=271)**	**MRD (n=220)**	**X²**	**P-value**
Age (years)[a]		-	40.6±11.1	40.0±9.9	-	0.66	40.4±11.6	40.2±9.7	-	0.91
Weight (kg)[a]		-	65.4±24.5	70.3±15.9*	-	<.001	65.9±15.1	69.5±15.6•	-	0.01
Height (cm)[a]		-	163.9±6.2	164.6±6.3	-	0.18	164.0±6.0	164.4±6.5	-	0.78
BMI [a,b]		-	24.3±5.2	26.0±5.8*	-	<.001	24.4±5.4	25.8±5.7•	-	0.00
		Underweight	26 (7.8)	6 (2.8)†	13.96	0.03	18 (6.6)	10 (4.5)	7.46	0.28
		Normal weight	190 (56.7)	105 (49.3)			153 (56.5)	105 (47.7)		
		Pre-obesity /overweight	66 (19.7)	52 (24.4)			55 (20.3)	54 (24.5)		
		Obesity I	37 (11.0)	30 (14.1)			28 (10.3)	34 (15.5)		
		Obesity II	10 (3.0)	11 (5.2)			9 (3.3)	11 (5.0)		
		Obesity III	3 (0.9)	6 (2.8)			4 (1.5)	4 (1.8)		
Medical history [b]										
Autoimmune diseases	Types	Thyroid	14 (4.2)	17 (8.0)	0.08	0.77	14 (5.2)	15 (6.8)	0.00	0.99
		Dermatological	5 (1.5)	6 (2.8)	0.01	0.91	3 (1.1)	6 (2.7)	0.96	0.33
		Gastrointestinal	5 (1.5)	9 (4.2)	0.91	0.34	6 (2.2)	7 (3.2)	0.03	0.86
		Rheumatic / articular	3 (0.9)	4 (1.9)	0.06	0.81	2 (0.7)	4 (1.8)	0.60	0.44
		None of the above	4 (1.2)	3 (1.4)	0.32	0.57	4 (1.5)	3 (1.4)	0.24	0.62
Other clinical conditions	Types	Gynecological	21 (6.3)	9 (4.2)	1.05	0.31	15 (5.5)	13 (5.9)	0.03	0.86
		Cancer	13 (3.9)	6 (2.8)	0.44	0.51	11 (4.1)	7 (3.2)	0.27	0.61
		Neurological / mental	8 (2.4)	9 (4.2)	1.46	0.23	5 (1.8)	12 (5.5)†	4.73	0.03
		Thyroid	8 (2.4)	5 (2.3)	.001	0.98	6 (2.2)	5 (2.3)	0.00	0.97
		Gastrointestinal	7 (2.1)	8 (3.8)	1.36	0.24	7 (2.6)	5 (2.3)	0.05	0.83
		Respiratory	7 (2.1)	7 (3.3)	0.75	0.39	5 (1.8)	6 (2.7)	0.43	0.51
		Dermatological	6 (1.8)	2 (0.9)	0.66	0.42	4 (1.5)	4 (1.8)	0.09	0.77
		Cardiovascular	5 (1.5)	2 (0.9)	0.66	0.42	4 (1.5)	4 (1.8)	0.09	0.77
		Rheumatic / articular	5 (1.5)	12 (5.6)•	7.43	0.01	4 (1.5)	12 (5.5)•	6.10	0.01
		HPV	5 (1.5)	3 (1.4)	.006	0.94	2 (0.7)	3 (1.4)	0.47	0.49
		None of the above	20 (6.0)	18 (8.5)	1.24	0.27	11 (4.1)	21 (9.5)•	6.00	0.01
Allergies		-	106 (31.6)	84 (39.4)	3.49	0.06	94 (34.7)	74 (33.6)	0.06	0.81
COVID-19		-	43 (12.8)	29 (13.6)	0.07	0.79	19 (7.0)	13 (5.9)	0.24	0.62
Gynecological history [a,b]										
Menarcheal age		-	12.7±1.4	12.5±1.7	-	0.18	12.6±1.5	12.6±1.6	-	0.69
Current/past use of contraceptives	Time of use	< 10 years	88 (26.3)	73 (34.3)	0.32	0.57	61 (22.5)	87 (39.5)	3.43	0.06
		>10 years	25 (7.5)	17 (8.0)			21 (7.7)	15 (6.8)		
	Types	None	219 (65.4)	122 (57.3)	3.63	0.16	186 (68.6)	117 (53.2)•	12.68	.002
		Hormonal	84 (25.1)	66 (31.0)			60 (22.1)	77 (35.0)•		
		IUD (nonhormonal)	32 (9.6)	25 (11.7)			25 (9.2)	26 (11.8)		
Reproduction	Have you ever been pregnant?	Yes	204 (60.9)	126 (59.2)	0.165	0.69	156 (57.6)	134 (60.9)	0.562	0.45
		No	131 (39.1)	87 (40.8)			115 (42.4)	86 (39.1)		
	Nº pregnancies	0-2	290 (86.6)	180 (84.5)	0.399	0.53	234 (86.3)	189 (85.9)	0.014	0.91
		>2	44 (13.1)	32 (15.0)			36 (13.3)	6 (2.7)		

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	Nº children	0-2	314 (93.7)	209 (98.1)•	5.765	0.02	255 (94.1)	214 (97.3)	2.863	0.09
		>2	21 (6.3)	4 (1.9)			16 (5.9)	6 (2.7)		
Amenorrhea	Types	Perimenopause	41 (12.2)	51 (23.9)*	17.054	<0.001	33 (12.2)	47 (21.4)•	10.596	0.01
		Postmenopause	70 (20.9)	25 (11.7)*			57 (21.0)	29 (13.2)•		
		None of the above	224 (66.9)	137 (64.3)			181 (66.8)	144 (65.5)•		
Diseases	Types	Adenomyosis	7 (2.1)	3 (1.4)	0.337	0.56	5 (1.8)	5 (2.3)	0.111	0.74
		PCOS	67 (20.0)	43 (20.2)	0.003	0.96	61 (22.5)	40 (18.2)	1.392	0.24
		Heavy menstrual bleeding	57 (17.0)	48 (22.5)	2.562	0.11	44 (16.2)	53 (24.1)†	4.726	0.03
		Endometriosis	30 (9.0)	27 (12.7)	1.934	0.16	21 (7.7)	29 (13.2)†	3.918	0.05
		Fibroids	20 (6.0)	10 (4.7)	0.420	0.52	16 (5.9)	13 (5.9)	0.000	0.99
		Menorrhagia	14 (4.2)	7 (3.3)	0.282	0.60	13 (4.8)	7 (3.2)	0.811	0.37
		Uterine bleeding	7 (2.1)	2 (0.9)	1.067	0.30	7 (2.6)	2 (0.9)	1.891	0.17
		None of the above	37 (11.0)	16 (7.5)	1.860	0.17	28 (10.3)	20 (9.1)	0.212	0.65
Side effects [b]	Types	Arm pain	231 (69.0)	167 (78.4)†	5.847	0.02	180 (66.4)	159 (72.3)	1.946	0.16
		Fatigue	126 (37.6)	101 (47.4)†	5.160	0.02	139 (51.3)	133 (60.5)†	4.126	0.04
		Headache	78 (23.3)	81 (38.0)*	13.863	<0.001	88 (32.4)	95 (43.4)†	5.957	0.02
		Fever	41 (12.2)	33 (15.5)	1.181	0.28	76 (28.0)	68 (30.9)	0.481	0.49
		Swollen glands	15 (4.5)	27 (12.7)*	12.367	<0.001	18 (6.6)	29 (13.2)•	6.000	0.01
		Nauseas	8 (2.4)	20 (9.4)*	13.166	<0.001	20 (7.4)	26 (11.9)	2.817	0.09
		Breast lumps	0 (0.0)	7 (3.3)*	11.125	0.001	0 (0.0)	9 (4.1)*	11.293	0.001
		None of the above	19 (5.7)	19 (8.9)	2.129	0.15	22 (8.1)	20 (9.1)	0.147	0.70

Values are expressed as: [a] mean ± standard deviation; [b] n (%). *P≤.001; • P≤0.01; † P≤0.05 vs. n-MRD. Abbreviations: BMI, Body Mass Index; HPV, Human Papillomavirus; IUD, Intrauterine Device; MRD, menstrual-related disturbances subgroup; n-MRD, non-menstrual-related disturbances subgroup; PCOS, Polycystic Ovary Syndrome.

Subsequently, in the case of the first dose (Table 4), the binary logistic regression analysis showed that those who have a higher weight and receive the vaccine during perimenopause are much likely to suffer from MRD. Conversely, not suffering from a rheumatic / articular condition, not having experienced other side effects − such as arm pain, headache, swollen glands, and nausea, and having 2 or more children decreases the probability of this unexpected event. For the second dose (Table 5), suffering MRD after receiving the first dose of the SARS-CoV-2 vaccine increases the probability of this event. In addition, the use of hormonal contraceptives was found to be an influential factor in experiencing MRD. Finally, as was observed for dose 1, being a perimenopausal woman increases the probability of the occurrence of this phenomenon.

Table 4. Factors associated with the occurrence of MRD after receiving the first dose of COVID-19 vaccine (formerly menstruating women, N= 548): binary logistic regression.

		Dose 1	
Variable	**Category**	**MRD (n=213) AOR (CI 95%)**	**P-value**
Weight	-	1.02 (1.01-1.03)	<.001
OCC rheumatic/articular	No	0.31 (0.10-1.00)	0.05
	Yes	1	
Nº children	≥ 2	0.25 (0.07-0.82)	0.02
	0-2	1	
Amenorrhea	Perimenopause	2.28 (1.37-3.77)	.001
	Postmenopause	0.74 (0.43-1.28)	0.28
	None of the above	1	
Side effects			
Arm pain	No	0.61 (0.39-0.95)	0.03

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	Yes	1	
Headache	No	0.53 (0.35-0.80)	.003
	Yes	1	
Swollen glands	No	0.29 (0.15-0.59)	.001
	Yes	1	
Nauseas	No	0.35 (0.14-0.86)	0.02
	Yes	1	

Note: reference subgroup n-MRD. AOR: adjusted odd radio; CI 95%: confidence interval. MRD: menstrual-related disturbances subgroup; OCC: other clinical conditions.

Table 5. Factors associated with the occurrence of MRD after receiving the second dose of COVID-19 (formerly menstruating women, N = 491): binary logistic regression.

		Dose 2	
Variable	**Category**	**MRD (n=220) AOR (CI 95%)**	**P-value**
Alterations dose 1	Yes	13.88 (8.66-22.08)	<.001
	No	1	
Contraceptives	Hormonal	2.70 (1.54-4.76)	.001
	IUD	1.85 (0.86-3.99)	0.12
	None	1	
Amenorrhea	Perimenopause	2.07 (1.06-4.06)	0.03
	Postmenopause	1.17 (0.60-2.29)	0.64
	None of the above	1	
Arm pain	No	0.64 (0.40-1.02)	0.06
	Yes	1	

Note: reference subgroup n-MRD. AOR: adjusted odd radio; CI 95%: confidence interval. MRD: menstrual-related disturbances subgroup; IUD: intrauterine device.

4. Discussion

FMW may experience long-term MRD after receiving COVID-19 vaccine. Interindividual factors influencing this unexpected event may include weight, perimenopause, pre-existing diagnoses of rheumatic/articular conditions, hormonal contraceptive use, suffering from other vaccine side effects and the number of previous pregnancies.

Concurring with previous studies in menstruating women [14,20,27], the occurrence of MRD seems to be significantly higher following the second dose of the vaccine. As Male et al. [31] point out, any change that affects the post-dose 1 period could potentially still be in effect for the post-dose 2 period; in turn, it would depend on the time between doses established by the competent national body (in the case of Spain, 3-4 weeks) [32]. In this sense, it has been observed that the those who received both doses in the same cycle were more prone to suffer from more substantial and long-lasting MC [16,24]. Other possible explanations include that people who experienced MRD following dose 1 may be more likely to report it again.

Focusing on the menstrual bleeding disturbances, those related with the length and flow stand out. This observation is consistent with that found in previous studies reporting longer-lasting menstruation [14,20], and/or heavier flow [14,21,25,33,34] than usual in vaccinated women. Unexpectedly, increased bleeding has also been observed in women using hormonal contraception [14,21,25,33], which may indicate that certain immunological processes may be also involved. As for the duration of the changes, several studies agree that MC are of transient nature [14,16,20,23,24,27]. However, our results show far from negligible percentages of women who suffer from it more than 12 months, which has not been described previously. Moreover, abnormal bleeding was mostly experienced in the two weeks post-vaccination, which extends beyond the typical 7 days for the adverse symptoms monitoring established in vaccine trials, as observed by Lee et al. [25]. Similar percentages of vaccinated women experiencing MCD was found in this and other studies [20,28], independently of their previous history of SARS-CoV-2 infection. For their part, Li et al. [35] conclude that the average sex hormone concentrations and the ovarian reserve did not significantly change in

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those COVID-19 women of child-bearing age who experienced self-resolve menstrual changes. Altogether, this evidence would support the theory about the role of unknown immunological/inflammatory mechanisms as triggers of the MC resulting from both infection and vaccination, including thrombocytopenia [36,37] and changes in lymphocytes subpopulation patterns [38].

Other aspects must also be considered. Firstly, our results show that the presence of side effects was higher after receiving the second dose of the vaccine. Other authors [20,26] report a greater likelihood of MCD when such side effects are experienced; on the contrary, those who did not experience it had better premenstrual syndrome, whose symptoms seem to change following COVID-19 vaccination [17] Secondly, we agree with previous studies [20,22–24,27] that the occurrence of MCD seems not to be brand specific (data not shown) and, therefore, independent of the technology approach (adenovirus-vectored or mRNA vaccines). At this point, it should be emphasized that vaccine-induced immune thrombotic thrombocytopenia has been reported as a rare side effect associated to adenovirus-vectored vaccines [7,8,39–41]. It mostly affects individuals aged between 20-50 years old and occurs between 4-30 days after the exposure. Our study population was mostly inoculated with the Pfizer-BioNTech vaccine, as in similar researches [23,24]; thus, the non-detection of statistically significant differences may be due to the overrepresentation of this brand compared to others. Therefore, sociodemographic factors may also underlie the different rates of menstrual disturbances among nations. In this sense, the country of residence could be an influencing factor, resulting not only from the predominant use of certain brands of vaccines − and the corresponding schedule – but also from cultural reasons, which may discourage help-seeking behavior among young women [20,42,43].

As regards the factors influencing the occurrence of MRD in FMW after vaccination, the study performed by Popkin et al. [44] concludes that overweight/obesity increases the risk of SARS-CoV-2 infection, worsen COVID-19 outcomes, and may influence the effectiveness of the therapeutic treatments and the vaccines because of immune impairments, among other factors. Since individuals with BMIs higher than 35 are known to have greater baseline variations in menstrual cyclicity [16], it is not surprising that this feature also influences the risk of suffer from MC post-vaccination. On the other hand, no study has explored the potential influence of perimenopause, therefore, we cannot properly compare our results. Only Lee et al. [25] has described an increased chance of breakthrough bleeding after being vaccinated for those premenopausal respondents who were Hispanic/Latinx, had been pregnant in the past but had not given birth, had a diagnosed reproductive condition, were on long-acting reversible contraceptives only, or experienced fever after vaccination. Emerging evidence suggests that the endocrine transition that characterized perimenopause is associated with a rise in chronic low-grade inflammation, which in turn accelerates ovarian failure [45].

This is one of the few studies conducted worldwide to focus on the impact of COVID-19 vaccination on FMW, as well as on the potential influence of health-related factors. The sample analyzed was large enough to be statistically representative. Nevertheless, the experimental design could have influenced the detection of significant differences and causal relationships cannot be properly determined. On the other hand, the online questionnaire was not previously validated. At this point, we would like to make the following comments: first of all, the historic events derived from an unprecedented health crisis compels us to be less rigorous from a methodological point of view. By this way we and other research groups were able to sample a target population at real time and to collect a variety of variables related to women health, when official reports did not yet reflect the emerging phenomenon. Second, self-reported data potentially represent errors such as recall bias or self-selection; nevertheless, self-reports are considered the gold standard for menstrual cycle data and useful for rapidly identifying potential signals or rare adverse events. As in similar studies, our findings here might not be applicable to other countries than Spain, due to some of the above-mentioned reasons. Taking into account all of the above, we agree that a longitudinal and multinational study could help to establish the cause-effect relationship and to clarify the factors influencing the occurrence of alterations in the menstrual cycle after COVID-19 vaccination.

5. Conclusions

Our findings, together with previous evidence, highlight the need to further investigate the current prevalence of menstrual disturbances in women after receiving COVID-19 vaccine or any other vaccine, as well as the physiological mechanisms involved and their relationship with the hypothalamus-pituitary-gonadal axis. From our point of view, such investigations should in no case feed anti-vaccine theories, but rather serve to confirm the need that this and other side effects can no longer be overlooked in clinical trials. Research on the safety of COVID-19 vaccination on women´s health, whatever their reproductive circumstances, is necessary to apply a gender perspective in clinical research, an issue largely neglected. This evidence could help health professionals to provide scientifically up-to-date information to their patients, empowering them to choose the best option for their reproductive decisions, especially in those societies where menstrual health is still a taboo.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of University of Extremadura (ref. 180/2021).

Informed Consent Statement: An informed consent was only obtained from those who agree to be contacted via email by the research group for additional data collection.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethical restrictions.

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