



Universiteit
Leiden
The Netherlands

Sex hormones and sex hormone-targeting therapies in systemic sclerosis: a systematic literature review

Ciaffi, J.; Leeuwen, N.M. van; Schoones, J.W.; Huizinga, T.W.J.; Vries-Bouwstra, J.K. de

Citation

Ciaffi, J., Leeuwen, N. M. van, Schoones, J. W., Huizinga, T. W. J., & Vries-Bouwstra, J. K. de. (2020). Sex hormones and sex hormone-targeting therapies in systemic sclerosis: a systematic literature review. *Seminars In Arthritis And Rheumatism*, 50(1), 140-148.
doi:10.1016/j.semarthrit.2019.07.007

Version: Publisher's Version

License: [Creative Commons CC BY 4.0 license](#)

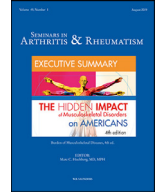
Downloaded from: <https://hdl.handle.net/1887/3182277>

Note: To cite this publication please use the final published version (if applicable).



Contents lists available at ScienceDirect

Seminars in Arthritis and Rheumatism

journal homepage: www.elsevier.com/locate/semarthrit

Sex hormones and sex hormone-targeting therapies in systemic sclerosis: A systematic literature review

Jacopo Ciaffi^{a,b,c,*}, Nina M. van Leeuwen^a, Jan W. Schoones^d, Tom W.J. Huizinga^a,
Jeska K. de Vries-Bouwstra^a

^a Department of Rheumatology, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands

^b Struttura Complessa di Reumatologia, Rheumatology Unit, Azienda Policlinico of Modena, University of Modena and Reggio Emilia, Largo del Pozzo 71, 41125 Modena, Italy

^c Medicine and Rheumatology Unit, IRCCS Istituto Ortopedico Rizzoli, via Pupilli 1, 40136 Bologna, Italy

^d Walaeus Library, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands

ARTICLE INFO

Keywords:

Systemic sclerosis
Sex hormones
Androgens
Estrogens
Hormone therapy

ABSTRACT

Background: The pathophysiology of systemic sclerosis (SSc) is complex and elusive, however, considering the strong female preponderance and different clinical characteristics between men and women, a contribution of sex hormones has been proposed.

Objectives: We undertook this systematic literature review to investigate: (1) the role played by male and female sex hormones in the pathogenesis of SSc; (2) how sex hormone levels change in SSc patients and how hormonal variations modify the progression of SSc; (3) the effect of therapies targeting sex hormones on the disease course.

Methods: A literature search was performed in Pubmed, Embase, Web of Science, and Cochrane library databases. Given the heterogeneity in study design, different quality assessment tools were applied where appropriate.

Results: We retrieved 300 articles and 30 were included in the review. The available evidence points to a fibrogenic, but also a vasodilatory, role of estrogens in SSc. With the limitation of small sample sizes, women with SSc tend to have lower levels of androgens and non-significantly higher levels of estradiol compared to healthy controls, while in men we found increased levels of estradiol and discordant results for androgens. After menopause the skin score seems to decrease and prevalence of pulmonary artery hypertension seems to rise, which might be prevented by the use of hormone replacement therapy. No recent high-quality trial evaluated the efficacy of hormone-targeting therapies in SSc.

Conclusions: Few translational studies of varying quality evaluated the role of sex hormones in SSc showing possible profibrotic and vasodilatory effects of estrogens, but more research is needed to elucidate the extent of this contribution. Insights on the influence of sex hormones, along with the availability of new compounds acting on estrogen pathways, might provide ideas for additional studies on the application of sex hormone-targeting therapies in SSc.

© 2019 Elsevier Inc. All rights reserved.

Introduction

Systemic sclerosis (SSc) is a rare autoimmune connective tissue disease characterized by vasculopathy, generalized fibrosis and deregulation of innate and adaptive immune system. The pathogenesis is complex, multifactorial, and incompletely understood, with

evidence suggesting contribution from genetic, environmental, and occupational factors [1,2]. As in most autoimmune diseases, SSc has a strong female preponderance, with women being involved between 3 and 14 times more frequently than men [1]. Moreover, clinical and immunological differences between male and female patients have been observed [3,4]. The prevalence of diffuse cutaneous SSc is higher in men [3,4], as well as cardiac involvement [3,4] and anti-topoisomerase I antibody (ATA) positivity, while women more often present anti-centromere antibodies (ACA). One study showed that overall peripheral vascular involvement is more common in female patients than in men [4], but male patients have higher risk of developing digital tip ulcers or gangrene [4]. However, contrasting results have been reported about the association between gender and digital

* Corresponding author at: Stafcentrum Reumatologie C1-R, Department of Rheumatology, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands.

E-mail addresses: j.ciaffi@lumc.nl (J. Ciaffi), n.m.van_leeuwen@lumc.nl (N.M. van Leeuwen), j.w.schoones@lumc.nl (J.W. Schoones), t.w.j.huizinga@lumc.nl (T.W.J. Huizinga), j.k.de_vries-bouwstra@lumc.nl (J.K. de Vries-Bouwstra).

ulcers in SSc [5]. Interstitial lung disease (ILD) prevails in men [3,4], for which it represents the leading cause of death [3,4]. In women, who have a better cumulative survival [3,4], the leading cause of death is pulmonary arterial hypertension (PAH) [3,4].

To account for the gender gap and disease dissimilarities, a role of sex hormones has been proposed [6], but few studies tried to assess their relevance in SSc, while it would be valuable, especially considering that hormone pathways can be modulated. The most important sex hormones in women are estrogens, with a predominance of estradiol (E2) and estrone, respectively before and after menopause, while in men testosterone is the main androgen. Dihydrotestosterone and androstenedione are the other two important androgens, while dehydroepiandrosterone (DHEA) and the sulphated form dehydroepiandrosterone sulphate (DHEAS) are considered as a reservoir for the production of both androgens and estrogens [7].

In men, androgen levels peak after puberty and then progressively decline with aging. In women the physiology of sex hormones is more complex and liable to high variability, especially during childbearing age, when estrogen and progesterone levels influence, and are influenced by, the different phases of menstrual cycle, pregnancy, and breastfeeding. Furthermore, medical interventions [8,9], genetic disorders [10], and daily life habits [11–14] can alter sex hormone levels.

Both androgens and estrogens affect the immune system and inflammation [15], have been implicated in autoimmunity, and have been reviewed in other rheumatic diseases [16,17]. Despite their putative role in SSc, their involvement has not been thoroughly explored and in particular the extent of contribution to fibrosis and vasculopathy, the disease hallmarks, is largely unknown. The aim of this systematic literature review is to summarize the available evidence on the association of androgens and estrogens with SSc, categorizing the research question into 3 areas of interest: (1) the role of sex hormones in the pathophysiology of SSc; (2) how sex hormone levels vary in men and women with SSc compared to healthy controls and how changes in sex hormone levels modify the SSc course; (3) the effect of sex hormone-targeting therapies on SSc onset, activity, and progression.

Materials and methods

Eligibility criteria

Articles were deemed eligible if they investigated the association between male or female steroid sex hormones and the pathogenesis, clinical presentation, or disease progression of patients with SSc. The following sex hormones were entered in the original search strategy: DHEA, DHEAS, estradiol, estrone, estriol, progesterone, testosterone, dihydrotestosterone, androstenedione, gonadotropin releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), pituitary gonadotropins. Prolactin was encompassed by the MeSH category “pituitary gonadotropins”, thus was part of the search string, but articles only describing prolactin were not included in the review. Complete details about the MeSH terms and synonyms used are shown in Appendix.

Studies were included if they:

- (1) Involved adult patients, or biological samples derived from adult patients, with a diagnosis of SSc. According to publication date, a statement reporting the fulfillment of classification criteria proposed either by the American College of Rheumatology (ACR) in 1980 [18], by LeRoy in 1988 [19], or by ACR and European League Against Rheumatism (EULAR) in 2013 [20], was considered valuable, but not essential for inclusion. Indeed, in order not to miss relevant studies, research published before the availability of classification criteria, or in which the fulfillment of criteria was not clearly stated, could be included in the review after evaluation of clinical and laboratory characteristics of the patients,

consistency of the diagnosis, and absence of diagnostic biases. In studies where individual patients' characteristics were described, we also retrospectively applied the 2013 ACR/EULAR criteria [20].

- (2) Measured levels of steroid sex hormones, FSH or LH, or if applied therapies targeting sex hormones, as agonists or antagonists.

For clinical studies, we utilized the PICO (population, intervention, comparator, outcome) framework [21]. We included randomized controlled trials (RCTs), explorative non-randomized and open-label trials, case-control studies, case series, case reports. Prospective and retrospective studies were eligible. Exclusion criteria were: animal studies; juvenile SSc; mixed population where a sub-cohort of SSc patients could not be identified or a sub-analysis had not been performed. Conferences' proceedings and non-original publications were excluded, as well as papers written in a language different from English.

Search strategy and study selection

Articles were retrieved undertaking literature search in PubMed, Medline, Cochrane Library and Web of Science databases. The original search strategy string can be found in Appendix. No date restriction was applied and all studies published up to 18th April 2019 underwent title and abstract screening by two independent authors (JC and NvL). Both researchers then performed full text reading of eligible articles and search of references to identify additional relevant papers. Disagreements were discussed together, with the opinion of a third investigator (JdV) when consensus could not be achieved.

Data extraction and quality assessment

Data were extracted and summarized by the first author (JC) and revised by the second author (NvL). From each selected article, the following information was gathered: first author, date of publication, country, population size, inclusion and exclusion criteria, type of intervention, duration of eventual follow-up, outcomes, statistical methods, summary of key findings. Given the heterogeneity in design of selected studies, different quality assessment methods were used. The Cochrane risk of bias tool [22] was used for randomized trials, the Newcastle-Ottawa scale [23] for non-randomized studies, and the Johanna-Briggs Institute critical appraisal tools [24] for case reports and case series.

Results

The search strategy identified 228 articles in PubMed, 185 in Embase, 141 in Web of science, and 19 in Cochrane library. After removal of duplicates, 300 unique articles were selected for title and abstract screening. Fifty-three were considered potentially relevant for full text evaluation. No additional papers were identified through manual search of references. The full article review identified 30 studies that proceeded to data extraction and analysis (Fig. 1).

In 12 studies [25–36] the ACR 1980 preliminary criteria for SSc were fulfilled. Of these, a study from the Canadian Scleroderma Research Group [35] was included on the basis that, although not mentioned in that article, we retrieved another manuscript [37] indicating that 90% of the patients in the same cohort fulfilled the classification criteria. In 3 studies [9,38,39] ACR/EULAR 2013 criteria were met, while Le Roy 1988 criteria were satisfied in other 2 [40,41]. Additionally, 9 studies [42–50] were included after retrospective application of the 2013 ACR/EULAR criteria. In 7 [42–48] of these studies, patients were classified as having SSc on the basis of skin thickening extending proximal to the metacarpophalangeal joints. In one manuscript [49], all patients presented Raynaud's phenomenon, dermal sclerosis, pitting scars, puffy fingers and teleangiectasia. One case report [50] described a patient with sclerodactyly, Raynaud's

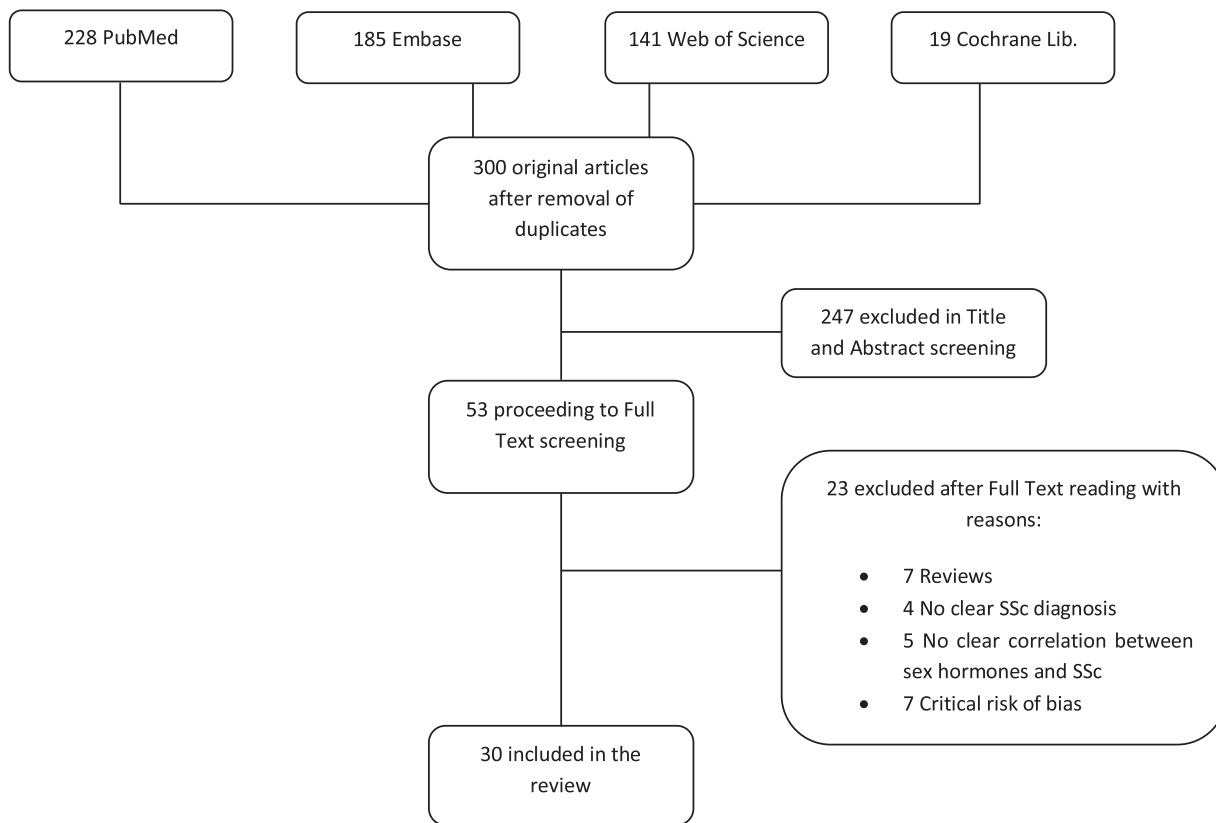


Fig. 1. Flowchart showing the article selection process.

phenomenon, nailfold abnormalities, and ILD, thus retrospectively fulfilling the 2013 ACR/EULAR classification criteria for SSc. Finally, 4 articles [51–54], in which all patients had the diagnosis of SSc, but fulfillment of classification criteria was not clearly specified, and ACR/EULAR 2013 criteria could not be retrospectively applied due to lack of sufficient clinical and laboratory information at individual patient-level, were included in the review after evaluation of relevance of the results, consistency of the diagnosis, and exclusion of diagnostic biases.

Characteristics of included studies and description of populations

The geographical origin of the included studies was inhomogeneous, with a contribution of 24 publications from European centers, 4 studies undertaken in North America, and 2 by Asian teams. Ten articles described only female patients, 10 only men, and in 7 both sexes were included. In 2 laboratory studies, patients' gender was not clearly stated and one case series reported male to female transgender patients. Overall, the selected literature, reports about 1842 SSc patients. For the first research question – (1) – seven articles [25,27,31,32,39,52,53] describing the potential role of sex hormones, and in particular of estrogens, in the pathogenesis of SSc, were retrieved. These 7 studies had a case-control design, 4 [25,39,52,53] were performed ex-vivo on cultured fibroblasts obtained from skin biopsies, while 3 [27,31,32] were in-vivo.

For the second research question – (2) – we found 9 articles assessing sex hormone levels in patients with SSc [9,25,29,30,33,41,49,51,54], 6 case reports [36,42,44,47,48,50] about the association of male hypogonadism and SSc, and 2 retrospective cohort studies evaluating the effect of menopause on SSc [34,35]. Finally, for the third research question – (3) – we included 7 studies on sex hormone-targeting therapies and their potential effect on SSc [26,28,38,40,43,45,46].

Quality score

Quality assessment yielded heterogeneous results. Fourteen case-control [9,25,27,29–31,33,39,41,49,51–54] and three cohort studies [34,35,40] were evaluated through the Newcastle-Ottawa scale. Six studies [9,29,30,34,41,54] were of high quality and 11 [25,27,31,33,35,39,40,49,51–53] were of moderate quality. Five randomized trials [26,28,32,45,46] were assessed using the Cochrane risk of bias tool. Two studies were considered at moderate risk of bias [28,32], and 3 were scored as high risk of bias [26,45,46]. Seven case reports [36,42–44,47,48,50] and 1 case series [38] were deemed eligible after the application of Johanna-Briggs critical appraisal tools.

Sex hormones in the pathophysiology of SSc {1}

Fibrosis {1a}

In 2013 Aida-Yasuoka et al. [25] evaluated the effects of E2, and estrogen-receptors alpha ($ER\alpha$) and beta ($ER\beta$), on fibronectin expression in fibroblasts derived from skin biopsies of 6 SSc patients and their 5 healthy twins. The authors found that physiological levels of E2 induced fibronectin production in dermal fibroblasts of both SSc patients and controls. This profibrotic influence was mainly regulated through $ER\alpha$, while treating the cells with genistein, an $ER\beta$ agonist, resulted in an only modest fibronectin production. Levels of $ER\beta$ were also lower in fibroblasts derived from SSc patients than in controls. As a possible explanation of their findings, the authors hypothesized a protective role of $ER\beta$ in SSc.

These results are in accordance with those described by Soldano et al. in 2009 [52] and 2010 [53]. In the first study [52], fibroblasts obtained from skin biopsies of 6 SSc patients and 6 healthy controls, were treated with physiological concentrations of either E2 or testosterone. E2 was found to enhance cell growth and extracellular matrix protein (ECM) expression both in SSc and in normal fibroblasts,

whereas testosterone had no effect on cell growth and inhibited laminin and fibronectin production in cells from healthy controls, but unexpectedly increased ECM protein synthesis in SSc fibroblasts, possibly due to enhanced aromatase activity. In a subsequent manuscript [53] the same team demonstrated that when Tamoxifen, a selective estrogen receptor modulator (SERM), was added to the cell culture of normal or SSc fibroblasts, ECM protein synthesis was inhibited compared to fibroblasts treated with E2 alone, and it was similar to that observed in fully untreated fibroblasts.

In 2013 a study on the role of anti-ERα antibodies in SSc was published by Giovannetti et al. [27]. The immunoreactivity of IgG to ERα in the serum of 71 patients with SSc and 90 age- and sex-matched controls was tested. Anti-ERα antibodies, that exert an agonistic behavior, were not detected in healthy controls but were present in 42% of SSc patients. Anti-ERα positivity correlated with diffuse disease subset, European Scleroderma Study Group (EScSG) activity index, presence of ATA, and late pattern at nailfold videocapillaroscopy. Emphasising the similar association between anti-ERα and disease activity both in SSc and in systemic lupus erythematosus, the investigators proposed a potential application of therapies targeting estrogen and the use of these antibodies as biomarkers in both diseases.

Interestingly, a recent article [39], presented the inhibitory action on collagen production of 2-methoxyestradiol (2-ME), a natural endogenous metabolite of E2 with low binding affinity for estrogen receptors. Fibroblasts from affected skin of 3 SSc patients were harvested and cultured under hypoxic conditions. Hypoxia had been proposed as a consequence of Raynaud’s phenomenon and tissue fibrosis in the skin of SSc patients, and the authors aimed to investigate whether 2-ME could inhibit the hypoxic pathways, mediated mainly by hypoxia-inducible factor 1α (HIF-1α), responsible for fibroblasts activation and collagen I synthesis. 2-ME resulted to have an anti-fibrogenic action, attenuating fibroblast growth and the profibrotic effect of hypoxia, thus suggesting a possible therapeutic application of 2-ME in SSc and other fibrotic diseases.

Vasculopathy {1b}

Together with fibrosis, vasculopathy is the distinguishing hallmark of SSc, and vascular manifestations are seen early and frequently over the disease course. The effect of hormones on endothelial dysfunction of patients with Raynaud’s phenomenon and SSc, was investigated in two studies conducted by Lekakis et al. on 12 [31] and 9 [32] female patients, treated respectively with short-term and long-term estrogen, and compared with healthy controls. In the first study [31], basal vasodilator responses of the brachial artery were evaluated with high-resolution echo-doppler ultrasound and, in post-menopausal women, the assessment was repeated the next

day after intravenous administration of conjugated estrogen. The infusion induced flow-mediated dilatation in 6 of the 10 patients and 7 of the 8 controls, with a similar percentage of improvement in the two groups. Thus, estrogen rapidly improved the endothelium-dependent abnormal vascular reactivity secondary to the disease, but had no effect on the functionality of smooth muscle cells. In the second study [32], 9 post-menopausal patients were randomized to 4 weeks of either placebo or oral conjugated estrogens, with a crossover design after 4 weeks of washout. Vasoreactivity studies on the brachial artery were performed with high-resolution echo-doppler ultrasound to measure vasodilation at baseline and after each 4-weeks period. Long-term conjugated estrogens significantly improved flow-mediated vasodilation compared to placebo. In both studies the authors suggested that estrogen, either acutely or chronically administered, enhanced endothelial relaxation.

Sex hormone levels and SSc {2}

How sex hormone levels vary in men and women with SSc {2a}

Overall, sex hormone levels were evaluated in 127 men, 152 women in post-menopause, and 52 in childbearing age. Differences in measurements between patients and healthy controls are represented in Table 1 for men and in Table 2 for women. All reported studies, with a case-control design, were assessed as moderate or high quality. In the already mentioned article by Aida-Yasuoka et al. [25], levels of E2 and estrone were measured in the sera of 68 post-menopausal patients with SSc and compared to 35 healthy controls. The mean values of hormone levels in patients and controls were not indicated in the manuscript, and thus are not reported in Table 2. The individuals were categorized as having low, intermediate, or high concentrations of estrogens. The authors found significantly higher percentages of SSc patients in the “high estrogen concentration” group compared to healthy controls ($p = 0.04$ for E2 and $p = 0.006$ for estrone) and no association with any specific disease manifestation. No other study investigating E2 levels observed significant differences between women with SSc and controls [29,33], while in men higher concentrations of E2 were found in 2 studies. In a recently published manuscript, Baker Frost et al. [54] measured E2 levels in 83 diffuse SSc male patients older than 50 years, and compared the results with 37 healthy controls and with a previously reported, and abovementioned, cohort of 68 post-menopausal women. Mean serum E2 concentration in the reference population was significantly higher than in the two control groups (respectively $p < 0.0001$ and $p = 0.0063$). Higher E2 levels were found in male patients with heart involvement ($p = 0.037$), while no association with ILD and renal disease was observed. Serum E2 also correlated with the risk of death

Table 1
Summary table of hormone variations in male SSc patients compared to healthy matched controls in the reviewed studies

Study	Population	MEN												
		DHEAS		SHBG		Total testosterone		DHT		Bioactive testosterone		E2		
		μmol/L	p-value	nmol/L	p-value	nmol/L	p-value	nmol/L	p-value	nmol/L	p-value	pmol/L	p-value	
Jemec, 1991 [49]	Patients	10	4.5 ± 2.8	n.s.	54.8 ± 11.6	<0.01	24.7 ± 7.2	<0.01	2 ± 0.5	<0.01			106.1 ± 55.6	<0.05
	Controls	14	7.6 ± 4		38.9 ± 14.7		17.4 ± 6.2		1.3 ± 0.5				95.8 ± 42.5	
Mirone, 2006 [51]	Patients	5	1.7 ± 1.4	0.005	37.5 ± 19.6	n.s.	15.3 ± 4.8 ^a	n.s.						
	Controls	8	5.3 ± 1.6		43.2 ± 18.4		17.7 ± 4.2 ^a							
Arnaud, 2017 [9]	Patients	29			36 (14–64)	n.s.	10 (4.8–16)	0.03			4.8 (2.2–7.2)	0.02		
	Controls	17			39 (9.8–63)		13 (7–17)				5.9 (2.4–8.2)			
Baker-Frost, 2019 [54]	Patients	83											112.3 ± 63.9	
	Controls	37											47.4 ± 22.4	<0.0001
	Controls ^b	68											88.8 ± 61.3	0.0063

All reported values have been converted to SI units and rounded to one decimal place. The values represent means ± standard deviations or medians (interquartile ranges); DHEAS= dehydroepiandrosterone sulphate; SHBG= sex hormone-binding globulin; DHT= dihydrotestosterone; E2= estradiol; n.s.= non-significant.

^a Not clear if total or free testosterone was measured.

^b Post-menopausal women.

Table 2

Summary table of hormone variations in female SSc patients compared to healthy matched controls in the reviewed studies

Study	Population		WOMEN										
			DHEAS		SHBG		Testosterone ^a		A4		E2		
			$\mu\text{mol/L}$	<i>p</i> -value	nmol/L	<i>p</i> -value	nmol/L	<i>p</i> -value	nmol/L	<i>p</i> -value	pmol/L	<i>p</i> -value	
La Montagna, 2001 [30]	CB	Patients	11	2.4 ± 2.3	<0.001								
		Controls	11	7.5 ± 1.1									
	PM	Patients	29	1.2 ± 1.1	n.s.								
		Controls	29	1.4 ± 0.7									
La Montagna, 2001 [29]	CB	Patients	12							309.9 ± 42.2	n.s.		
		Controls	12							238.6 ± 32.3			
	PM	Patients	6							48.8 ± 6.2	n.s.		
		Controls	6							36.7 ± 3.3			
Mirone, 2006 [51]	CB	Patients	12	2.7 ± 2.1	0.001	53 ± 20.5	n.s.	0.8 ± 0.7	0.005				
		Controls	14	5.7 ± 2		41.8 ± 17.5		1.5 ± 0.4					
	PM	Patients	22	1.8 ± 1	n.s.	41.5 ± 20.4	n.s.	0.6 ± 0.3	0.03				
		Controls	23	1.7 ± 0.7		46 ± 21.7		0.9 ± 0.6					
Imrich, 2006 [41]	CB	Patients	17	3.5 ± 2.7	n.s.								
		Controls	18	4.7 ± 3.3									
Perkovic, 2014 [33]	PM	Patients	27	1.2 ± 1	0.008			0.8 ± 0.6	<0.001	3.3 ± 3.1	0.004	180 ± 84	n.s.
		Controls	27	2 ± 1.1				1.6 ± 1		5.8 ± 2.8		160 ± 60	

All reported values have been converted to SI units and rounded to one decimal place. The values represent means ± standard deviations.

CB= childbearing age; PM= postmenopausal; DHEAS= dehydroepiandrosterone sulphate; SHBG= sex hormone-binding globulin; A4= androstenedione; E2= estradiol; n.s.= non-significant.

^a Not clear if total or free testosterone was measured.

(HR 1.18, 95% CI 1.01, 1.39 for a 10 pg/ml increase in serum E2, $p = 0.042$) and with skin thickness progression (10% increase in skin thickness (95% CI -0.4, 21.5%) for a 10 pg/ml increase in serum E2, $p = 0.062$ with significance set at $p = 0.10$ in multivariable model), and lower levels were noted in patients with anti-RNA polymerase III antibody positivity ($p = 0.027$). Also in a previous study published by Jemec and Sindrup [49], E2 levels in SSc male patients were higher than in controls ($p < 0.05$). The authors evaluated sex hormones in 10 men, and also levels of sex hormone-binding globulin ($p < 0.01$), total testosterone ($p < 0.01$) and dihydrotestosterone ($p < 0.01$), resulted to be significantly higher than in matched controls. Furthermore, androgen levels correlated with urinary excretion of collagen metabolites, supporting the idea of a pathogenetic role played by sex hormones in the development of SSc. These results are in contrast with those obtained in a cohort of 29 men with SSc [9], where total testosterone ($p = 0.03$) and bioactive testosterone ($p = 0.02$) levels were found to be significantly lower than in healthy controls. Bioactive testosterone deficiency was associated with the use of cyclophosphamide in the 12 months before the study, while no correlation with the use of prednisolone was noted. In the same patients, the presence of ILD was associated with higher FSH levels and both FSH, LH, and low testosterone, were correlated with the EScSG activity index. Similarly, Mirone et al. [51] evaluated androgen levels in SSc patients, including men and women in pre- or post-menopause. DHEAS concentrations were significantly reduced in men ($p = 0.005$) and women in childbearing age ($p = 0.001$) compared to healthy controls. The authors did not state whether total testosterone, or free testosterone, was measured, however low testosterone levels were not found in male patients, but were present in both childbearing age ($p = 0.005$) and post-menopause women ($p = 0.03$). These results are at least partially concordant with those obtained by Perkovic et al. [33] who observed, in 27 women in post-menopausal age, significantly lower levels of testosterone ($p < 0.001$), DHEAS ($p = 0.008$) and androstenedione ($p = 0.004$) compared to healthy controls. Again, it was not indicated if total testosterone, or free testosterone, was assessed. No correlation of hormone levels with disease activity or modified Rodnan Skin Score (mRSS) was detected, while androstenedione levels were negatively associated with ACA and positively with ATA, and a positive correlation between testosterone and ATA was found. A reduction of DHEAS levels was also found in 11 premenopausal SSc patients compared to healthy fertile women

($p < 0.001$) in a study published in 2001 [30], while no difference was detected in the group of 29 women in post-menopausal status compared with matched controls. Low DHEAS in fertile age was not associated with disease duration, disease subset, or organ involvement, but an impairing effect of glucocorticoid treatment on DHEAS concentration was noted. Low basal levels of DHEA ($p < 0.05$), but not of DHEAS, were also observed by Imrich et al. [41] in 17 childbearing age patients, who additionally presented androstenedione and 17-OH progesterone concentrations similar to healthy controls.

Furthermore, a possible association between low testosterone levels and SSc was identified in 6 reports describing cases of male hypogonadism predating the onset of SSc. Five men with Klinefelter syndrome [36,44,47,48,50], a condition of congenital hypogonadism, developed SSc in adulthood, and another report [42] described the case of a man diagnosed with SSc soon after being treated with orchidectomy and androgen deprivation therapy (ADT) for prostate adenocarcinoma, a form of acquired hypogonadism.

How changes in sex hormone levels modify the SSc course {2b}

After menopause, even with some fluctuations, both estrogens and progesterone decline, and two cohort studies retrospectively explored the effect of this hypoestrogenic state on women with SSc. In the Canadian multicentre CSRG cohort [35], 72% of the 1070 women were already in menopause at cohort entry and, after correction for confounders, their mRSS was lower than in pre-menopausal patients. This difference was substantial in the group with diffuse SSc (-2.62 units, 95% CI -4.44, -0.80), while the effect of post-menopausal status on mRSS was less evident, and not significant, in patients with limited SSc (-0.58 units, 95% CI -1.50, 0.34). Notably when only women with a disease duration of less than 5 years were analyzed, the impact of menopause on mRSS was stronger in both disease subsets. The authors suggested that the beneficial effect of hypoestrogenism on skin involvement could be explained by the stimulatory role played by estrogens on the profibrotic cytokines platelet derived growth factor (PDGF) and transforming growth factor-beta1 (TGF-beta 1).

At the same time, the positive influence of menopause on skin involvement is offset by the possible detrimental effect on the risk of developing pulmonary vascular disease revealed by a retrospective cohort study of 189 female patients [34]. During the observation period, 33% of patients were diagnosed with PAH, with a cumulative

probability progressively increasing in post-menopausal women while remaining stable in pre-menopausal patients. The authors concluded that post-menopausal status was the main risk factor for development of PAH, supporting the argument with the protective role of estrogens in mediation and inhibition of pathogenic mechanisms responsible for endothelial damage.

Effects of therapies targeting sex hormones on SSc [3]

The interest on a potential role of therapies targeting sex hormones, on SSc, dates back to the seventies. We found 1 case report [43] describing the improvement of a male patient with progressive SSc, and 3 RCTs on the efficacy of Cyclofenil, a SERM with weak estrogenic properties and partial agonist behavior. All 3 studies were scored as high risk of bias. Two double-blind placebo-controlled trials [45,46] were designed to assess superiority of the studied therapy compared to placebo, and 1 open-label placebo-controlled trial [26], was conceived to compare the efficacy of Cyclofenil to placebo and to Penicillamine. Skin involvement, lung and heart function, gastrointestinal complications, and laboratory tests, were assessed in all 3 studies, which showed a high withdrawal rate and failed to demonstrate an overall efficacy of Cyclofenil. In all 3 trials, the main reason for withdrawal was significant hypertransaminasemia. Lack of efficacy and potential liver toxicity lead to abandonment of Cyclofenil in SSc.

In 1991 the results of a double-blind placebo-controlled trial investigating the efficacy of the anabolic steroid Stanozolol in patients with SSc were published [28]. This study was assessed as moderate risk of bias. Twenty-four patients were assigned to receive either Stanozolol or placebo for 24 weeks, and then to cross-over for other 24 weeks. Seventeen patients completed both study phases. Periodic assessment did not reveal significant changes in mRSS and in frequency of Raynaud's phenomenon attacks. Side effects were more frequent during active treatment period, and one case of possible treatment-related fatal acute renal failure and hepatocellular damage was recorded. No further studies on Stanozolol in SSc were found.

Campochiaro et al. [38] recently reported a small case series of 3 patients with no pre-existing autoimmune disease, who received the diagnosis of SSc after being treated with feminizing hormone therapy for gender transition purposes. Type and duration of hormone treatment were different for each patient, but all underwent androgen-lowering procedures, either surgical or pharmacological, before disease onset. Hormonal imbalance has been implicated in the pathogenesis of autoimmune diseases, but the role of hormone therapy used for male-to-female transition in the onset of SSc is not clear, neither it is the benefit of interrupting the transition path once SSc has been diagnosed.

However, while the feminization process commonly combines the use of female sex hormones with anti-androgens or orchiectomy, the most frequent use of hormone replacement therapy (HRT) alone is to mitigate the unpleasant symptoms experienced during the climacteric period. Beretta et al. [40] retrospectively analyzed a cohort of 61 female patients with limited cutaneous SSc, who at inclusion were in post-menopause and had no signs or symptoms of PAH. In the attempt to evaluate the effect of HRT on PAH development, the authors defined the observation period as the time between menopause and last negative echocardiography or last year of HRT use. After a mean of 7.2 ± 3.5 and 7.5 ± 3.9 years respectively, none of the 20 women in the HRT group had developed PAH, while 19.5% of the non-HRT patients had been diagnosed with PAH ($p < 0.05$). No other relevant clinical or laboratory differences were present between the two groups to explain a higher PAH risk in the non-HRT cohort. A possible limitation of the study is that right heart catheterization was not performed to confirm PAH, however the diagnosis was robustly supported by echocardiographic parameters, presence of symptoms, and decline of diffusing lung capacity for carbon monoxide (DLCO) at pulmonary function tests. The authors thus

concluded that the use of HRT in early post-menopause might be protective against the development of PAH.

Discussion

Female preponderance in autoimmune diseases [55] raises the question of a pathogenetic role of sex hormones. Whereas the involvement of estrogens and androgens in rheumatoid arthritis [16] and in systemic lupus erythematosus [17] has been extensively investigated, the contribution to SSc is more difficult to define.

We reviewed the available literature to summarize: (1) the role of sex hormones in the pathophysiology of SSc; (2) how sex hormone levels vary in men and women with SSc compared to healthy controls and how changes in sex hormone levels modify the SSc course; (3) the effect of sex hormone-targeting therapies on SSc onset, activity, and progression.

Categorizing the results, for the first research question we found that E2 plays a profibrotic role and at the same time exerts a vasodilatory action at macrovascular level. For the second research question we found that levels of DHEAS and testosterone are lower in women with SSc compared to healthy controls, while in male patients the limited sample size and the discordant results don't allow to draw conclusions about imbalances in androgen levels, but significantly higher levels of E2 have been found at least in older men, and are associated with heart involvement, skin progression, and death. In women, after menopause, skin thickening seems to be less severe and the risk of development of PAH seems to increase. In line with this and concerning our third research question, there might be a role for HRT in the prevention of PAH. Other attempts to treat SSc patients with therapies modulating sex hormones, have not shown promising results so far.

Considering the role of steroid sex hormones in the pathophysiology of SSc, we could not find data about androgens, while estrogens seem to be profibrotic. The results, however, derive from small studies, with limited number of patients, and approaching the topic from different perspectives. Estrogens have been demonstrated to be vasodilatory in large vessels, but there is no evidence about effects on microvasculature. Studies exploring how steroid sex hormone levels vary in SSc compared to healthy controls, were frequently limited by small sample sizes, especially in men, and not all of them explored the same hormones. Past experiences with hormone-targeting therapies in SSc were not positive, but no high-quality study has been conducted, while deeper knowledge of the role played by sex hormones in SSc might provide ideas for further research.

In our first research question, we aimed at assessing how sex hormones contribute to SSc fibrogenesis, as evidenced by ex-vivo studies on cultured fibroblasts [25,52,53] and by the association of anti-estrogen receptor antibodies with the diffuse disease subset [27]. As the authors propose, these functional autoantibodies, full estrogen agonists, could be useful as biomarkers [27] but, in our opinion, also to identify patients who could eventually be candidates to assess the efficacy of estrogen-targeting therapies. The fibrogenic role of female sex hormones is corroborated by the finding that after menopause, when estrogens decline, skin involvement is less severe than in child-bearing age [35]. This is in agreement with what is observed in the general female population, in which menopause entails skin thinness and decreased collagen content [56]. However, it is interesting to note that a recent study [39] explored the antifibrotic effect of 2-ME in skin biopsies obtained from SSc patients. For its anti-proliferative and anti-angiogenic properties, 2-ME has been studied in the treatment of advanced malignancies and has been shown to inhibit fibrosis in bleomycin-induced SSc mice models [57]. The authors of the reviewed article propose 2-ME as a prospective treatment for SSc but our opinion is that the results attained ex-vivo in cultured SSc fibroblasts, would be difficult to replicate in SSc patients, especially

considering the hazard of using an anti-angiogenic medication in a disease frequently burdened by vasculopathy.

Whether estrogens affect vasculopathy of SSc patients has been evaluated in two studies [31,32], both pointing to an endothelium-dependent vasodilatory action, that can be retrieved also in the evidence that HRT use after menopause might be protective against the onset of PAH [40], for which the hypoestrogenic state of menopause has been hypothesised to represent a major risk factor [34]. In the first [40] of the latter two studies, no statistically significant differences were found in the clinical or demographic characteristics of the compared groups, while in the second [34] patients who developed PAH were significantly older at SSc onset. However, the results were not adjusted for age and disease duration. The prevalence of PAH is higher in women not only if we consider patients with SSc, but also in the general population, although estrogen has been demonstrated to be protective against the disease, leading to the idea of an “estrogen paradox” [58]. In this regard it is remarkable that, while HRT has been shown to reduce the risk of PAH in SSc patients, a cross-sectional study [59] demonstrated an association between unopposed estrogen therapy and primary Raynaud’s phenomenon. No association with combined HRT could be observed, suggesting that the action on the vasculature might be dependent on hormonal formulation.

Concerning the second research question, although recent findings indicate an association between elevated E2 levels and disease manifestations or progression in older men, it is difficult to draw firm conclusions about how sex hormone levels vary in SSc, and in particular it is impossible to say if the observed imbalances are a consequence of the disease, or if they played a pathogenetic role in its development. It is conceivable that in the future, measuring sex hormones in early patients identified through the application of VEDOSS (Very Early Diagnosis Of Systemic Sclerosis) criteria [60], and monitoring them over time, could help to elucidate how estrogens and androgens change during the disease course and their potential contribution to SSc progression. The finding of a possible association between male hypogonadism and SSc onset, suggested by few cases of patients with Klinefelter syndrome [36,44,47,48,50], cannot be considered conclusive, although the occurrence of different autoimmune rheumatic diseases has been described in patients with Klinefelter syndrome [61]. The report of a patient developing SSc soon after treatment with ADT [42], does not find confirmation in a recent study comparing the risk of autoimmune diseases between over 11,000 prostate cancer patients treated with ADT or not, where no case of SSc was diagnosed in either group [62].

Finally, addressing the third research question, we encountered the major limitation of this review, which was the lack of high-quality research about the efficacy of therapies targeting sex hormones in SSc. Furthermore, with the exception of an exploratory study on Tamoxifen [63], excluded from this review due to unacceptably high risk of bias, we could not find any study conducted in recent years. In particular no RCTs have been published since the early nineties.

Conclusions

In conclusion, this systematic review of the literature observed possibly profibrotic and vasodilatory effects of estrogens in SSc. Sex hormone imbalances are not uncommon in patients with SSc, but their significance and role in disease onset or progression need to be further investigated, preferably with a longitudinal approach. Clinical trials are old and did not show any efficacy of the SERM Cyclofenil or of the anabolic steroid Stanozolol. However, the striking female prevalence, and the clinical and autoantibody differences observed between men and women with SSc, might be contributed by sex hormones, but also by an interplay of other factors that, to date, have not been thoroughly determined. In addition, although the results of past attempts have not been promising, new insights on the profibrotic influence of sex hormones and on the role of anti-estrogen receptor

antibodies, along with the availability of compounds acting on estrogen pathways, might worth additional clinical research.

Acknowledgments

The first author (JC) wishes to acknowledge the European League Against Rheumatism (EULAR) for the opportunity to work on the present manuscript with the support of a bursary awarded for scientific training.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare they have no conflict of interest with respect to this manuscript.

Appendix. Search strategies in databases

((“Scleroderma, Systemic”[Mesh] OR “systemic sclerosis”[tw] OR “systemic scleroderma”[tw] OR “diffuse scleroderma”[tw] OR “limited scleroderma”[tw] OR “scleroderma”[tw] OR scleroderm*[tw] OR “CREST Syndrome”[tw] OR (“SSc”[tw] AND (scleroderm*[tw] OR “sclerosis”[tw]))) AND (“Gonadal Steroid Hormones”[mesh] OR “gonadal steroid hormone”[tw] OR “gonadal steroid hormones”[tw] OR “sex hormone”[tw] OR “sex hormones”[tw] OR “Estradiol Congeners”[tw] OR “Equilenin”[tw] OR “Equilin”[tw] OR “Estradiol”[tw] OR “Estriol”[tw] OR “Estetrol”[tw] OR “Alkylated Estrogenic Steroids”[tw] OR “Ethinyl Estradiol”[tw] OR “Ethinyl Estradiol-Norgestrel Combination”[tw] OR “Mestranol”[tw] OR “Quinestrol”[tw] OR “Catechol Estrogens”[tw] OR “Conjugated Estrogens”[tw] OR “Esterified Estrogens”[tw] OR “Estrone”[tw] OR “Progesterone Congeners”[tw] OR “Pregnenolone”[tw] OR “17-alpha-Hydroxypregnenolone”[tw] OR “Progesterone”[tw] OR “20-alpha-Dihydroprogesterone”[tw] OR “5-alpha-Dihydroprogesterone”[tw] OR “Hydroxyprogesterones”[tw] OR “17-alpha-Hydroxyprogesterone”[tw] OR “Pregnanetriol”[tw] OR “Pregnanediol”[tw] OR “Testosterone Congeners”[tw] OR “Androstane-3,17-diol”[tw] OR “Androstenediol”[tw] OR “Androstenedione”[tw] OR “Androsterone”[tw] OR “Dehydroepiandrosterone”[tw] OR “DHEA-S”[tw] OR “DHEA”[tw] OR “Dihydrotestosterone”[tw] OR “Etiocolanalone”[tw] OR “Nandrolone”[tw] OR “Testosterone”[tw] OR “Epitestosterone”[tw] OR “Hydroxytestosterones”[tw] OR “Methenolone”[tw] OR “Methyltestosterone”[tw] OR “Testosterone Propionate”[tw] OR “Catechol Estrogen”[tw] OR “Conjugated Estrogen”[tw] OR “Esterified Estrogen”[tw] OR “Estrones”[tw] OR “Pregnenolones”[tw] OR “Progesterones”[tw] OR “Hydroxyprogesterone”[tw] OR “Androstenediones”[tw] OR “Androsterones”[tw] OR “Dehydroepiandrosterones”[tw] OR “Dihydrotestosterones”[tw] OR “Etiocolanolones”[tw] OR “Testosterones”[tw] OR “Epitestosterones”[tw] OR “Hydroxytestosterone”[tw] OR “Estrogens”[mesh] OR “Estrogens”[pharmacological action] OR “estrogen”[tw] OR “estrogens”[tw] OR “oestrogen”[tw] OR “oestrogens”[tw] OR “phytoestrogen”[tw] OR “phytoestrogens”[tw] OR “2,3-bis(3'-hydroxybenzyl)butane-1,4-diol”[Supplementary Concept] OR “2,3-bis(3'-hydroxybenzyl)butyrolactone”[Supplementary Concept] OR “4-octylphenol”[Supplementary Concept] OR “8-prenylnaringenin”[Supplementary Concept] OR “biochanin A”[Supplementary Concept] OR “bisphenol A”[Supplementary Concept] OR “Chlorotrianisene”[mesh] OR “Coumestrol”[mesh] OR “daidzein”[Supplementary Concept] OR “Dienestrol”[mesh] OR “Diethylstilbestrol”[mesh] OR “diethylstilbestrol dipropionate”[Supplementary Concept] OR “Epimestrol”[mesh] OR “Equal”[mesh] OR “Estradiol”[mesh] OR “estradiol enanthate”[Supplementary Concept] OR “Estrogenic Steroids, Alkylated”[mesh] OR “Estrogens, Conjugated

(USP)[mesh] OR “estrogens, conjugated synthetic A”[Supplementary Concept] OR “estrogens, conjugated synthetic B”[Supplementary Concept] OR “Estrogens, Esterified (USP)”[mesh] OR “Estrone”[mesh] OR “Ethiny! Estradiol”[mesh] OR “formononetin”[Supplementary Concept] OR “Genistein”[mesh] OR “glycitein”[Supplementary Concept] OR “glycitin”[Supplementary Concept] OR “Hexestrol”[mesh] OR “Infecundin”[Supplementary Concept] OR “Mestranol”[mesh] OR “nylestriol”[Supplementary Concept] OR “o,p’-DDT”[Supplementary Concept] OR “O-desmethylangolensin”[Supplementary Concept] OR “polyestradiol phosphate”[Supplementary Concept] OR “Quinestrol”[mesh] OR “secoisolariciresinol”[Supplementary Concept] OR “Zearalenone”[mesh] OR “Zeranol”[mesh] OR “2,3-bis(3’-hydroxybenzyl)butane-1,4-diol”[tw] OR “2,3-bis(3’-hydroxybenzyl)butyrolactone”[tw] OR “4-octylphenol”[tw] OR “8-prenylnaringenin”[tw] OR “biochanin A”[tw] OR “bisphenol A”[tw] OR “Chlorotrianisene”[tw] OR “Coumestrol”[tw] OR “daidzein”[tw] OR “Dienestrol”[tw] OR “Diethylstilbestrol”[tw] OR “diethylstilbestrol dipropionate”[tw] OR “Epimestrol”[tw] OR “Equol”[tw] OR “Estradiol”[tw] OR “estradiol enanthate”[tw] OR “Estrogenic Steroids, Alkylated”[tw] OR “Estrogens, Conjugated (USP)”[tw] OR “estrogens, conjugated synthetic A”[tw] OR “estrogens, conjugated synthetic B”[tw] OR “Estrogens, Esterified (USP)”[tw] OR “Estrone”[tw] OR “Ethiny! Estradiol”[tw] OR “formononetin”[tw] OR “Genistein”[tw] OR “glycitein”[tw] OR “glycitin”[tw] OR “Hexestrol”[tw] OR “Infecundin”[tw] OR “Mestranol”[tw] OR “nylestriol”[tw] OR “o,p’-DDT”[tw] OR “O-desmethylangolensin”[tw] OR “polyestradiol phosphate”[tw] OR “Quinestrol”[tw] OR “secoisolariciresinol”[tw] OR “Zearalenone”[tw] OR “Zeranol”[tw] OR “Proggestins”[mesh] OR “Proggestins”[Pharmacological Action] OR “Proggestin”[tw] OR “Proggestins”[tw] OR “11-hydroxyprogesterone”[Supplementary Concept] OR “17-alpha-hydroxyprogesterone caproate”[Supplementary Concept] OR “20-alpha-Dihydroprogesterone”[mesh] OR “Algestone”[mesh] OR “Algestone Acetophenide”[mesh] OR “Allylestrenol”[mesh] OR “altrenogest”[Supplementary Concept] OR “delmadinone”[Supplementary Concept] OR “Desogestrel”[mesh] OR “Dydrogesterone”[mesh] OR “Flurogesterone Acetate”[mesh] OR “Gestodene”[Supplementary Concept] OR “Gestrinone”[mesh] OR “Infecundin”[Supplementary Concept] OR “Progesterone”[mesh] OR “quingestron”[Supplementary Concept] OR “11-hydroxyprogesterone”[tw] OR “17-alpha-hydroxyprogesterone caproate”[tw] OR “20-alpha-Dihydroprogesterone”[tw] OR “Algestone”[tw] OR “Algestone Acetophenide”[tw] OR “Allylestrenol”[tw] OR “altrenogest”[tw] OR “delmadinone”[tw] OR “Desogestrel”[tw] OR “Dydrogesterone”[tw] OR “Flurogesterone Acetate”[tw] OR “Gestodene”[tw] OR “Gestrinone”[tw] OR “Infecundin”[tw] OR “Progesterone”[tw] OR “quingestron”[tw] OR “Androgens”[Pharmacological Action] OR “Androgens”[mesh] OR “Androgen”[tw] OR “Androgens”[tw] OR “19-nortestosterone 4-hexoxyphenylpropionate”[Supplementary Concept] OR “3-methyl-5-trifluoromethyl-3,4-dihydro-2H-pyrazole-3-carboxylic acid (4-cyano-3-trifluoromethylphenyl)amide”[Supplementary Concept] OR “acetothiolutamide”[Supplementary Concept] OR “Dihydrotestosterone”[mesh] OR “LG 121071”[Supplementary Concept] OR “Nandrolone”[mesh] OR “Oxandrolone”[mesh] OR “Oxymetholone”[mesh] OR “Stanozolol”[mesh] OR “Testosterone”[mesh] OR “testosterone 17 beta-cypionate”[Supplementary Concept] OR “testosterone enanthate”[Supplementary Concept] OR “tstosterone undecanoate”[Supplementary Concept] OR “tetrahydrogestrinone”[Supplementary Concept] OR “19-nortestosterone 4-hexoxyphenylpropionate”[tw] OR “3-methyl-5-trifluoromethyl-3,4-dihydro-2H-pyrazole-3-carboxylic acid (4-cyano-3-trifluoromethylphenyl)amide”[tw] OR “acetothiolutamide”[tw] OR “Dihydrotestosterone”[tw] OR “LG 121071”[tw] OR “Nandrolone”[tw] OR “Oxandrolone”[tw] OR “Oxymetholone”[tw] OR “Stanozolol”[tw] OR “Testosterone”[tw] OR “testosterone 17 beta-cypionate”[tw] OR “testosterone enanthate”[tw] OR “tstosterone undecanoate”[tw] OR “tetrahydrogestrinone”[tw] OR “Androstenedione”[Mesh] OR “Androstenedione”[tw] OR “Gonadotropin-

Releasing Hormone”[Mesh] OR “gonadotropin releasing hormone”[tw] OR “gonadotropin releasing hormones”[tw] OR “GnRH”[tw] OR “GnRHs”[tw] OR “Buserelin”[tw] OR “Goserelin”[tw] OR “Leuproli-
de”[tw] OR “Nafarelin”[tw] OR “Triptorelin”[tw] OR “Pamoate”[tw] OR “Follicle Stimulating Hormone”[Mesh] OR “follicle stimulating hormone”[tw] OR “follicle stimulating hormones”[tw] OR “FSH”[tw] OR “FSHs”[tw] OR “Follitropin”[tw] OR “Luteinizing Hormone”[Mesh] OR “luteinizing hormone”[tw] OR “luteinizing hormones”[tw] OR “luteinising hormone”[tw] OR “luteinising hormones”[tw] OR “Prolactin”[Mesh] OR “prolactin”[tw] OR “prolactins”[tw] OR “male hypogonadal disorders”[tw] OR “male hypogonadal disorder”[tw] OR hypogonad* [tw] OR “Klinefelter Syndrome”[Mesh] OR Klinefelter* [tw] OR “Hormone Replacement Therapy”[Mesh] OR “hormone replacement therapy”[tw] OR “HRT”[tw] OR “Estrogen Replacement Therapy”[tw] OR “Contraceptives, Oral, Hormonal”[Mesh] OR “Contraceptives, Oral, Hormonal”[Pharmacological Action] OR “hormonal contraception”[tw] OR “Aromatase Inhibitors”[Mesh] OR “Aromatase Inhibitors”[Pharmacological Action] OR “aromatase inhibitors”[tw] OR “aromatase inhibitor”[tw] OR “transgender hormone therapy”[tw] OR “Transgender Persons”[mesh] OR transgender* [tw] OR “Menopause”[mesh] OR Menopaus* [tw] OR Postmenopaus* [tw] OR Premenopaus* [tw] OR “Cyclofenil”[Mesh] OR “cyclofenil”[tw] OR cyclofenil* [tw] OR “Cyclophenil”[tw] OR “4-((4-(Acetyloxy)phenyl)cyclohexylidene)methyl)phenol Acetate”[tw] OR “Ondogyne”[tw] OR “F-6066”[tw] OR “F 6066”[tw] OR “F6066”[tw] OR “Fertility Agents, Female”[mesh] OR “Fertility Agents, Female”[Pharmacological Action] OR “Buserelin”[tw] OR “cetorelix”[tw] OR “Clomiphene”[tw] OR “Enclomiphene”[tw] OR “Pituitary Gonadotropins”[tw] OR “Pituitary Gonadotropin”[tw] OR “Leuproli-
de”[tw] OR “Menotropins”[tw] OR “Menotropin”[tw] OR “Nafarelin”[tw] OR “onapristone”[tw] OR “Zuclomiphene”[tw] AND (english [la] OR dutch[la]) NOT (“Animals”[mesh] NOT “Humans”[mesh]) NOT (“child”[mesh] OR “infant”[mesh]) NOT (“Adult”[mesh] OR “Adolescent”[mesh]))

References

- Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. *N Engl J Med* 2009;360 (19):1989–2003. doi: 10.1056/NEJMra0806188.
- Marie I, Gehanno JF. Environmental risk factors of systemic sclerosis. *Semin Immunopathol* 2015;37(5):463–73. doi: 10.1007/s00281-015-0507-3.
- Freire M, Rivera A, Sopena B, Tolosa Vilella C, Guillen-Del Castillo A, Colunga Arguelles D, et al. Clinical and epidemiological differences between men and women with systemic sclerosis: a study in a spanish systemic sclerosis cohort and literature review. *Clin Exp Rheumatol* 2017;35(Suppl 106(4)):89–97.
- Peoples C, Medsger Jr. TA, Lucas M, Rosario BL, Feghali-Bostwick CA. Gender differences in systemic sclerosis: relationship to clinical features, serologic status and outcomes. *J Scleroderma Relat Disord* 2016;1(2):177–240. doi: 10.5301/jsrd.5000209.
- Hughes M, Herrick AL. Digital ulcers in systemic sclerosis. *Rheumatology (Oxford)* 2017;56(1):14–25. doi: 10.1093/rheumatology/kew047.
- D’Amico F, Skarmoutsou E, Mazzarino MC. The sex bias in systemic sclerosis: on the possible mechanisms underlying the female disease preponderance. *Clin Rev Allergy Immunol* 2014;47(3):334–43 [doi]. doi: 10.1007/s12016-013-8392-9.
- Rutkowski K, Sowa P, Rutkowska-Talipska J, Kuryliszyn-Moskal A, Rutkowski R. Dehydroepiandrosterone (DHEA): hypes and hopes. *Drugs* 2014;74(11):1195–207. doi: 10.1007/s40265-014-0259-8.
- Wetzels JF. Cyclophosphamide-induced gonadal toxicity: a treatment dilemma in patients with lupus nephritis? *Neth J Med* 2004;62(10):347–52.
- Arnaud L, Nordin A, Lundholm H, Svenungsson E, Hellbacher E, Wikner J, et al. Effect of corticosteroids and cyclophosphamide on sex hormone profiles in male patients with systemic lupus erythematosus or systemic sclerosis. *Arthritis Rheumatol* 2017;69(6):1272–9 [doi]. doi: 10.1002/art.40057.
- Goodarzi MO. Genetics of common endocrine disease: the present and the future. *J Clin Endocrinol Metab* 2016;101(3):787–94. doi: 10.1210/jc.2015-3640.
- Tweed JO, Hsia SH, Lutfy K, Friedman TC. The endocrine effects of nicotine and cigarette smoke. *Trends Endocrinol. Metab* 2012;23(7):334–42. doi: 10.1016/j.tem.2012.03.006.
- Sierksma A, Sarkola T, Eriksson CJ, van der Gaag MS, Grobbee DE, Hendriks HF. Effect of moderate alcohol consumption on plasma dehydroepiandrosterone sulfate, testosterone, and estradiol levels in middle-aged men and postmenopausal women: a diet-controlled intervention study. *Alcohol Clin Exp Res* 2004;28 (5):780–5.

- [13] Marcocchia D, Pellegrini M, Fiocchetti M, Lorenzetti S, Marino M. Food components and contaminants as (anti)androgenic molecules. *Genes Nutr* 2017;12(1):6. doi: 10.1186/s12263-017-0555-5.
- [14] Behr M, Oehlmann J, Wagner M. Estrogens in the daily diet: in vitro analysis indicates that estrogenic activity is omnipresent in foodstuff and infant formula. *Food Chem Toxicol Int J Published Br Ind Biol Res Assoc* 2011;49(10):2681–8. doi: 10.1016/j.fct.2011.07.039.
- [15] Bereshchenko O, Bruscoli S, Riccardi C. Glucocorticoids, sex hormones, and immunity. *Front Immunol* 2018;9:1332. doi: 10.3389/fimmu.2018.01332.
- [16] Cutolo M, Villaggio B, Craviotto C, Pizzorni C, Seriola B, Sulli A. Sex hormones and rheumatoid arthritis. *Autoimmun Rev* 2002;1(5):284–9.
- [17] Petri M. Sex hormones and systemic lupus erythematosus. *Lupus* 2008;17(5):412–5. doi: 10.1177/0961203308090026.
- [18] Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association diagnostic and therapeutic criteria committee. *Arthritis Rheum* 1980;23(5):581–90.
- [19] LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger Jr. TA, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988;15(2):202–5.
- [20] van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. *Arthritis Rheum* 2013;65(11):2737–47. doi: 10.1002/art.38098.
- [21] Schardt C, Adams MB, Owens T, Keitz S, Fontelo P. Utilization of the PICO framework to improve searching PubMed for clinical questions. *BMC Med Inform Decis Mak* 2007;7(1):16. doi: 10.1186/1472-6947-7-16.
- [22] Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011;343:d5928. doi: 10.1136/bmj.d5928.
- [23] Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle–Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analysis. 2019 [Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (Accessed 26 April 2019)]
- [24] Johanna-Briggs Institute. Critical appraisal tools 2017 [Available from: <http://joannabriggs.org/research/critical-appraisal-tools.html> (Accessed 26 April 2019)]
- [25] Aida-Yasuoka K, Peoples C, Yasuoka H, Hershberger P, Thiel K, Cauley JA, et al. Estradiol promotes the development of a fibrotic phenotype and is increased in the serum of patients with systemic sclerosis. *Arthritis Res Ther* 2013;15(1):R10. ar4140 [pii][doi]. doi: 10.1186/ar4140.
- [26] Akesson A, Blombulow B, Scheja A, Wollmer P, Valind S, Wollheim FA. Long-term evaluation of penicillamine or cyclofenil in systemic sclerosis – results from a 2-year randomized study. *Scand J Rheumatol* 1992;21(5):238–44.
- [27] Giovannetti A, Maselli A, Colasanti T, Rosato E, Salsano F, Pisarri S, et al. Autoantibodies to estrogen receptor alpha in systemic sclerosis (SSc) as pathogenetic determinants and markers of progression. *PLoS One* 2013;8(9):e74332. [doi]; PONE-D-13-18878 [pii]. doi: 10.1371/journal.pone.0074332.
- [28] Jayson MI, Holland CD, Keegan A, Illingworth K, Taylor L. A controlled study of stanozolol in primary Raynaud's phenomenon and systemic sclerosis. *Ann Rheum Dis* 1991;50(1):41–7.
- [29] La Montagna G, Baruffo A, Pasquali D, Bellastella A, Tirri G, Sinisi AA. Assessment of pituitary gonadotropin release to gonadotropin releasing hormone/thyroid-stimulating hormone stimulation in women with systemic sclerosis. *Rheumatology (Oxford)* 2001;40(3):310–4.
- [30] La Montagna G, Baruffo A, Buono G, Valentini G. Dehydroepiandrosterone sulphate serum levels in systemic sclerosis. *Clin Exp Rheumatol* 2001;19(1):21–6.
- [31] Lekakis J, Mavrikakis M, Papamichael C, Papazoglou S, Economou O, Scotinotiis I, et al. Short-term estrogen administration improves abnormal endothelial function in women with systemic sclerosis and Raynaud's phenomenon. *Am Heart J* 1998;136(5):905–12 S0002870398000532 [pii].
- [32] Lekakis J, Papamichael C, Mavrikakis M, Voutsas A, Stamatielopoulou S. Effect of long-term estrogen therapy on brachial arterial endothelium-dependent vasodilation in women with Raynaud's phenomenon secondary to systemic sclerosis. *Am J Cardiol* 1998;82(12):1555–7 A8S0002-9149(98)00708-5 [pii].
- [33] Perkovic D, Martinovic KD, Jurisic Z, Lalovac M, Radic M. Androgens in post-menopausal patients with systemic sclerosis. *Rheumatology (Oxford)* 2015;54(4):744–6 keu432 [pii][doi]. doi: 10.1093/rheumatology/keu432.
- [34] Scorza R, Caronni M, Bazzi S, Nador F, Beretta L, Antonioli R, et al. Post-menopause is the main risk factor for developing isolated pulmonary hypertension in systemic sclerosis. *Ann N Y Acad Sci* 2002;966:238–46.
- [35] Vinet E, Bernatsky S, Hudson M, Pineau CA, Baron M. Effect of menopause on the modified Rodnan skin score in systemic sclerosis. *Arthritis Res Ther* 2014;16(3):R130. R130ar4587 [pii][doi]. doi: 10.1186/ar4587.
- [36] De Keyser F, Mielantes H, Veys EM. Klinefelter's syndrome and scleroderma. *J Rheumatol* 1989;16(12):1613–4.
- [37] Fan X, Pope J, Baron M. What is the relationship between disease activity, severity and damage in a large Canadian systemic sclerosis cohort? Results from the Canadian scleroderma research group (CSRG). *Rheumatol Int* 2010;30(9):1205–10. doi: 10.1007/s00296-009-1129-7.
- [38] Campochiaro C, Host LV, Ong VH, Denton CP. Development of systemic sclerosis in transgender females: a case series and review of the literature. *Clin Exp Rheumatol* 2018;36(Suppl 113(4)):50–2 12276 [pii].
- [39] Zhou X, Liu C, Lu J, Zhu L, Li M. 2-Methoxyestradiol inhibits hypoxia-induced scleroderma fibroblast collagen synthesis by phosphatidylinositol 3-kinase/Akt/mTOR signalling. *Rheumatology (Oxford)* 2018;57(9):1675–84 5037742 [pii][doi]. doi: 10.1093/rheumatology/key166.
- [40] Beretta L, Caronni M, Origgi L, Ponti A, Santaniello A, Scorza R. Hormone replacement therapy may prevent the development of isolated pulmonary hypertension in patients with systemic sclerosis and limited cutaneous involvement. *Scand J Rheumatol* 2006;35(6):468–71.
- [41] Imrich R, Lukac J, Rovensky J, Radikova Z, Penesova A, Kvetnansky R, et al. Lower adrenocortical and adrenomedullary responses to hypoglycemia in premenopausal women with systemic sclerosis. *J Rheumatol* 2006;33(11):2235–41 0315162X-33-2235 [pii].
- [42] Giordano N, Magaro L, Fattorini L, Marcucci P, Battisti E, Fioravanti A, et al. Systemic sclerosis following anti-androgenic treatment for prostatic adenocarcinoma. *Clin Rheumatol* 1993;12(1):81–4.
- [43] Herbai G. Treatment of progressive systemic sclerosis with a synthetic weak estrogen: cyclofenil (Sexovid). Report of a case. *Acta Med Scand* 1974;196(6):537–40.
- [44] Nowlin NS, Zwillich SH, Brick JE, Carlson HE. Male hypogonadism and scleroderma. *J Rheumatol* 1985;12(3):605–6.
- [45] Blom-Bulow B, Oberg K, Wollheim FA, Persson B, Jonson B, Malmberg P, et al. Cyclofenil versus placebo in progressive systemic sclerosis. A one-year double-blind crossover study of 27 patients. *Acta Med Scand* 1981;210(5):419–28.
- [46] Gibson T, Grahame R. Cyclofenil treatment of scleroderma – a controlled study. *Br J Rheumatol* 1983;22(4):218–23.
- [47] Kobayashi S, Shimamoto T, Taniguchi O, Hashimoto H, Hirose S. Klinefelter's syndrome associated with progressive systemic sclerosis: report of a case and review of the literature. *Clin Rheumatol* 1991;10(1):84–6.
- [48] O'Donoghue DJ. Klinefelter's syndrome associated with systemic sclerosis. *Postgrad Med J* 1982;58(683):575–6.
- [49] Jemec GB, Sindrup JH. Circulating androgens in male patients suffering from systemic scleroderma. *Arch Dermatol Res* 1991;283(5):289–91.
- [50] Bargagli E, Bartalini G, Galeazzi M, Maggiorelli C, Anichini C, Rottoli P. Chromosome 5 inversion in two siblings, one with Klinefelter syndrome and systemic sclerosis, the other with rheumatoid arthritis. *Rheumatol Int* 2008;28(7):725–6 [doi]. doi: 10.1007/s00296-007-0501-8.
- [51] Mirone L, Barini A, Barini A. Androgen and prolactin (Prl) levels in systemic sclerosis (SSc): relationship to disease severity. *Ann N Y Acad Sci* 2006;1069(1):257–62 1069/1/257 [pii][doi]. doi: 10.1196/annals.1351.023.
- [52] Soldano S, Montagna P, Villaggio B, Parodi A, Gianotti G, Sulli A, et al. Endothelin and sex hormones modulate the fibronectin synthesis by cultured human skin scleroderma fibroblasts. *Ann Rheum Dis* 2009;68(4):599–602 ar2008.097378 [pii][doi]. doi: 10.1136/ard.2008.097378.
- [53] Soldano S, Montagna P, Brizzolaro R, Sulli A, Parodi A, Seriola B, et al. Effects of estrogens on extracellular matrix synthesis in cultures of human normal and scleroderma skin fibroblasts. *Ann N Y Acad Sci* 2010;1193(1):25–9 NYA55296 [pii][doi]. doi: 10.1111/j.1749-6632.2009.05296.x.
- [54] Baker Frost D, Wolf B, Peoples C, Fike J, Silver K, Laffoon M, et al. Estradiol levels are elevated in older men with diffuse cutaneous SSc and are associated with decreased survival. *Arthritis Res Ther* 2019;21(1):85. doi: 10.1186/s13075-019-1870-6.
- [55] Gonzalez DA, Diaz BB, Rodriguez Perez Mdel C, Hernandez AG, Chico BN, de Leon AC. Sex hormones and autoimmunity. *Immunol Lett* 2010;133(1):6–13. doi: 10.1016/j.imlet.2010.07.001.
- [56] Raine-Fenning NJ, Brincat MP, Muscat-Baron Y. Skin aging and menopause: implications for treatment. *Am J Clin Dermatol* 2003;4(6):371–8. doi: 10.2165/00128071-200304060-00001.
- [57] Zhu L, Song Y, Li M. 2-Methoxyestradiol inhibits bleomycin-induced systemic sclerosis through suppression of fibroblast activation. *J Dermatol Sci* 2015;77(1):63–70 S0923-1811(14)00251-5 [pii][doi]. doi: 10.1016/j.jdermsci.2014.10.007.
- [58] Umar S, Rabinovitch M, Eghbali M. Estrogen paradox in pulmonary hypertension current controversies and future perspectives. *Am J Respir Crit Care Med* 2012;186(2):125–31.
- [59] Fraenkel L, Zhang Y, Chaisson CE, Evans SR, Wilson PW, Felson DT. The association of estrogen replacement therapy and the raynaud phenomenon in postmenopausal women. *Ann Intern Med* 1998;129(3):208–11.
- [60] Avouac J, Fransen J, Walker UA, Ricciardi V, Smith V, Muller C, et al. Preliminary criteria for the very early diagnosis of systemic sclerosis: results of a Delphi consensus study from the EULAR scleroderma trials and research group. *Ann Rheum Dis* 2011;70(3):476–81. doi: 10.1136/ard.2010.136929.
- [61] Lazurova I, Rovensky J, Imrich R, Blazickova S, Lazurova Z, Payer J. Autoimmune rheumatic diseases and Klinefelter syndrome autoimmune reumaticke choroby a klinefelterov syndrom. *Eur Pharm J* 2016;63(2):18–22.
- [62] Liu JM, Yu CP, Chuang HC, Wu CT, Hsu RJ. Androgen deprivation therapy for prostate cancer and the risk of autoimmune diseases. *Prostate Cancer Prostatic Dis* 2019 [doi];10.1038/s41391-019-0130-9 [pii]. doi: 10.1038/s41391-019-0130-9.
- [63] Thomas-Golbanov CK, Wilke WS, Fessler BJ, Hoffman GS. Open label trial of tamoxifen in scleroderma. *Clin Exp Rheumatol* 2003;21(1):99–102.