Short-term treatment with a gonadotropin-releasing hormone antagonist, cetrorelix, in rheumatoid arthritis (AGRA): a randomized, double-blind, placebo-controlled study

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Objectives: Gonadotropin-releasing hormone (GnRH) stimulates immune responses; therefore, antagonizing GnRH with cetrorelix may have anti-inflammatory effects. The aim of this study was to assess short-term cetrorelix therapy in rheumatoid arthritis (RA) patients.

Method: In this proof-of-concept, randomized, double-blind study involving 99 patients with active, long-standing RA, 48 patients received subcutaneous cetrorelix (5 mg/day on days 1 and 2; 3 mg/day on days 3–5) and 51 received placebo. The primary end-point was the change in the 28-joint Disease Activity Score based on C-reactive protein (DAS28-CRP) by day 5, when the greatest GnRH suppression was anticipated. Secondary end-points included the change in tumour necrosis factor (TNF)-α, and achievement of American College of Rheumatology (ACR) responses and DAS28-CRP < 2.6 by day 5. Patients were followed up on days 10 and 15.

Results: By day 5, DAS28-CRP was non-significantly reduced by 0.82 in the cetrorelix group compared to a 0.57 reduction in the placebo group ($p = 0.091$), TNF-α (log pg/mL) was significantly reduced in the cetrorelix group compared with the placebo group [0.55, 95% confidence interval (CI) 0.08–1.01, $p = 0.023$], and more patients on cetrorelix achieved ACR20 responses (40% vs. 18%, $p = 0.015$) and DAS28-CRP < 2.6 (13% vs. 0%, $p = 0.009$). Inflammatory markers increased towards baseline levels after withdrawal of treatment. Rates of adverse events were similar in both groups.

Conclusions: Although there was no significant difference in the primary end-point between groups, antagonizing GnRH led to significant improvements in key secondary end-points. Thus, GnRH antagonists may have rapid anti-inflammatory effects in RA, already occurring within 5 days. The data suggest a novel mode of action for TNF-α inhibition in RA, and potentially in other autoimmune diseases.

Rheumatoid arthritis (RA) may develop, flare, or subside during hormonal changes in the hypothalamic–pituitary–gonadal (HPG) axis; for example, during pregnancy, postpartum, menopause, or aromatase inhibition therapy (1–3). These observations have prompted research into the effects of gonadal hormones of the HPG axis, such as oestrogen and testosterone in RA; but the results have been inconclusive.

Hypothalamic and pituitary hormones of the HPG axis, which control gonadal hormones, have not yet been studied in RA. This is surprising, as these hypothalamic and pituitary hormones are also profoundly involved in pregnancy, menopause, and postpartum. Gonadal hormones in both sexes are stimulated by pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH secretion are stimulated by the hypothalamic, gonadotropin-releasing hormone (GnRH). GnRH, LH, and FSH have important physiological roles in both male and female reproduction. Therefore, these hormones may be involved in pathological processes in males as well as females.

In vitro and animal studies in both sexes suggest that GnRH is secreted not only in the hypothalamus; but also in peripheral T cells. GnRH interacts with T cells, thus regulating immune responses (4–6). GnRH may also act indirectly on the immune system through LH (7) and/or FSH (8, 9). GnRH agonists have been associated with RA onset (10) and with polymyositis associated with vasculitis onset (11), whereas GnRH antagonists have shown anti-inflammatory effects in vitro and in animal studies (12, 13), suggesting therapeutic potential in autoimmunity.

We hypothesized that antagonizing GnRH in RA may have beneficial effects on disease activity compared to

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placebo. In the Antagonist to Gonadotropin-Releasing Hormone in RA (AGRA)-study, we aimed to investigate short-term clinical and biochemical effects and the safety of a GnRH antagonist, cetrorelix (Cetrotide, Aeterna Zentaris, Frankfurt, Germany), in RA patients. As hypothalamic reproductive suppression has not been investigated in RA before, the intervention was limited to a short period.

Method

In this investigator-initiated, proof-of-concept, randomized, double-blind, placebo-controlled, single-centre study, we enrolled males and females aged $\geq$ 18 years, with RA according to the 1987 revised American College of Rheumatology (ACR) criteria, and with a 28-joint Disease Activity Score based on C-reactive protein (DAS28-CRP) > 3.2 (full inclusion/exclusion criteria can be seen in the online Supplementary Material).

Ethical committee approval was obtained. A blinded independent committee, the Oslo University Hospital Data Monitoring Committee, regularly reviewed source documents against case record forms. Safety and efficacy visits, with blood sampling, were between 0730 and 0930 h on days 1 (baseline), 2, 3, 4, 5 (visit 5a), 10, and 15. There was an additional visit (visit 5b) between 1930 and 2130 h when maximum GnRH suppression was anticipated. Using computer-generated allocation, patients were randomly assigned 1:1 to cetrorelix (5 mg/day s.c. on days 1 and 2, 3 mg/day on days 3−5) or corresponding volumes of placebo. The cetrorelix doses were chosen to achieve rapid reductions in GnRH, LH, a surrogate marker for GnRH, and FSH. TNF-α was measured using multiplex technology with a high sensitivity (0.5 pg/mL) assay (for details see the online Supporting Information).

The predefined primary end-point was the baseline-adjusted between-group difference in DAS28-CRP by visit

Table 1. Baseline characteristics.*

	Cetrorelix (n = 48)	Placebo (n = 51)
Demographics		
Age (years)	54.9 ± 11.4	55.0 ± 11.7
Female sex (%)	73	71
Disease duration (years)	11.5 ± 10.6	12.0 ± 12.9
Anti-CCP antibody positive, n (%)	28 (58)	35 (69)
Current smoker, n (%)	13 (27)	20 (39)
Clinical and laboratory measures		
DAS28-CRP	5.0 ± 1.0	5.2 ± 1.0
CRP (mg/L)	18.9 ± 24.5	17.3 ± 22.5
ESR (mm/h)	22.0 ± 18.3	25.8 ± 27.0
TNF-α (pg/mL)†	21.2 ± 291	49.6 ± 290
LH (IU/L)	20.4 ± 16.3	19.6 ± 16.3
FSH (IU/L)‡	36.9 ± 30.0	32.9 ± 31.1
Cortisol (nmol/L)	392 ± 151	401 ± 200
Current medication		
None, n (%)	11 (23)	12 (24)
Stable NSAIDs, n (%)	9 (19)	14 (27)
Stable prednisolone ≤ 7.5mg, n (%)	24 (50)	22 (43)
Stable DMARDs, n (%)	19 (40)	27 (53)
MTX	16 (33)	17 (33)
LEF	2 (4)	2 (4)
SSZ	0	1 (2)
HCQ	1 (2)	2 (4)
MTX + SLZ	0	3 (6)
MTX, SSZ, + HCQ	0	2 (4)
Previous failure to DMARD and biologic therapy§		
Previous failure with DMARDs, n (%)	40 (83)	45 (88)
One previous DMARD	13 (27)	13 (25)
Two previous DMARDs	10 (21)	9 (18)
Three or more previous DMARDs	17 (35)	23 (45)
Previous failure with biologics, n (%)	21 (44)	23 (45)
One previous biologic	9 (19)	9 (18)
Two previous biologics	6 (13)	3 (6)
Three or more previous biologics	6 (13)	11 (22)

CCP, Cyclic citrullinated peptide; DAS28-CRP, 28-joint Disease Activity Score calculated with C-reactive protein levels; ESR, erythrocyte sedimentation rate; TNF-α, tumour necrosis factor-α; LH, luteinizing hormone; FSH, follicle-stimulating hormone; NSAID, non-steroidal anti-inflammatory drug; DMARD, disease-modifying anti-rheumatic drug; MTX, methotrexate; SSZ, sulfasalazine; HCQ, hydroxychloroquine; LEF, leflunomide.

* Values are given as mean ± standard deviation unless otherwise indicated.

† Data are median (IQR), based on detectable TNF-α values > 0.5 pg/mL; n = 21 (cetrorelix), n = 30 (placebo).

‡ Based on detectable FSH values < 256 IU/L; n = 48 (cetrorelix), n = 50 (placebo).

§ Previous failure includes inefficacy or intolerability.

5b. Predefined secondary end-points included the baseline-adjusted between-group difference in TNF-α, ACR 20/50/70 responses, European League Against Rheumatism (EULAR) responses, DAS28-CRP < 2.6 and ≤ 3.2, and adverse events. Continuous end-points were assessed with regression using day 5 as the response variable, and treatment and baseline measurement as covariates (ANCOVA). No adjustments for multiple analyses were made because of the highly correlated variables. Statistical tests were two-sided ($\alpha = 0.05$) using Stat12/StatXact9, and performed by an offsite statistician who received the locked database from the blinded investigators, and the allocation key from the offsite central office (further statistics are provided in the online Supporting Information).

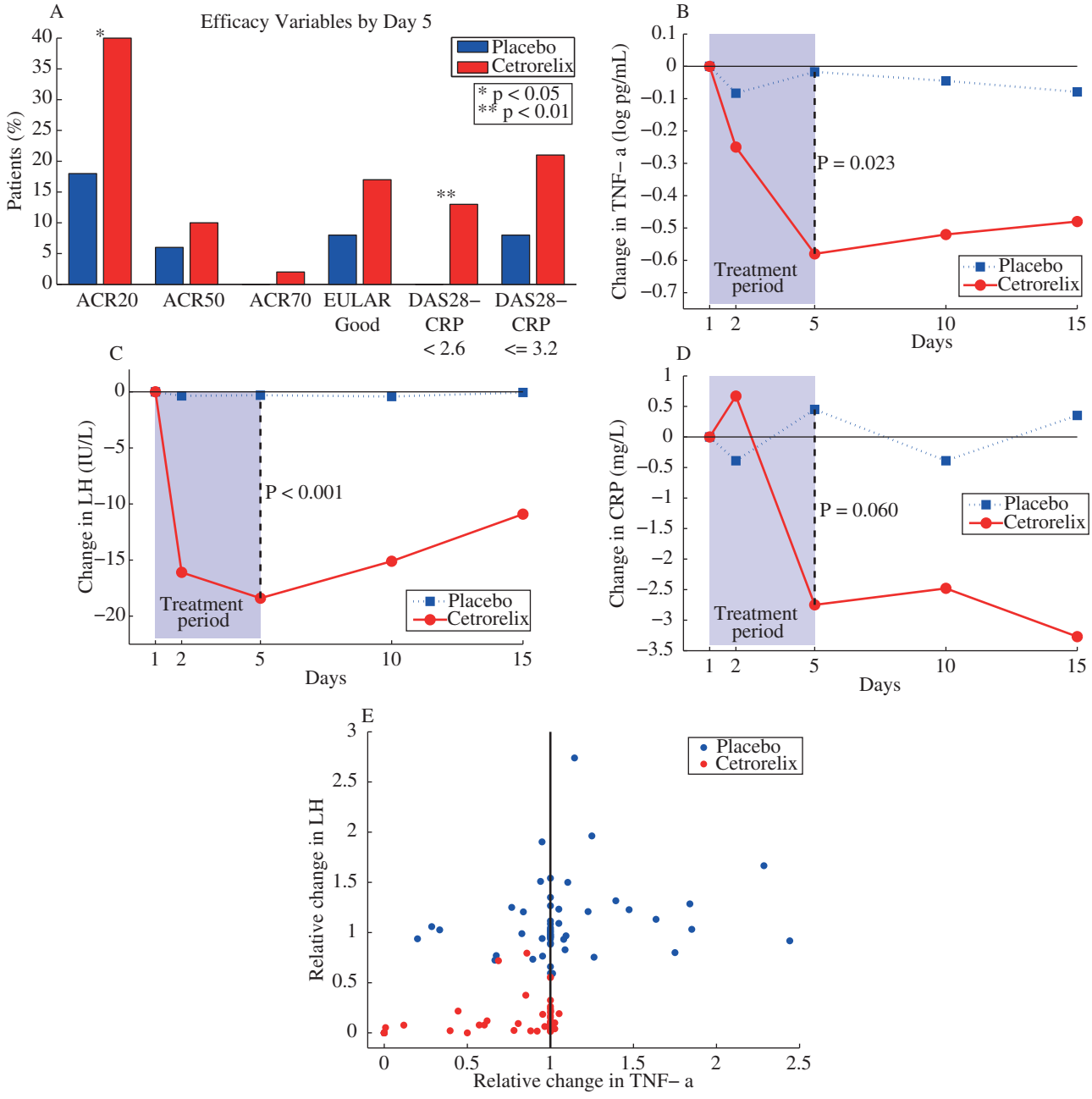

Figure 1. (A) Efficacy variables by day 5. Change from baseline in the cetrorelix and placebo groups for (B) tumour necrosis factor-α (TNF-α), (C) luteinizing hormone (LH) and (D) C-reactive protein (CRP). (E) Scatterplot of relative change of LH and TNF-α from baseline to day 5 in the cetrorelix and placebo groups. On both axes, 1.0 denotes no change. On the left side of the vertical line are patients whose levels of TNF-α decreased, and on the right side are patients whose levels of TNF-α increased.

Table 2. Correlations of relative changes from baseline to day 5 in TNF-α and hormones, and in DAS28-CRP and hormones.

Variables*	n	Spearman's rho	p-value
TNF-α† and hormones			
Relative change in TNF-α related to relative change in LH	51	0.48	0.0004
Relative change in TNF-α related to relative change in FSH‡	50	0.30	0.034
Relative change in TNF-α related to relative change in oestradiol	51	0.30	0.035
Relative change in TNF-α related to relative change in testosterone	51	0.22	0.12
Relative change in TNF-α related to relative change in cortisol	51	–0.12	0.40
DAS28-CRP and hormones			
Relative change in DAS28-CRP related to relative change in LH	99	0.20	0.045
Relative change in DAS28-CRP related to relative change in FSH‡	98	0.13	0.22
Relative change in DAS28-CRP related to relative change in oestradiol	99	0.01	0.94
Relative change in DAS28-CRP related to relative change in testosterone	99	< 0.01	0.98
Relative change in DAS28-CRP related to relative change in cortisol	99	0.10	0.32

TNF-α, Tumour necrosis factor-α; DAS28-CRP, 28-joint Disease Activity Score calculated with C-reactive protein levels; LH, luteinizing hormone; FSH, follicle-stimulating hormone.
* Relative change calculated as the change by day 5 divided by the baseline level.
† Based on detectable TNF-α values > 0.5 pg/mL; n = 21 (cetrorelix), n = 30 (placebo).
‡ Based on detectable FSH values < 256 IU/L; n = 48 (cetrorelix), n = 50 (placebo).

Results

The predefined intent-to-treat population comprised 99 patients who received at least one dose of cetrorelix (n = 48) or placebo (n = 51) (see online Supplementary Figure). Patients' baseline characteristics were similar between groups (Table 1).

DAS28-CRP reduction by day 5 was non-significantly greater in the cetrorelix group (0.82) compared with placebo (0.57) [between-group difference 0.26, 95% confidence interval (CI) –0.04 to 0.57, p = 0.091]. More patients achieved DAS28-CRP < 2.6 (13% vs. 0%, p = 0.009) and ACR20 responses (40% vs. 18%; p = 0.015) by day 5 in the cetrorelix group compared with placebo. More patients reached ACR50/70 responses in the cetrorelix group, although the numbers were too small for valid conclusions (Figure 1A).

Baseline TNF-α levels were comparable with other studies. Fifty-one of the 99 patients had detectable levels of TNF-α > 0.5 pg/mL. TNF-α (log pg/mL) reduction was greater in the cetrorelix group (–0.58) than in the placebo group (–0.02) by day 5 (between-group difference 0.55, 95% CI 0.08–1.01, p = 0.023) (Figure 1B). TNF-α percentage change from baseline was also significantly reduced in the cetrorelix group compared with placebo by day 5 (–28.2% vs. 11.1%, p = 0.0028).

There was a significant correlation between the relative changes in both TNF-α and LH, a surrogate marker for GnRH, from baseline to day 5 [rho = 0.48, p < 0.001, n = 51 (both groups included). Relative changes between TNF-α and LH from baseline to day 5 are illustrated in Figure 1E. There were weaker associations between relative changes in TNF-α and FSH, and TNF-α and oestradiol, but none between TNF-α and testosterone, or TNF-α and cortisol]. These findings were supported by a significant correlation between relative changes in DAS28-CRP and LH (p = 0.045), but not with FSH, oestradiol, testosterone, or cortisol (Table 2).

Non-significant reductions of CRP (day 5, p = 0.060, Figure 1D) followed by erythrocyte sedimentation rate (ESR) (day 15, p = 0.051) were observed in the cetrorelix group compared to placebo.

Cortisol changes were not significantly different between groups by day 5 (p = 0.80). In the cetrorelix group, DAS28-CRP reduction did not differ between prednisolone users (n = 24) and non-users (n = 24) (p = 0.40).

LH, FSH, and inflammatory markers generally increased towards baseline levels after withdrawal of treatment; however, CRP levels remained lowered by day 15. (Figures 1B–1D).

Adverse events arose at similar frequencies in both groups (see online Supplementary Table).

Discussion

It is possible that our study did not have enough power to show a significant reduction in the primary end-point, DAS28-CRP. However, significant improvements in key secondary end-points, representing important disease activity markers, suggest that pathways targeted by GnRH could be beneficial in inflammatory disease. Our data indicate that these pathways may involve TNF-α as a key molecule. As TNF-α inhibition is a common mode of action in RA therapy, antagonizing GnRH may have substantial therapeutic potential.

It is noteworthy that our study detected significant results, despite being a proof-of-concept trial with relatively few patients and a substantial proportion of them having long-standing therapy-resistant RA.

GnRH antagonists are used in other indications with a good safety profile over longer periods, and we did not observe any serious side-effects in this study.

The observed changes in disease activity and TNF-α may be due to direct cellular effects of GnRH, or indirect effects through other hormones. Although the exact mechanisms are unknown, the highly significant association between changes in LH, a surrogate marker for GnRH, and TNF-α suggests there is a close relationship between endocrinological and immunological responsiveness to GnRH.

A plausible mechanism for TNF-α inhibition and anti-inflammatory effects in our study may be through the direct effects of GnRH on T cells, through binding of GnRH to its receptor on T cells. Human peripheral T cells can secrete GnRH, which acts upon these T cells in a cytokine-like way, stimulating T-cell proliferation and maturation (4–6). A second mechanism may be through the effects of GnRH on B cells. GnRH administration led to increased immunoglobulin G levels in diabetes-prone rats (14). Few data exist on the effects of GnRH on other immune cells. Indirect mechanisms include LH reduction, as LH itself stimulates T-cell proliferation (7), or FSH reduction, as FSH stimulates macrophage TNF-α production (8).

Our findings do not indicate that cetrorelix ameliorates disease activity by effects on cortisol or oestradiol. Increased risk of presenting symptoms of RA and flares of disease activity are associated with the postpartum period, menopause, and aromatase inhibitor therapy (2, 3), when LH and FSH increase. By contrast, RA amelioration is associated with pregnancy and fasting (1, 15), when LH and FSH decrease. Our findings support the notion these relationships may be related to changes in upstream hormones (GnRH, LH, or FSH) of the HPG axis rather than downstream gonadal hormones. This is also supported by our previous study, which showed that changes in disease activity and key cytokines, such as TNF-α, were significantly associated with changes in LH and FSH (but not with oestradiol, testosterone, prolactin, or cortisol) in RA (9). Women have more frequent and more substantial HPG axis fluctuations than men, which may contribute to an explanation of why RA is more frequent and severe in women than men.

The rapid offset observed was expected because of the short half-life of cetrorelix. This suggests that the effects of GnRH in RA are short-lived and reversible.

This study was not designed to assess long-term efficacy. The short duration of the study and the relatively small sample size might have led to an underestimation of the effects of cetrorelix. Despite these limitations, the study has generated important data. A larger, longer, and dose-ranging study is now feasible. Similar trends were observed for several parameters, suggesting that cetrorelix has an anti-inflammatory effect in RA. Our study was strengthened by its double-blind, placebo-controlled design with central randomization, with no significant baseline differences between groups, and predefined end-points. Exclusion criteria and missing values were few, providing good generalizability of the results.

These results justify research on the effects of GnRH and GnRH antagonism on immune system regulation, specific blockade of immunologically active GnRH, and gender- or disease-specific immune responsiveness to upstream HPG hormones,

In summary, antagonizing GnRH may represent a novel mode of action for TNF-α inhibition with rapid anti-inflammatory effects in RA, and potentially in other autoimmune diseases.

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References

1. Ostensen M, Aune B, Husby G. Effect of pregnancy and hormonal changes on the activity of rheumatoid arthritis. Scand J Rheumatol 1983;12:69–72.
2. Pikwer M, Bergstrom U, Nilsson JA, Jacobsson L, Turesson C. Early menopause is an independent predictor of rheumatoid arthritis. Ann Rheum Dis 2012;71:378–81.
3. Tan AL, Emery P. Role of oestrogen in the development of joint symptoms? Lancet Oncology 2008;9:817–18.
4. Chen A, Ganor Y, Rahimipour S, Ben-Aroya N, Koch Y, Levite M. The neuropeptides GnRH-II and GnRH-I are produced by human T cells and trigger laminin receptor gene expression, adhesion, chemotaxis and homing to specific organs. Nat Med 2002;8:1421–6.
5. Azad N, La Paglia N, Jurgens KA, Kirsteins L, Emanuele NV, Kelley MR et al. Immunoactivation enhances the concentration of luteinizing hormone-releasing hormone peptide and its gene expression in human peripheral T-lymphocytes. Endocrinology 1993;133:215–23.
6. Morale MC, Batticane N, Bartoloni G, Guarcello V, Farinella Z, Galasso MG, et al. Blockade of central and peripheral luteinising hormone-releasing hormone (LHRH) receptors in neonatal rats with a potent LHRH-antagonist inhibits the morphofunctional development of the thymus and maturation of the cell-mediated and humoral immune responses. Endocrinology 1991;128:1073–85.
7. Sabharwal P, Varma S, Malarkey WB. Human thymocytes secrete luteinising hormone: an autocrine regulator of T-cell proliferation. Biochem Biophys Res Commun 1992;187:1187–92.
8. Iqbal J, Sun L, Kumar TR, Blair HC, Zaidi M. Follicle-stimulating hormone stimulates TNF production from immune cells to enhance osteoblast and osteoclast formation. Proc Natl Acad Sci USA 2006;103:14925–30.
9. Kass AS, Lea TE, Torjesen PA, Gulseth HC, Forre OT. The association of luteinizing hormone and follicle-stimulating hormone with cytokines and markers of disease activity in rheumatoid arthritis: a case-control study. Scand J Rheumatol 2010;39:109–17.
10. Pope J, Joneja M, Hong P. Anti-androgen treatment of prostatic carcinoma may be a risk factor for development of rheumatoid arthritis. J Rheumatol 2002;29:2459–62.
11. Lazúrova I, Angelovicova J, Petríkova J, Calfova A, Oetterova M, Švajdler M. A case of polymyositis and vasculitis induced by ovulation induction therapy with gonadotropin-releasing hormone. Clin Rheumatol 2010;29:1327–30.

12. Jacobson JD, Nisula BC, Steinberg AD. Modulation of the expression of murine lupus by gonadotropin-releasing hormone analogs. Endocrinology 1994;6:2516–23.
13. Ansari MA, Dhar M, Spieker S, Bakht N, Rahman AM, Moore WV, et al. Modulation of diabetes with gonadotropin-releasing hormone antagonists in the nonobese mouse model of autoimmune diabetes. Endocrinology 2004;1:337–42.
14. Jacobsen JD, Ansari MA, Mansfield ME, McArthur CP, Clement LT. Gonadotropin-releasing hormone increases CD4+ T-lymphocyte numbers in an animal model of immunodeficiency. J Allergy Clin Immunol 1999;104:653–58.
15. Kjeldsen-Kragh J, Haugen M, Borchgrevink CF, Laerum E, Eek M, Mowinkel P, et al. Controlled trial of fasting and one year vegetarian diet in rheumatoid arthritis. Lancet 1991;338:899–902.

Supporting Information

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Appendix

CONSORT 2010 Checklist

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