Methods: A rat model for RA was used with repeated intra-articular methylated bovine serum albumin (mBSA) injections in one knee ("arthritic" knee), the contralateral knee serving as internal control. Recombinant human AP (200 µg, s.c.) was administered twice (spaced 4 days) before mBSA injections (*prophylactic setting*) or after arthritis induction (4x, 2x/wk, *therapeutic setting*), or combined with MTX (0.3 mg/kg or 1 mg/kg, i.p.) in 4 rats/group. Plasma pharmacokinetics of AP in arthritic and healthy rats was monitored by colorimetric enzymatic assay. As an endpoint of AP/MTX treatment outcome, macrophage infiltration (marking arthritic conditions) in knee sections, liver and spleen was assessed by immunohistochemistry (ED1 and ED2-macrophage specific antibodies), immunofluorescence (macrophage marker; Folate Receptor-β, FRβ), and positron emission tomography (PET) scans and *ex vivo* tissue distribution with the macrophage tracer [¹⁸F] fluoro-PEG-folate targeting FRβ.

Results: After AP administration, both in healthy and arthritic rats, plasma AP levels increased over 1 hour to reach a maximum of 50%–70% above baseline. Increased plasma AP levels in healthy rats were retained for at least 4 hours, whilst in arthritic rats AP plasma levels steadily returned to baseline levels within this time frame, suggesting consumption of available AP by conjugating to its ITM substrates. Prophylactic and therapeutic schedules of AP treatment, either as single agent or in combination with MTX, were well tolerated. Both prophylactic and therapeutic AP markedly reduced synovial macrophage infiltration in arthritic knees (ED1; 3.5–4 fold, ED2; 3.5–6 fold), comparable with MTX treatment effects. AP/MTX combinations slightly improved on single agent effects. PET monitoring and *ex-vivo* tissue distribution studies corroborated the impact of AP, MTX and AP/MTX on reducing synovial macrophage infiltration. Beyond localised articular effects, AP also displayed systemic anti-inflammatory effects by a 2-fold reduction of ED1, ED2 and FRβ-positive macrophages in liver and spleen of arthritic rats.

Conclusions: AP as single agent and combined with MTX elicits local and systemic anti-arthritis activity in arthritic rats and appears promising as a new therapeutic compound against arthritic conditions.

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AB0116

ADIPOSE DERIVED STEM CELLS SUPPRESSED SYNOVIAL INFLAMMATION IN SKG/JCL MICE IN VIVO AND INFLAMMATORY CYTOKINE IN SYNOVIAL FIBROBLAST IN VITRO

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Background: Adipose derived stem cell (ADSC) is one of the stem cells produced by adipose tissue which can be collected easily and in large quantities. It has been reported the anti-inflammatory effect of ADSC in some disease models.¹ However, the effect of ADSC for synovitis such as rheumatoid arthritis (RA) is unknown.

Objectives: The aim of this study is to investigate the effects of ADSC for joint synovitis and cartilage degeneration in SKG/Jcl mice *in vivo* and the effects of ADSC for synovial fibroblast *in vitro*.

Methods: SKG/Jcl mice which developed auto-immune arthritis by adjuvant stimulation were used as RA animal model.² *In vivo*, the intra-articular injections of ADSC (ADSC group; n=10) or PBS (PBS group, n=10) were performed to the bilateral knee of SKG mice with arthritis. The knee joint was histologically assessed with synovitis score and Mankin score at 2 weeks after ADSC injection. *In vitro*, the anti-inflammatory effect of ADSC for stimulated human synovial fibroblast was analysed by real-time RT-PCR.

Results: *In vivo*, the synovitis score and Mankin score were statistically lower in ADSC group (synovitis score 2.0±0.7 vs 6.0±1.6, p<0.01 and Mankin score 2.2±0.8 vs 4.9±0.8, p<0.01) (figure 1). *In vitro*, the expression of tumour necrosis factor-stimulated gene-6 (TSG-6), the anti-inflammatory cytokine, was significantly higher in ADSC than in synovial cell (p<0.01). The inflammatory cytokine levels in

stimulated synovial cell were significantly decreased by ADSC treatment (p<0.01) (table 1).

Abstract AB0116 – Table 1. Gene expressions in each cell by real-time RT-PCR

	Relative gene expressions referenced GAPDH as 1 (mean ±SD)		
	IL-6	TNF-α	TSG-6
ADSC	0.39±0.03	0.37±0.1	0.77 ±0.06*
Synovial fibroblast	0.21±0.07	0.35±0.1	0.08±0.03
Stimulated synovial fibroblast (non-treatment, 24 hour)	77.8±1.5	38.9±0.1	5.2±0.7
Stimulated synovial fibroblast (non-treatment, 48 hour)	34.9±0.5	39.9±0.1	4.2±0.3
Stimulated synovial fibroblast (ADSC treatment, 48 hour)	5.2 ±0.06**	9.8 ±0.1**	1.9±0.1

ADSC; adipose derived stem cell, IL; interleukin, TNF; tumour necrosis factor, TSG; tumour necrosis factor-stimulated gene.

*p<0.01 (the comparison to synovial cell by Mann-Whitney U test)

**p<0.01 (the comparison to non-treatment group by Mann-Whitney U test)

Abstract AB0116 – Figure 1. The anti-inflammatory effects of intra-articular ADSC injection. The H-E stain of knee joint in SKG/Jcl mice (a) and PBS treated SKG/Jcl mice after induction of synovitis (b). The synovial proliferation was suppressed in ADSC treated SKG/Jcl mice after induction of synovitis (c). The Safranin-O stain in SKG/Jcl mice without synovitis (d), PBS treated SKG/Jcl mice (e) and ADSC treated SKG/Jcl mice (f) after induction of synovitis. The cartilage staining was reduced in SKG/Jcl mice with inflammatory synovitis (yellow arrows).

Conclusions: This is the first report to show the anti-inflammatory effect of ADSC for synovitis in RA animal model. ADSC can be collected with a minimally invasive technique more easily than other mesenchymal stem cells. ADSC might have potential to be one of the RA treatment.

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AB0117

ASSOCIATION OF VITAMIN D DEFICIENCY WITH CRP/ACPA POSITIVITY IN ALGERIAN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Vitamin D (VD) is a steroid hormone belonging to the class of secosteroids with myriad immune functions and seems to be involved in the development and severity of rheumatoid arthritis (RA).^{1–3}

Objectives: The aim of this study was to estimate the prevalence of vitamin D deficiency in patients with rheumatoid arthritis as compared to healthy controls and to analyse the association between levels of vitamin D and CRP (C-reactive protein)/ACPA (anti citrullinated protein antibodies) positivity.

Methods: Serum 25(OH)D levels were measured in 115 RA patients and 104 age- and gender-matched healthy controls using the chemiluminescent immuno-assay method (CLIA).

CRP (mg/dl) was measured by the nephelometric method and ACPA antibodies were evaluated using enzymatic linked immuno-assay (ELISA).

Results: There was no statistically significant difference between levels of 25 (OH)D in RA (25.08±9.22 ng/ml) and healthy controls (15.62±5.34 ng/ml).

The vitamin D deficiency (<10 ng/ml) was found in 10% of RA patients, while 60% were insufficient (<20 ng/ml).

Low 25-OHD levels were negatively associated with CRP (23 ng/ml vs 29 ng/ml; $p=0.023$) and ACPA positivity (23 ng/ml vs 28 ng/ml; $p=0.03$).

Conclusions: The 25(OH)D levels are inversely related to CRP and ACPA values in Algerian RA patients.

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AB0118

ORAL CONTRACEPTIVES AND THE RISK OF DEVELOPING RHEUMATOID ARTHRITIS: RESULTS FROM THE MALAYSIAN EPIDEMIOLOGICAL INVESTIGATION OF RHEUMATOID ARTHRITIS CASE-CONTROL STUDY

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Background: Hormonal and reproductive factors are implicated in the etiology of rheumatoid arthritis (RA). The association between oral contraceptive (OC) use and the risk of RA has been reported, but the findings are inconsistent across different populations.

Objectives: We investigated the association between OC use and risk of RA stratified by anti-citrullinated peptide antibody (ACPA) status in the Malaysian female population.

Methods: Data from the Malaysian Epidemiological Investigation of rheumatoid Arthritis (MyEIRA) population-based case control study involving 902 female early RA and 906 age and residential area-matched female controls were analysed. OC use data was assessed through a questionnaire. Never users of OC were compared with ever users of OC to estimate the risk of developing ACPA-positive and ACPA negative RA. The odds ratio (OR) with 95% confidence interval (CI) was calculated.

Results: In this study, a total of 28.4% (n=254) RA cases and 34.2% (n=310) controls had reported as ever OC users. Compared with never users, ever OC users had a decreased risk of developing RA in the Malaysian population particularly in ACPA-negative RA (OR 0.68, 95% CI 0.51–0.90, $p<0.001$). No significant

association was observed between ever OC users and ACPA-positive RA (OR 0.80, 95% CI 0.64–1.01). Further stratification analysis by duration of OC use (less than or more than 7 years versus never OC use) demonstrated significant decreased risk of developing ACPA-positive RA (OR 0.66, 95% CI 0.51–0.86, $p<0.01$) and ACPA-negative RA (OR 0.57, 95% CI 0.41–0.80, $p<0.001$), respectively.

Conclusions: Oral contraceptives decreased the risk of RA, particularly ACPA-negative RA in the Malaysian population. A long duration of OC use was however decreased the risk in both ACPA-positive and ACPA-negative RA subsets.

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AB0119

RHEUMATOID ARTHRITIS (RA)-ASSOCIATED AUTOANTIBODIES ARE PRESENT IN THE PERIODONTAL EXUDATE OF PATIENTS WITH AND WITHOUT RA

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Background: Seropositivity for anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF) is a hallmark of RA and can be present years before clinical disease onset. Environmental factors, including smoking and chronic inflamed mucosal tissues of the lungs, gastro-intestinal tract or oral cavity (i.e., the periodontium) are suggested to contribute to initiation of these autoantibodies. While it is known that the inflamed periodontium contains citrullinated proteins and peptides, only Harvey et al. (2013) showed that IgG ACPA is indeed present in periodontal exudate (n=9).

Objectives: As the IgA isotype is specific for mucosal immunity, we assessed in patients with and without RA whether IgA RF and IgA ACPA are present gingivocrevicular fluid (GCF), i.e., the periodontal inflammatory exudate.

Methods: RA patients fulfilling the ACR/EULAR 2010 classification criteria were recruited at the Rheumatology department of the Dr. Sardjito General Hospital, Yogyakarta, Indonesia. Patients without RA (non-RA) were recruited from the Oral and Maxillofacial Surgery department of the same hospital. In both groups, patients with diabetes or cardiovascular disease were excluded. Periodontitis was defined as periodontal inflamed surface area (PISA) >130 mm² (Leira et al. 2017). RF and ACPA were determined by ELISA in serum (IgM RF, IgA RF, IgG ACPA, IgA ACPA) and GCF (IgA RF, IgA ACPA). Total IgG and IgA were determined in GCF. IgA ACPA seropositivity and IgA RF- and IgA ACPA positivity in GCF were defined as >mean +2 SD of healthy controls (non-RA patients, never smokers, no periodontitis, n=88).

Abstract AB0119 – Figure 1. Non-RA patients (n=151)