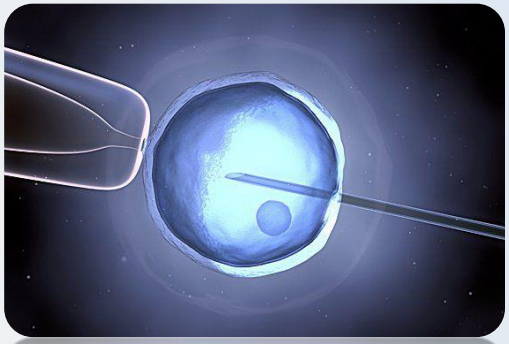
UNIVERSITÀ DEGLI STUDI DI PADOVA

University of Padua
Woman and Child Health Department
Gynecologic and Obstetric Unit



MODIFICAZIONI IMMUNOMODULATORIE ACUTE DURANTE STIMOLAZIONE OVARICA CONTROLLATA: EFFETTI A BREVE TERMINE DELL'ESTRADIOLO SUI BIOMARKER COINVOLTI NELL'AUTOIMMUNITA' E SUL FENOTIPO DELLE CELLULE-B

M. Noventa M.D.

Scuola di Specializzazione in Ginecologia e Ostetricia

BACKGROUND

Steroid hormones play a crucial role in the correct functioning of the reproductive system; however, they also greatly affect many non-reproductive tissues, including the **IMMUNE SYSTEM**

Contents lists available at SciVerse ScienceDirect

Hormones and Behavior

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

Review

Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis

Dionne P. Robinson [a], Sabra L. Klein [a,b,*]

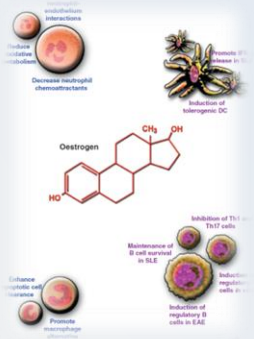
Contents lists available at ScienceDirect

Cellular Immunology

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

Estrogen receptors regulate innate immune cells and signaling pathways

Susan Kovats *



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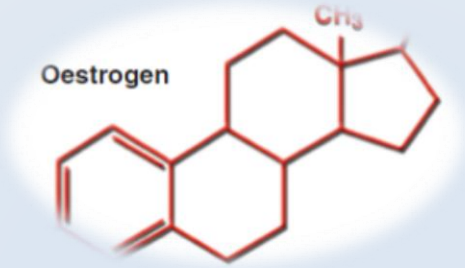
Current Opinion in Pharmacology

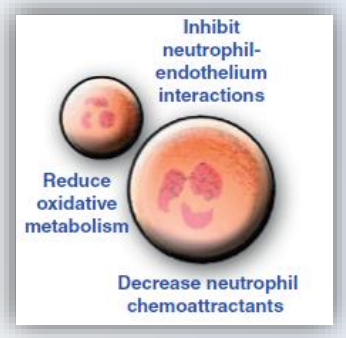
Oestrogen and immunomodulation: new mechanisms that impact on peripheral and central immunity

Suchita Nadkarni and Simon McArthur

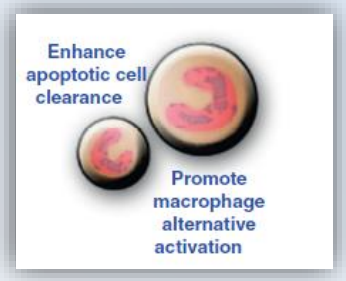
BACKGROUND

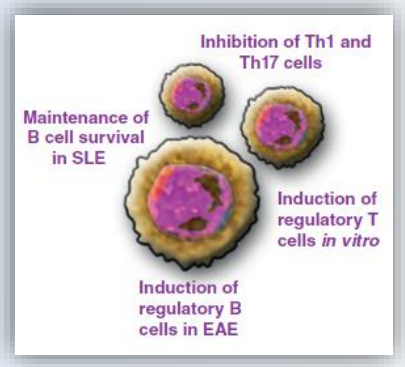


17 beta estradiol



Innate immune response

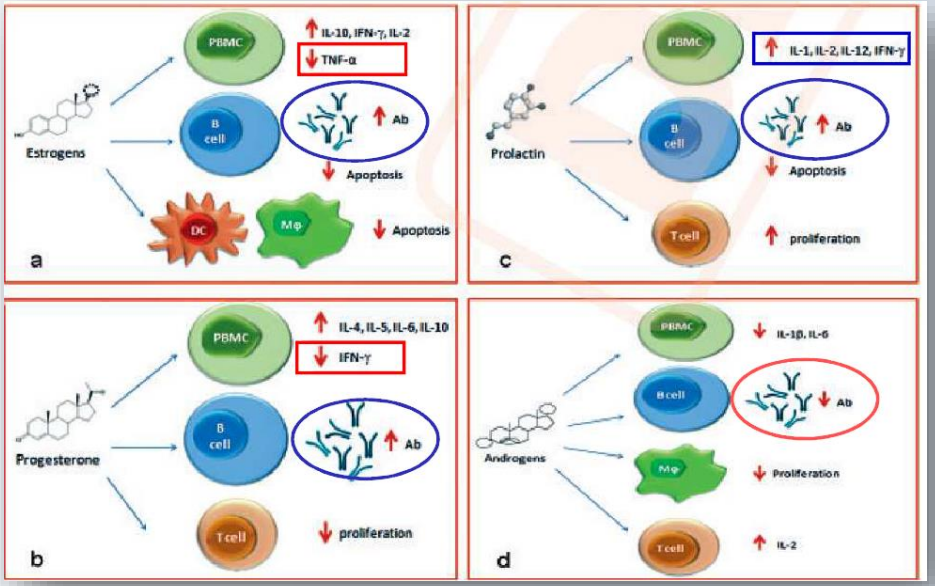




Adaptatative immune response

Suchita Nadkarni and Simon McArthur, Current Opinion in Pharmacology, 2013

BACKGROUND



REVIEWS

Gonadal steroids and humoral immunity

Sanaz Sakiani, Nancy J. Olsen and William J. Kovacs

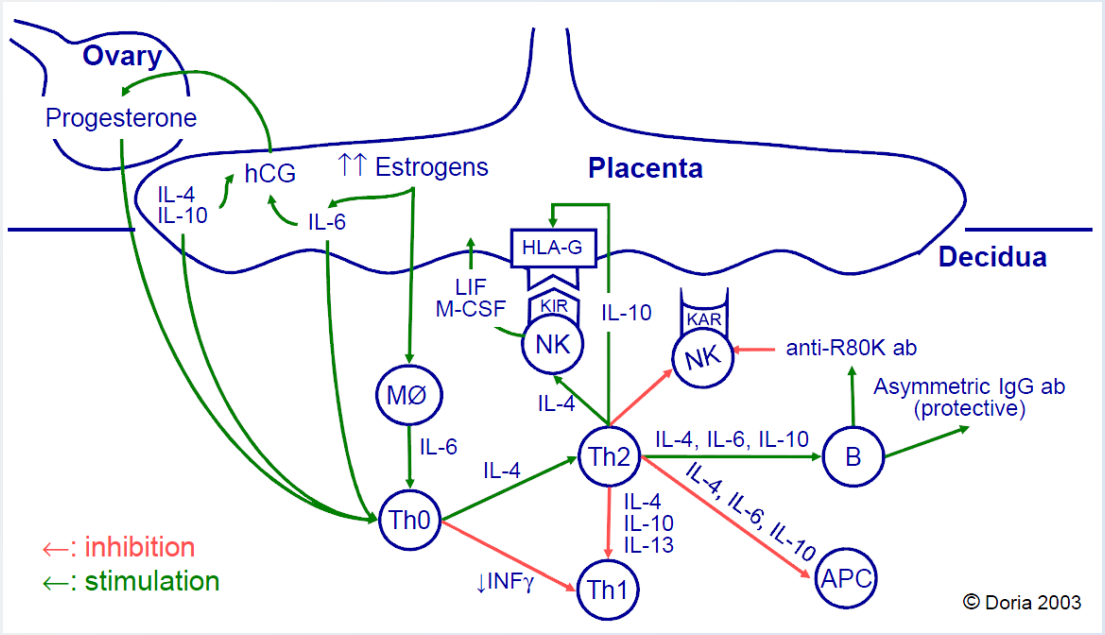
Table 1 | Molecular targets of gonadal steroid actions on humoral immunity

Hormone	Target cell	Target gene	Regulation	Expected physiological effect
Oestrogens	Immature B cell	*BCL2*[55] *PTPN6*[55] *CD22*[55]	Increased	Diminished apoptosis; increased emergence of autoreactive cells
Androgens	Immature B cell	*BCL2*[52]	Decreased	Increased B cell apoptosis
Oestrogens	Marrow stromal cell	SFRP1[50]	Increased	Suppression of early stage lymphopoiesis
Androgens	Marrow stromal cell	*TGFB1*[53]	Increased	Increased B cell apoptosis
Oestrogens	Mature B cell	*AICDA*[64,65] *HOXC4*[65]	Increased	Increased somatic hypermutation and class switch recombination
Progestins	Mature B cell	*AICDA*[67]	Decreased	Diminished somatic hypermutation and class switch recombination

Table 2 | Postulated effects of gonadal steroids on B lymphocytes

Hormone	Regulation	Potential consequences
B lymphopoiesis		
Oestrogens	Suppression	Unknown
Androgens	Suppression	Unknown
Checkpoints for autoreactivity		
Oestrogens	Impairment	Increased propensity for autoimmunity
Androgens	Enhancement	Diminished propensity for autoimmunity
Immunoglobulin class switching		
Oestrogens	Enhancement	Enhancement of vaccine responses; increased propensity for pathogenic autoimmunity
Androgens	Inhibition	Attenuation of vaccine responses; decreased propensity for pathogenic autoimmunity
Progestins	Inhibition	Attenuation of vaccine responses; decreased propensity for pathogenic autoimmunity

BACKGROUND



↑Anti-inflammatory factors	↓Proinflammatory factors
IL-4	IL-12
IL-10	IL-2
TGF-β	IFN-γ
PIBF	TNF-α
Tolerogenic DCs	NK cells
M2 Macrophages	M1 Macrophages
Th2 Cells	Th1 Cells
Regulatory T cells	Th17 Cells
Antibody	

↑Successful Pregnancy
↓Susceptibility to Inflammatory Diseases
↑Susceptibility to Infectious Diseases

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Hormones and Behavior

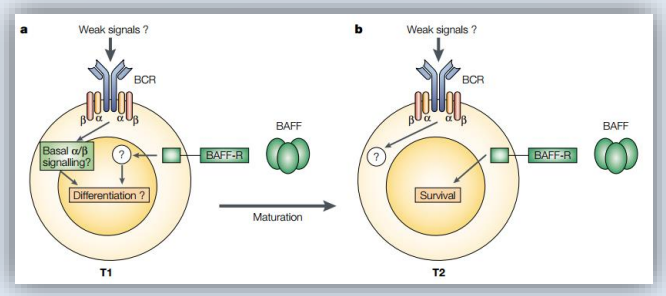
journal homepage: www.elsevier.com/locate/yhbeh

Review

Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis

Dionne P. Robinson [a], Sabra L. Klein [a,b,*]

BACKGROUND



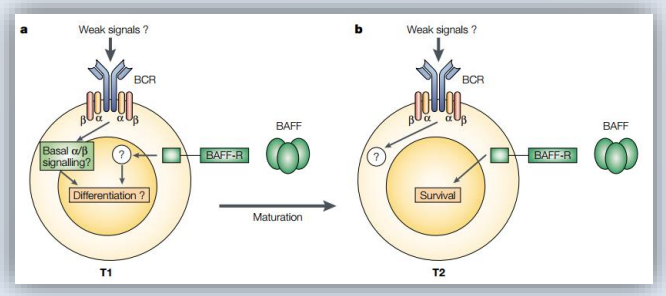
BAFF/Blys

Member of the tumor necrosis factor (TNF) ligand superfamily

BAFF is primarily a myeloid-derived cytokine, either soluble (active) or cell surface expressed, which physiologically **promotes immature and mature B cell survival in the periphery**

Plays critical role in physiologic B-cell development and induces B cells to **secrete immunoglobulins** and it is involved in **T cell co-stimulation**

BACKGROUND



BAFF/Blys

Increasing evidence suggests that **BAFF** is essentially implicated in the pathogenesis of **B cell-mediated autoimmune diseases**

Increased serum levels of BAFF were reported in both non-organ-specific autoimmune diseases (such as systemic lupus erythematosus and Sjögrens's syndrome) and organ-specific conditions

Both E2 and BAFF are implicated in B cell-mediated autoimmunity.

STUDY DESIGN

PROBLEM…

To our knowledge, no existing studies have reported data on the in vivo **short-term immunomodulatory effects** of **E2** on **BAFF** levels and other immunological related changes

AIM

The aim of the study was to evaluate if the short-term increase in **E2** levels (and subsequent **establishment of pregnancy**) may modulate serum levels of **BAFF**, **immunoglobulins (Ig)**, **antinuclear antibodies (ANA)**, and **peripheral B cell phenotype** in women without any prior history of clinical or biochemical features of autoimmune disease.

STUDY DESIGN

Prospective case control study on infertile women scheduled for fresh non-donor in vitro fertilization treatment at the Assisted Reproduction Unit of Gynecology and Obstetrics Clinic

Group-A
(63 patients)

18-43 years, Idiopathic infertility
Normo-responders

Group-B
(39 patients)

Normo-ovulatory
age-matched healthy women

Enclusion criteria

history of personal or familial autoimmune and/or other immunological disorders, diagnosis or suspicion of endometriosis based on clinical symptoms associated with ultrasound features and an increasing Ca125 serum value, abnormalities in karyotype, mutations of the cystic fibrosis gene, acquired or inherited thrombophilia, previous chemotherapy and/or radiotherapy for neoplasia, and cancellation of COS prior to oocyte retrieval due to poor ovarian response

Initial evaluation of all study patients included a **detailed family and personal history specifically aimed to exclude any suspicion of autoimmune disorder**. Serum screening for markers of the most common autoimmune disorders: **thyroid autoantibody screening** (antitireoglobulin antibodies, anti-tiroperoxidase antibodies, anti-TSH receptor antibodies), **anti-nuclear antibodies** (ANA), **anti-cardiolipin antibodies**, **β2-glycoprotein antibodies**, **lupus anti-coagulant** (LAC), **anti-neutrophil cytoplasmic antibodies** (ANCA), **extractable nuclear antigens** (ENA), **anti-DNA antibodies**, and **rheumatoid factor** (RF).

STUDY DESIGN

Group-A
(63 patients)

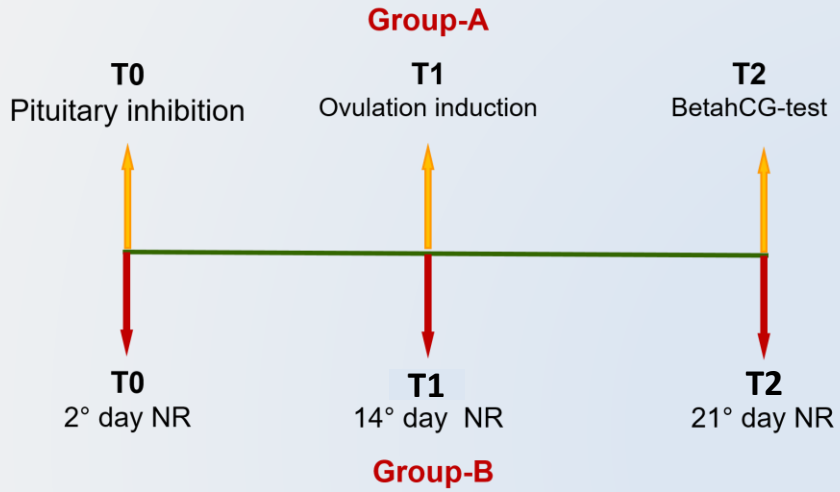
Pre-treatment basal ovarian reserve testing by biochemical assays of **FSH** and **AMH** levels in association with **AFC**

COS cycles were performed by a long protocol using **gonadotropin-releasing hormone agonist** 0.1 mg daily initiated in the mild-luteal phase of the previous cycle, and **recombinant-FSH** at a starting dose of 200 IU daily

When at least three follicles with mean diameter larger than 16 mm (or at least one follicle bigger than 18 mm) were observed at transvaginal sonography, we administrated **rhCG 250 µg**

All oocytes were fertilized by **ICSI technique**.
When obtained, **one or two embryos** were transferred 3 days after pickup.
All patients received high-dose progesterone supplementation (600 mg vaginally and 100 mg intramuscular per day) for **luteal phase support** until β-hCG assay was performed 14 days after embryo transfer (ET)

STUDY DESIGN



BAFF,
BAFF/E2 ratio
Levels of IgM, IgG, IgA
ANA titer
Circulating B cell subpopulations

E2 (nmol/l) : at **T0** and **T1** by electrochemiluminescent immunoassay

βhCG (IU/l): at **T2** by automatized chemiluminescent Immunoassay

BAFF (ng/ml): at **T0, T1, T2** by sandwich ELISA (range of measurability was 0.049–50 ng/ml)

Immunoglobulin (IgG, IgA, IgM) (g/l): at **T0, T1, T2** by automatized immunonephelometry

ANA: at **T0, T1, T2** semiquantitatively detected by indirect immunofluorescence on HEp-2 cells

B-cell subpopulations: at **T0, T1, T2 by f**low cytometry analysis with fluorochrome-conjugated monoclonal antibodies

STUDY DESIGN

ENDPOINTS

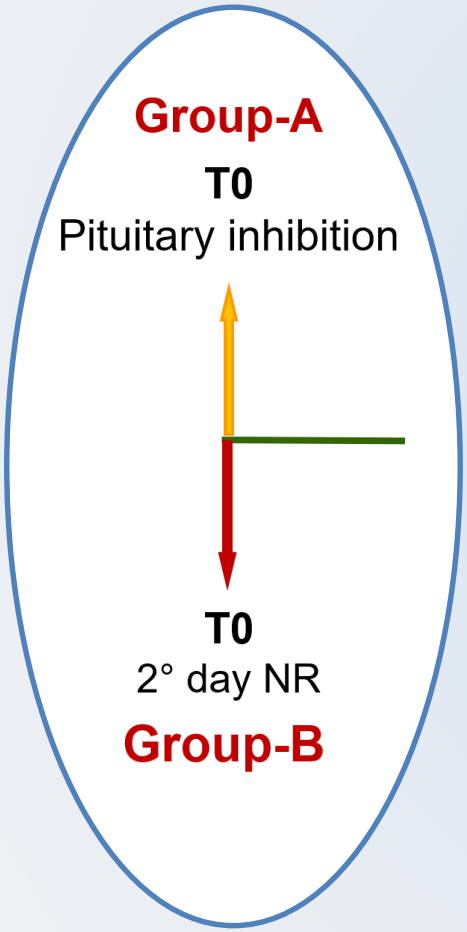
1) Primary endpoint was to compare **Group-A** vs **Group-B** in terms of absolute and normalized for E2 values of **BAFF** at baseline (**T0**) in order to evaluate whether **differences may exist between healthy and infertile women.**

2) Second endpoint was to compare **Group-A** vs **Group-B** in terms of absolute and normalized for E2 values of **BAFF** at **T1**, in order to evaluate if differences may exist between **spontaneous ovulation versus COS**.

3) Third endpoint was to evaluate whether differences exist in **BAFF** levels **between pregnant versus non-pregnant patients in Group-A** and between **non-pregnant women conceiving after spontaneous versus COS cycles** (**Group-B vs Group-A**) at **T2**.

4) Finally, in the **Group-A** women, we evaluated for variations in **immunoglobulin serum levels** (considering IgG, IgA, IgM) and **ANA titer**, at **T0** versus **T1** versus **T2**, and for **peripheral blood B cell subpopulation status** (considering the proportion of transitional, mature naïve, and memory CD19+BR3+ B cells) at **T0 versus T1.**

RESULTS

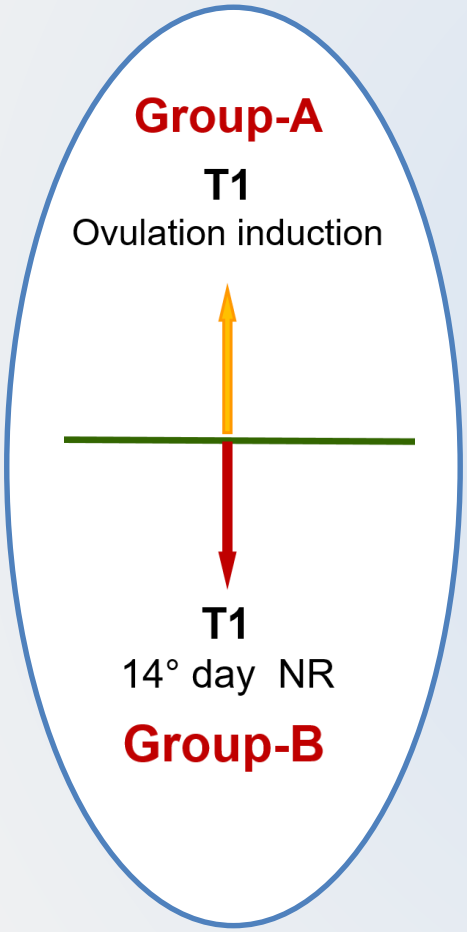


T0, baseline

At T0, the comparison between women in group-A versus group-B showed no significant differences in terms of absolute value of E_2 (mean±SD, 0.06±0.04 vs 0.08±0.06 nmol/l; p=n.s.) as well as absolute value of BAFF (median, 95° percentile, 0.85, 5.85 vs 0.53, 4.34 ng/ml; p=n.s.) (Fig. 1)

Considering the BAFF/E_2 ratio, no significant difference was observed between group-A and group-B: (median, 95° percentile, 19.0, 104.33 vs 16.3, 29.60; p=n.s.).

RESULTS



T1 evaluation

At T1, the comparison between group-A versus group-B women showed a significant difference in terms of absolute value of E_2 (mean±SD 4.63±3.19 vs 0.94±0.11 nmol/l; $p<0.0001$), but no difference in terms of absolute value of BAFF (median, 95° percentile, 0.74, 10.60 vs 0.44, 4.15 ng/ml; p=n.s.) (Fig. 1).

Considering the BAFF/E_2 ratio no difference was observed between group-A and group-B: (median, 95° percentile, 0.17, 1.84 vs 0.48, 4.15; p=n.s.).

RESULTS

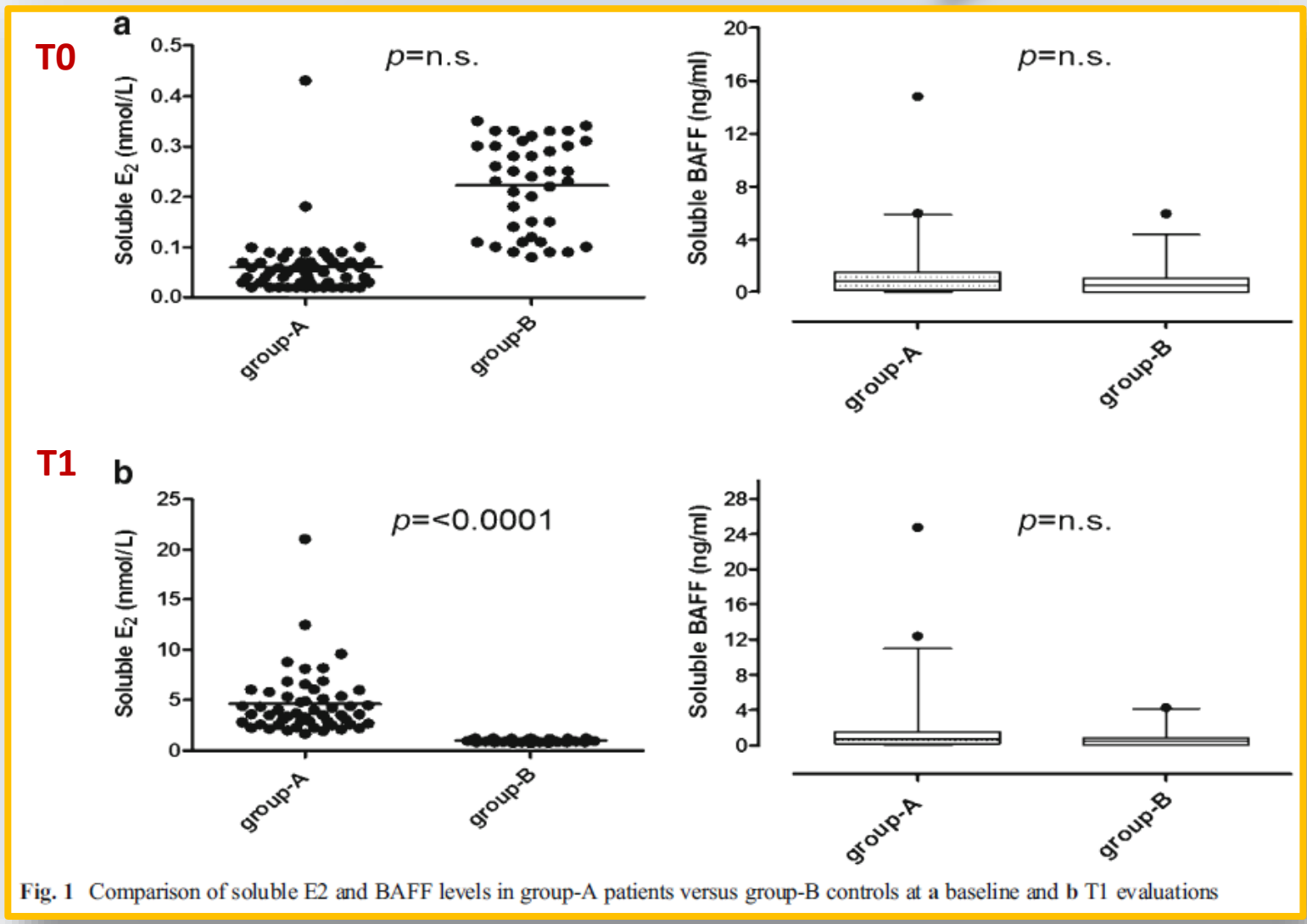
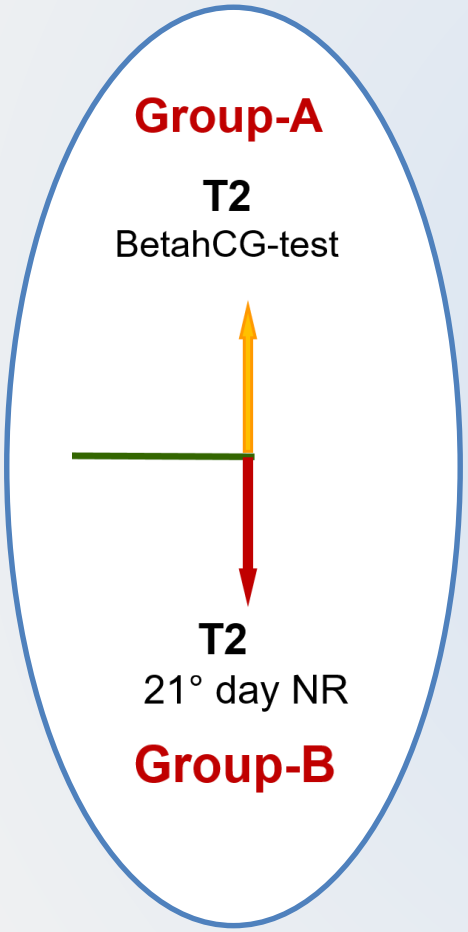


Fig. 1 Comparison of soluble E2 and BAFF levels in group-A patients versus group-B controls at a baseline and b T1 evaluations

RESULTS



T2 evaluation

Of the 63 women comprising group-A, 16 (25.4 %) had hematic βhCG levels ≥100 (mIU/ml), and 15/16 (93 %) were confirmed pregnant.

The comparison between the non-pregnant women of group-A versus those of group-B showed no differences in terms of absolute values of BAFF levels (median, 95° percentile, 0.68, 4.52 vs 0.81, 4.34 ng/ml; p=n.s.). The comparison between the non-pregnant versus the pregnant women of group-A showed no differences in terms of absolute values of BAFF levels (median, 95° percentile, 0.68, 4.52 vs 1.24, 3.20 ng/ml; p=n.s.)

Group-A
(63 patients)

Table 1 Circulating IgG, IgA, and IgM levels and ANA positivity and titer in group-A patients at T0, T1, and T2 evaluations

	Observation periods			
	T0	T1	T2	p
IgG (g/l) mean±SD	11.9±2.56	11.4±2.15	11.9±2.74	n.s.
IgA (g/l) mean±SD	2.0±0.90	1.9±0.82	1.9±0.74	n.s.
IgM (g/l) mean±SD	1.5±0.64	1.4±0.53	1.6±0.77	n.s.
ANA positivity number (%)	42 (67 %)	38 (61 %)	41 (66 %)	n.s.
ANA titer median (range)	80 (0–320)	80 (0–320)	80 (0–320)	n.s.

RESULTS

Group-A
(63 patients)

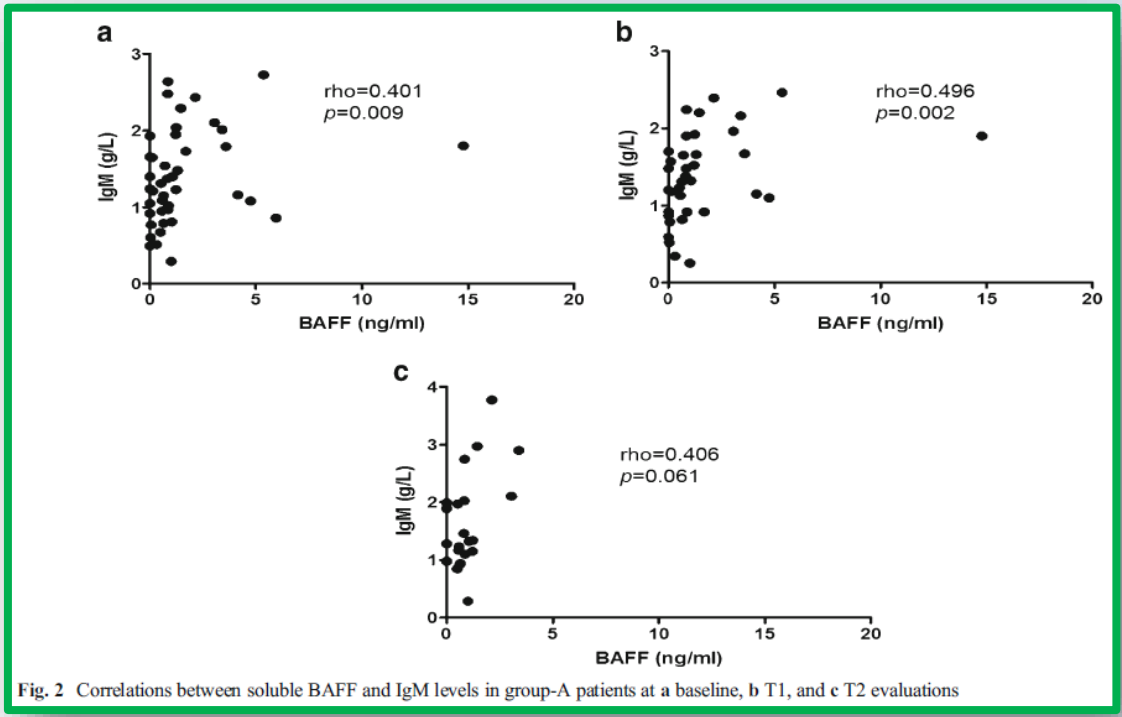


Fig. 2 Correlations between soluble BAFF and IgM levels in group-A patients at a baseline, b T1, and c T2 evaluations

A correlation was observed between **BAFF** levels at T0 and **IgM at T0** (rho= 0.401; p=0.009), **T1** (rho=0.496; p=0.002) and at **T2** (rho= 0.406; p=0.061)

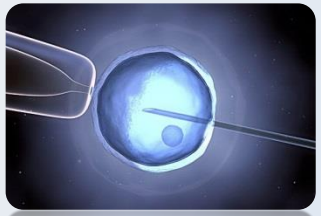
Group-A
(63 patients)

Table 2 Circulating B cell subpopulations in group-A patients at basal (T0) and periovulatory (T1) evaluations	Observation periods		
	T0	T1	*p*
Lymphocytes (106/ml, mean±SEM)	2.15±0.1	2.12±0.1	n.s.
CD19+ B cells (%, mean±SEM)	10.36±0.8	10.31±0.8	n.s.
B lymphocytes (106/ml, mean±SEM)	0.23±0.3	0.22±0.3	n.s.
CD19+BR3+ B cells (%, mean±SEM)	97.08±0.5	98.07±0.4	n.s.
MFI BR3+ B cells (mean±SEM)	3.76±0.1	3.73±0.1	n.s.
Marginal zone memory B cells CD19+CD27+IgD+ (%, mean±SEM)	14.35±3.0	26.39±8.1	0.083
Switched memory B cells CD19+CD27+IgD- (%, mean±SEM)	15.15±1.3	13.96±1.3	n.s.
Naïve B cells CD19+CD27-IgD+ (%, mean±SEM)	65.79±3.9	65.53±5.7	n.s.
Transitional B cells (%, mean±SEM)			
−Type 1 (CD19+CD27-IgDloCD21lo)	2.16±1.0	3.04±1.0	n.s.
−Type 2 (CD19+CD27-IgDhiCD21hi)	91.04±2.2	77.70±7.9	0.074

SEM standard error of the mean, *MFI* mean fluorescence intensity

note, a tendency was observed toward an expansion of the mature marginal zone $CD19^{+}CD27^{+}IgD^{+}$ of memory B cells at T1 in comparison with T0 (p=0.083), whereas the proportion of immature "transitional" type 2 B cells $CD19^{+}CD27^{-}IgD^{hi}CD21^{hi}$ was lower at T1 than at T0 (p=0.074). No significant difference was observed in the proportion of transitional type 1 B cells $CD19^{+}CD27^{-}CD21^{lo}IgD^{lo}$ at T1 versus T0, although a tendency toward an expansion at T1 was observed (data are summarized in Table 2). Notably,

CONCLUSIONS



Human Reproduction Update, pp. 1–16, 2016
doi:10.1093/humupd/dmw019

human reproduction update

The impact of thyroid autoimmunity on IVF/ICSI outcome: a systematic review and meta-analysis

Andrea Busnelli[1,2,*], Alessio Paffoni[1], Luigi Fedele[1,2], and Edgardo Somigliana[1]

Annals of NEUROLOGY

An Official Journal of the American Neurological Association and the Child Neurology Society

EDITORIAL

Assisted Reproduction Technology in Multiple Sclerosis: Giving Birth to a New Avenue of Research in Hormones and Autoimmunity

Obstet Gynecol Surv. 2015 Mar;70(3):196-210. doi: 10.1097/OGX.0000000000000160.

Ovarian function and reproductive outcomes of female patients with systemic lupus erythematosus and the strategies to preserve their fertility.

Oktem O[1,2], Guzel Y[3], Aksoy S[4], Aydin E[5], Urman B[6,7].

Lupus. 2004;13(9):669-72.

Autoimmunity, infertility and assisted reproductive technologies.

Lockshin MD[1].

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

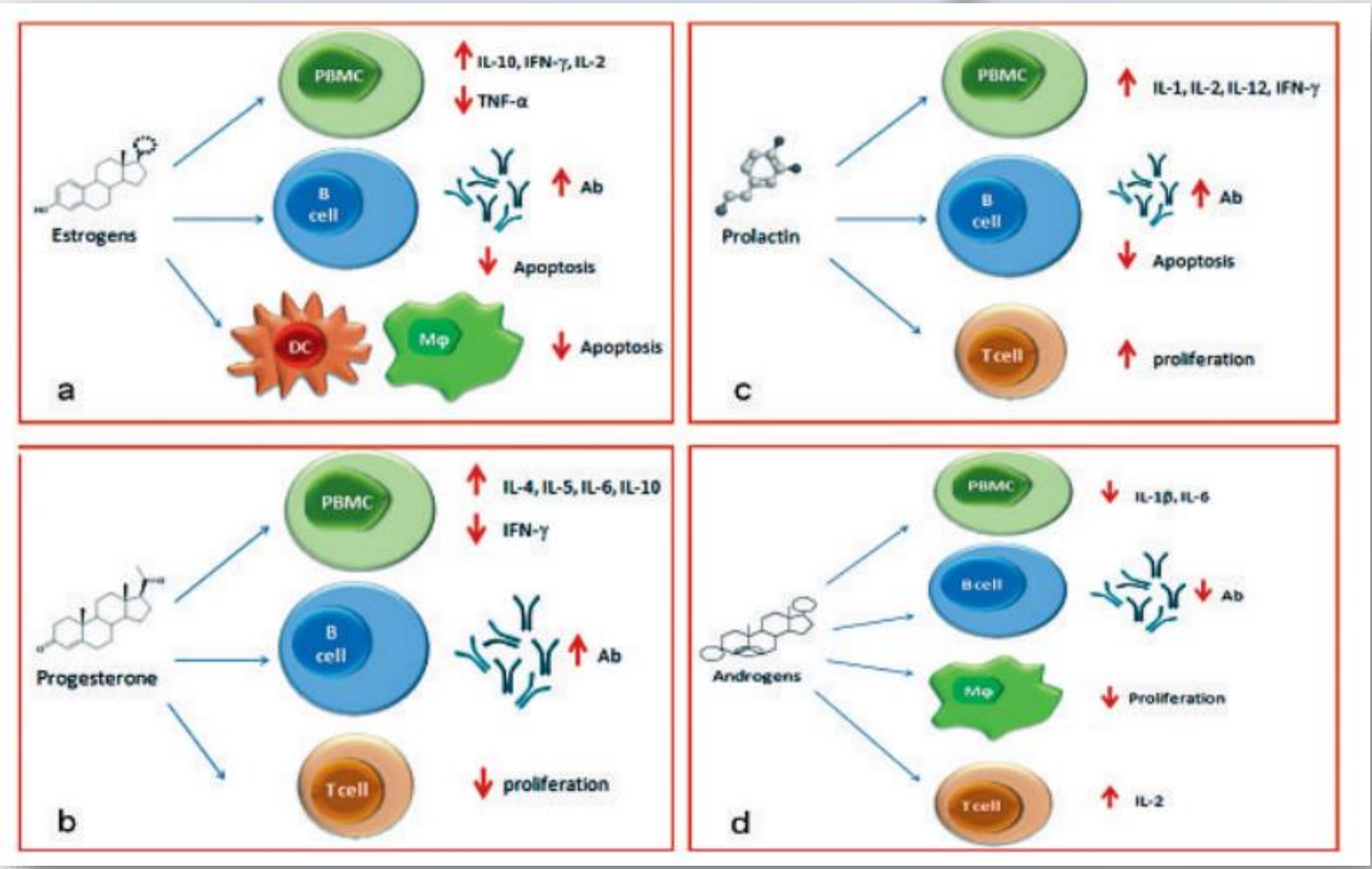
Original Article

Effect of thyroid autoimmunity *per se* on assisted reproduction treatment outcomes: A meta-analysis

Hui He [a, b], Shuang Jing [a, b], Fei Gong [a, b, c], Yue Qiu Tan [a, b, c], Guang Xiu Lu [a, b, c], Ge Lin [a, b, c, *]

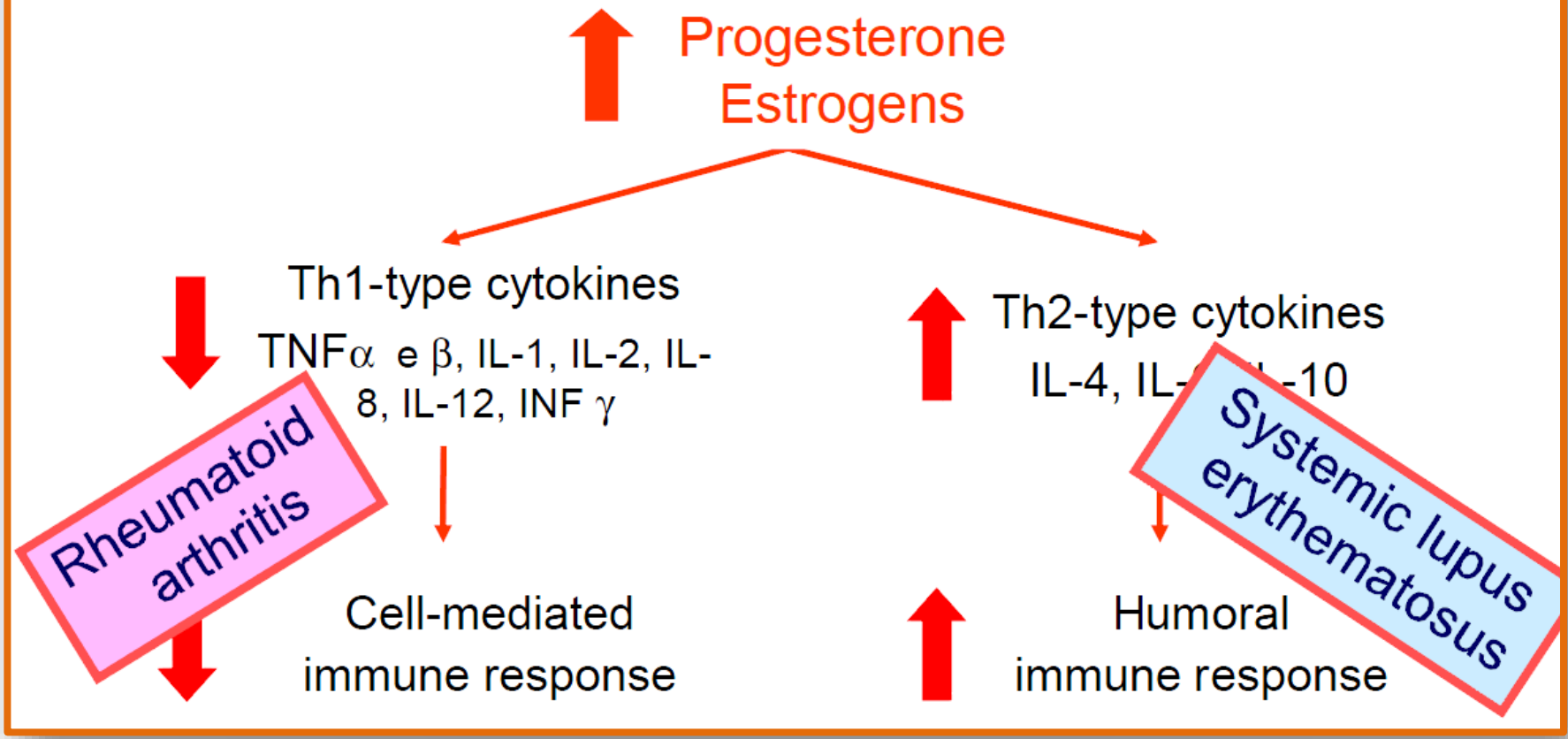
Unresolved issues pertaining to the application of ART in patients affected with autoimmune disorders have arisen specifically in regards to the **safety profile of rapidly increasing levels of E2 observed in a COS cycle**

CONCLUSIONS



CONCLUSIONS

Shift in Th1/Th2 balance during pregnancy at the maternal-foetal interface as well as in the maternal circulation



CONCLUSIONS

Our data suggested that COS in infertile women in the absence of immunological disorders does not exert significant immunomodulatory short-term effects on circulating BAFF and B cell biomarkers and phenotype in comparison with healthy normo-ovulatory women.

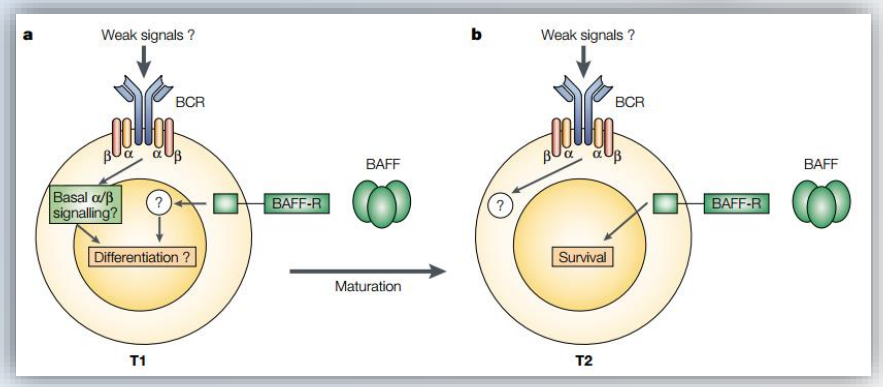
our findings suggest that a short-term in vivo E_2 increase might lead to a physiological expansion of marginal zone mature B cells, as observed in group-A patients at T1, which however does not influence biomarkers of B cell dysfunction, including BAFF, immunoglobulins, or ANA titer.

Conversely, our results confirmed that BAFF is constitutively involved in the activation of antigen-stimulated B cells, as demonstrated by the correlation between BAFF levels and IgM. Indeed, IgM is the first antibody produced by B cells after antigen stimulation [38]. Yet, such BAFF-induced immunomodulation seems to be independent from the short-term surge in E_2 levels.

REVIEWS

BAFF: A FUNDAMENTAL SURVIVAL FACTOR FOR B CELLS

Fabienne Mackay* and Jeffrey L. Browning‡



CONCLUSIONS

To our knowledge, our study was the first performed with the aim of clarifying the immediate effect of ART on one of the most important pathways involved in the development of immunological disorders

Since our data showed that in patients without immunological disorders, E2 has no short term effects on BAFF, the analysis and comparison of data in patients affected by immunological disorders undergoing ART may contribute to better clarify whether or not ART may represent a safe option in this subgroup of patients.

Despite several mechanistic and clinical studies supporting a stimulatory role of E2 on autoimmunity, the acute increase of E2 during COS for infertility treatment does not seem to have a major impact on immune system

University of Padua, Italy
Department of Woman and Child Health
Unit of Reproductive Medicne

NEW FINDINGS ON ACUTE IMMUNOMODULATORY CHANGES DURING CONTROLLED OVARIAN STIMULATION