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ANTI-LAMININ-1 ANTIBODIES IN SERA AND FOLLICULAR FLUID OF WOMEN WITH ENDOMETRIOSIS UNDERGOING *IN VITRO* FERTILIZATION

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There is increasing evidence that autoimmune phenomena, including auto-antibody production, may affect fertility in women with endometriosis. The aims of this study are to evaluate anti-laminin-1 antibody (aLN-1) presence in sera and in follicular fluids (FF) of women with endometriosis undergoing IVF and its impact on oocyte maturation and IVF outcome. aLN-1 were measured by a home-made enzyme linked immunosorbent assay in sera and FF obtained from 35 infertile women with endometriosis and in sera from 50 fertile controls and 27 infertile women without endometriosis (IWWE). aLN-1 serum levels were significantly higher in women with endometriosis in comparison with both fertile controls and IWWE ($P<0.001$ and $P<0.05$, respectively) and a positive correlation was found between serum- and FF- aLN-1 ($r = 0.47$, $P = 0.004$). According to the cut-off (mean+3 SD of fertile controls), 31% of women with endometriosis were aLN-1 positive. Metaphase II oocyte counts showed inverse correlation with FF-aLN-1 levels ($r = -0.549$, $P = 0.0006$). Ongoing pregnancy (i.e pregnancy progressing beyond the 12th week of gestation) occurred in 4/11 aLN-1 positive patients and in 7/24 aLN-1 negative with no significant difference ($P= 0.7$). In conclusion, our results highlight that aLN-1 are increased in women with endometriosis and their presence in FF may affect oocyte maturation leading to a reduced fertility. However, aLN-1 seem to have no effect on IVF outcome.

Laminin-1 (LN-1), the first identified and sequenced laminin, is a 900 kD multifunctional glycoprotein of basement membranes thought to enhance trophoblast adhesion in the peri-implantation period (1). Laminins and their specific integrins are important components of the extracelluar matrix (ECM), due to their role in cell adhesion processes and, in particular, LN-1 is involved in embryogenesis, implantation, and placentation (2-3).

The effects of anti-laminin antibodies (aLN-1) on conception and pregnancy outcome has been investigated in various animal models. In monkeys, aLN-1 antibodies were associated with reproductive failure (4). In mice, passive immunization with rabbit aLN-1 administered intraamniotically in pregnant animals, resulted in a higher abortion incidence, and/or fetal death (5), while active immunization with LN-1 induced high fetal resorption rate, and low fetal and placental weights (6).

In humans, aLN-1 were initially reported as auto-antibodies (auto-Abs) associated with recurrent miscarriages. In fact, Inagaki et al. (7) documented

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higher IgG aLN-1 levels in patients with history of 2-11 first-trimester miscarriages when compared with both healthy pregnant and non-pregnant women.

Endometriosis is a disease associated with reproductive failure and cellular and humoral immune abnormalities, including autoantibody production, which could play a detrimental role impairing fertility (8). Among the various auto-Abs described (9-10), recently aLN-1 have gained more attention due to their significantly higher prevalence in patients with endometriosis when compared with infertile patients without endometriosis (11). It has been suggested that aLN-1 affect early reproductive processes, leading to autoimmune-mediated infertility (12-13).

In the current literature, data concerning the presence and pathogenic relevance of aLN-1 in follicular fluid (FF) are lacking and the relationship between aLN-1 and pregnancy outcome in patients undergoing *in vitro* fertilization (IVF) has not yet been investigated. In addition, although ECM components, including LN-1, promote follicle maturation to produce meiotically competent oocytes (14), data concerning the possible effects of aLN-1 on oocyte maturation are lacking.

Therefore, the primary aims of this study are to evaluate: i) aLN-1 antibody levels in sera and in FF of patients with endometriosis undergoing IVF; ii) the relationship between serum- and/or FF- aLN-1 and oocyte maturation, iii) the impact of aLN-1 presence on ongoing pregnancy.

In a previous study dealing with the presence and clinical relevance of antiphospholipid antibody (APA) in infertile women undergoing IVF, we demonstrated significantly increased APA levels after IVF treatment and we hypothesized a possible role of hyperestrogenism in rising auto-Ab production (15). Since an interesting issue could be whether other auto-Abs are involved in this phenomenon, a further aim of this study is to analyze the effect of IVF treatment on aLN-1 levels.

MATERIALS AND METHODS

Patients

Thirty-five consecutive women affected by endometriosis (mean age 34.8±3.7 years, range 26-43) undergoing ovarian stimulation and IVF with or without intracytoplasmic sperm injection (ICSI) at the Centre of Reproductive Medicine of Bari, University Hospital were enrolled in the study which was approved by the Institutional Review Board of Bari University Hospital, and written informed consent was obtained from each patient. Endometriosis was diagnosed by laparoscopy and histological assessment of lesions. Eligibility criteria were: (1) first IVF attempt; (2) normal menstrual cycle (range of 26-32 days); (3) baseline FSH levels < 12 IU/ml; (4) body mass index (BMI) between 18-30 Kg/m^2; (5) no oral contraceptive pills taken in the last year. Exclusion criteria were: (1) concomitant autoimmune disease; (2) treatment with corticosteroids, immune-suppressive drugs or immune-modulators.

The patients were down-regulated with GnRH agonist (a-GnRH-Decapeptyl 0.1 mg, IPSEN, Italy), starting in the mid-luteal phase of the previous cycle. Ovarian stimulation was carried out with a fixed daily dose of 225 UI of recombinant FSH (recFSH-Gonal F, Serono, Switzerland), starting from day 2 to 3 of the cycle for the first 5 days. The dose was adapted depending on the ovarian response to the treatment. A dose of 10,000 IU hCG (Profasi, Serono, Switzerland) was given intramuscularly for ovulation induction when two or more follicles ≥ 18 mm in diameter were present and E_2 concentrations dropped to 150–200 pg/ml for each leading follicle.

Oocyte retrieval was carried out 35-36 h later by ultrasound-guided transvaginal puncture. Oocyte nuclear maturity was assessed by evaluating the polar body extrusion immediately before ICSI or on the day after oocyte retrieval in the IVF procedure.

Fourteen days after embryo-transfer, quantitative definition of serum βhCG was carried out. Clinical pregnancy was defined as fetal cardiac activity on transvaginal sonography. Pregnancy progressing beyond the 12th week of gestation was considered to be ongoing.

Sera and follicolar fluids

Patient sera were harvested on three different days: (i) between day 19 and 21 of the menstrual cycle, before starting triptorelin treatment (time 0; T0); (ii) on the day of oocyte retrieval (time 1, T1); (iii) 14 days after ET (time 2, T2), on the day of βhCG testing.

Oocyte retrieval was carried out by an Ovum Aspiration Needle Double Lumen (Cook Medical, Australia PTY LTD) and only the first aspirated FF was utilized for aLN-1 detection. Samples contaminated by blood cells were excluded. After centrifugation at 2000 x g, FFs were stored at –80°C until assay.

Sera were also obtained in the luteal phase of the menstrual cycle by two different control groups, i.e. 50 fertile women (fertile controls, FCTRs) and 27 infertile women without endometriosis (IWWE, 9 male factor, 13

tubal factor, and 5 polycystic ovary syndrome), matched for age, basal FSH, and BMI. They were included in the study according to the following criteria: (1) no oral contraceptive pill taken in the last year; (2) absence of autoimmune disease and/or treatments affecting immune response; (3) last pregnancy > 2 years before study entry in fertile women; (4) no previous IVF in IWWE.

Reagents

Laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane, affinity purified anti-laminin antibodies produced in rabbit, bovine serum albumin (BSA), *o*-phenylenediamine (OPD), Tween-20, and 30% H_2O_2, were purchased from Sigma-Aldrich, Milan, Italy. Peroxidase (HRP)-conjugated affinity purified goat antibodies to human IgG and to rabbit IgG were obtained from Jackson Immunoresearch , Avondale, PA, USA.

Enzyme linked immunosorbent assay (ELISA) for aLN-1 determination.

In the ELISA described in this study, phosphate buffered saline (PBS) containing 0.05% (v/v) Tween-20, PBS/3% (w/v) BSA, and PBS/1% BSA/0.05/Tween 20 were used as washing, blocking, and diluent buffer, respectively. Each step, except the coating of the plates, was performed at 25°C.

Half of the wells (alternate rows) of 96-well ELISA plates (Falcon ProBind, Becton Dickinson, Oxnard, CA, USA) were coated with 500ng/100 µL/well of laminin in 0.05 M carbonate/bicarbonate buffer, pH 9.6 (coating buffer), whilst the other half with 100 µL of coating buffer alone and left overnight at 4°C. The wells were washed 4 times (250 µL/well of washing buffer) and non-specific site on the plates were blocked by incubation for 2 h with 150 µL of blocking buffer. After washes, 100 µL/well of serum or FF specimens (diluted 1:100 and 1:20, respectively) were added in duplicate in antigen coated wells as well as in non-coated wells, the latter to assess non-specific binding. Sera from the same patient at T0, T1, and T2 were assessed on the same day and plate to minimize inter-assay variation. In each plate, rabbit anti-LN-1 antibody was incubated in 2 wells at 100 ng/mL concentration to confirm antigen binding and as positive control. The assay blank value was obtained by using 100 µL/well of diluent buffer in place of serum. After a 30 minute incubation, plates were washed 4 times and 100 µL/well of (HRP)-conjugated anti human IgG or anti-rabbit IgG, diluted 1:8,000 were applied for 30 mins. After washings, 100 µL/well of freshly prepared solution of OPD (0.4 mg/mL) and Hydrogen peroxide 30% (0.4 µL/mL) in citrate buffer (pH 5) were dispensed. The color reaction was stopped by addition of 50 µL/well of 2 M H_2SO_4 after a 10 min incubation in the dark. Optical densities (OD) were measured at 492 nm using an ELISA reader (Bio-Rad, Hercules, CA, USA).

The mean OD of the sample duplicates in the absence of antigen was subtracted from the mean OD achieved in the presence of antigen to obtain the net OD reading. aLN-1 values were expressed as percent of the positive control. Intra- and inter-assay coefficients of variation were always < 10%.

Statistical analysis

Statistical analysis was performed with version 4 of GraphPad prism software (GraphPad, San Diego, CA, USA). The results are reported as median and 25th-75th percentiles, numbers are presented as counts (percentage) for dichotomous data. Analyses were performed using the Mann-Whitney *U* test for unpaired two group analyses, Kruskal-Wallis ANOVA test for unpaired three group analyses (Dunn's *post hoc* test), Friedman ANOVA for paired three group analyses (Dunn's *post hoc* test).

Spearman rank correlation coefficient analysis was used to examine the correlation between serum and FF aL-1 and between aL-1 levels and oocyte count. Frequency data were compared with Fisher's exact test. P-values <0.05 were taken as statistically significant.

RESULTS

aLN-1 levels detected at T0 in sera of patients with endometriosis (median 22.3% of the positive control; 25-75th percentiles 14.9-35.5) were significantly higher in comparison with those found in both FCTRs (12; 8.9-15.7; P<0.001) and in IWWE (15; 9.4-22.6; P<0.05). The difference between FCTRs and IWWE was not significant (Fig. 1).

Since aLN-1 levels were normally distributed in FCTRs, cut-off was set at mean+3 SD of values obtained in FCTRs, i.e. 30.7. According to the cut-off, 11/35 (31.4%) patients with endometriosis, 1/50 (2%) of FCTRs and 2/27 (7.4%) of IWWE were aLN-1 positive. aLN-1 prevalence found in patients with endometriosis was significantly higher in comparison with that found in both FCTRs (P= 0.0002) and IWWE (P = 0.028) whereas the differences between FCTRs and IWWE was not significant.

aLN-1 levels detected at T2 (median 30.1; 25-75th percentiles 19.6-55) were significantly higher when compared with those found at T0 (22.3, 14.9-35.5; P<0.01) or T1 (21, 14.1-32.4; P<0.01) (Fig. 2). In particular, in 27/35 patients aLN-1 levels detected at

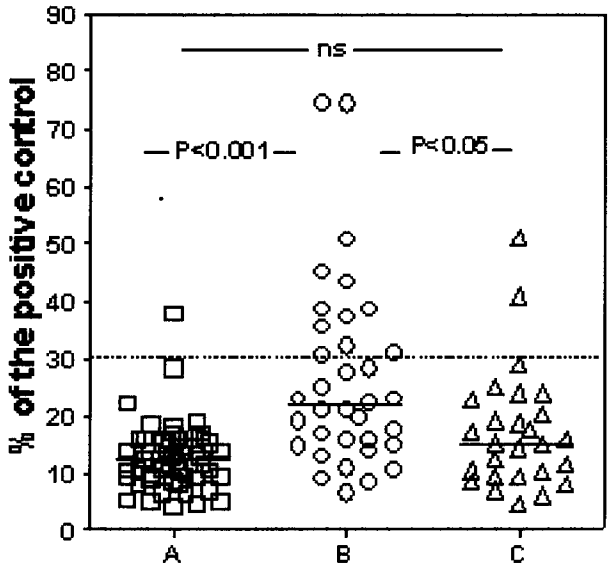

Fig. 1. *IgG- aLN-1 levels detected by ELISA in sera of 50 fertile women (A), 35 women with endometriosis undergoing the first cycle of IVF (B), and 27 infertile women without endometriosis (C). Horizontal lines represent median values, while dotted line represents the cut-off (mean+3 SD of values detected in fertile controls).*

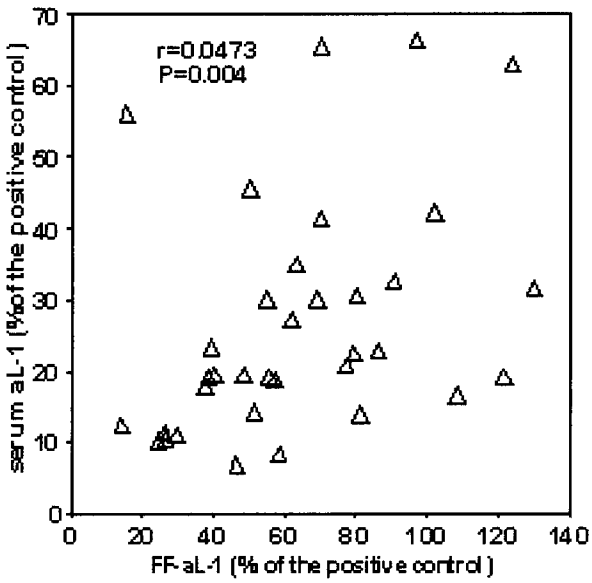

Fig. 3. *Positive correlation between aLN-1 levels detected in serum and FF of 35 women with endometriosis.*

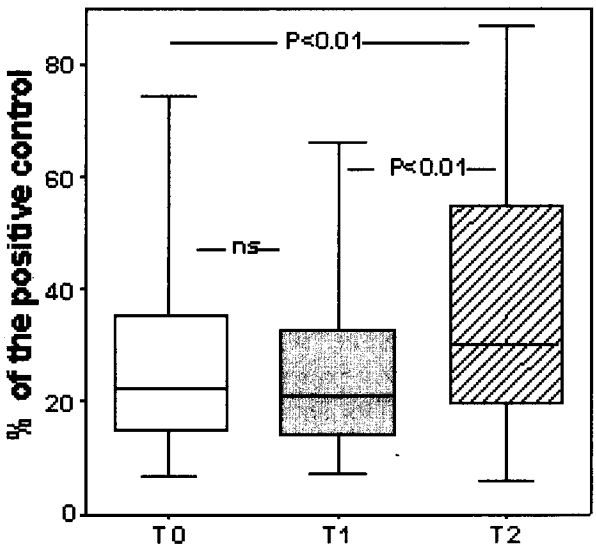

Fig. 2. *Differences in aLN-1 levels between T0, T1 and T2 in women with endometriosis. The data are depicted as box-plot diagrams, with the box encompassing the range of values from the 25th percentile (lower bar) to the 75th percentile (upper bar). The horizontal line within the box represents the median while the vertical lines represent maximum and minimum values.*

T2 were, to a varying extent, higher than those found at T0.

No significant correlation was found between E_2 levels evaluated at the hCG day administration and serum aLN-1 detected at T2 ($r = 0.29$, $P = 0.09$, data not shown).

In each woman with endometriosis, aLN-1 were also measured in FFs obtained from at least two different follicles and data reported represent the mean value for a single patient. The coefficient of variation among FF-aLN-1 in the same patient was always <15%.

FF-aLN-1 showed significant positive correlation with serum aLN-1 detected at T1, i.e the day when FFs were obtained ($r = 0.47$, $P = 0.004$) (Fig. 3). Also serum aLN-1 found at T0 and T2 significantly correlated with FF-aLN-1 ($r = 0.35$, $P = 0.04$, and $r = 0.41$, $P = 0.014$, respectively).

Metaphase II (MII) oocytes ranged from 1 to 12 (median 4) and a highly significant negative correlation was found between MII oocyte counts and FF-aLN-1 levels ($r = -0.549$, $P = 0.0006$) (Fig. 4 A), whereas total oocyte counts did not correlate

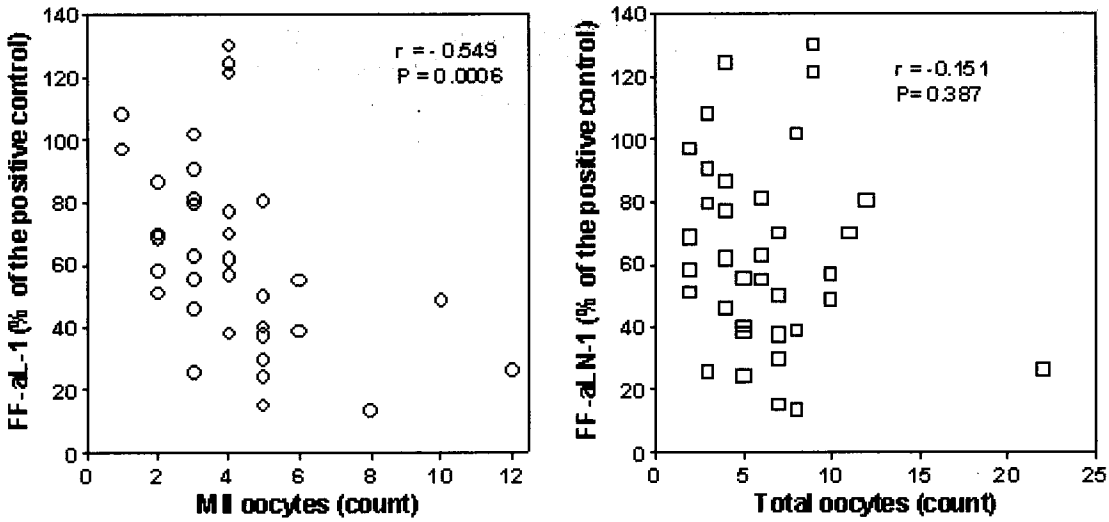

Fig. 4. *Correlations between MII oocyte counts and aLN-1 levels detected in FF (panel **A**) and sera (panel **B**) of 35 women with endometriosis.*

with FF-aLN-1 levels (r = -0.15, P = 0.387) (Fig. 4 B).

Ongoing pregnancy occurred in 4/11 aLN-1 positive patients (36%) and in 7/24 aLN-1 negative (29%) being the difference not significant (P= 0.7, statistical power 70%). There was no significant difference between FF-aLN-1 levels detected in women with (median 48.4 %; 25-75th percentiles 29.7-86.3) or without (62.5%; 48.15-79.7) ongoing pregnancy (P = 0.42, statistical power 55%). Moreover, MII oocyte counts did not differ significantly between women with (median 4; 25-75th percentiles 2-5) or without (4; 3-5) ongoing pregnancy.

DISCUSSION

Endometriosis, a disease characterized by extrauterine endometrial tissue development, is frequently associated with infertility likely caused by multifactorial mechanisms, including alterations of immune function, such as dysfunction of NK activity, enhanced production of pro-inflammatory cytokines, and polyclonal B cell activation (8, 16-18). Several auto-Abs, i.e. antibodies against endometrium, ovary, theca cells and granulosa cells, antinuclear antibodies, antibodies to ribonucleoproteins, histones, smooth muscle, lupus anticoagulant and anticardiolipin antibodies, have been described in women with endometriosis (9, 19-20), and autoimmunity is thought to play an important role in reproductive failure (21).

In this study, we show a significant increase of both aLN-1 levels and aLN-1 prevalence in patients affected by endometriosis when compared with both FCTRs or IWWE, providing further evidence that disturbance in B lymphocyte functions occurs in this disease. Our results are in agreement with Inagaki et al. (11), who demonstrated significantly higher aLN-1 levels in women with endometriosis when compared with IWWE.

LN-1 plays a crucial role in the initial migration of trophoblast cells into maternal decidua during implantation and its interaction with integrin receptors regulates both proliferation and differentiation of trophoblast during implantation and placentation (22). Therefore, it is conceivable that the inhibition of LN-1 functions exerted by aLN-1 may affect very early reproductive processes, leading to infertility.

We demonstrated for the first time the presence of aLN-1 in FFs of women with endometriosis. In addition, we found a positive correlation between serum- and FF-aLN-1 levels and no significant differences of aLN-1 concentrations among follicles which suggests that antibodies, like other molecules, diffuse from serum to follicular environment.

An interesting issue raised by our results is the

negative correlation between MII oocyte number and FF-aLN-1 antibody levels. This finding suggests that aLN-1 may exert a specific detrimental role in follicular milieu, indicating a possible relationship between FF-aLN-1 presence and impaired oocyte maturation.

The *in vitro* effects of ECM components, including LN-1, in the regulation of ovarian follicle development, as well as on oocyte quality have been thoroughly investigated by Kreeger et al. (14) in follicles isolated from C57BL/6 female mice. To this end, immature mouse follicles were cultured within unmodified alginate matrices or, alternatively, within alginate matrices modified with specific ECM components (e.g. LN-1, fibronectin) or RGD peptides. Culture in alginate matrices modified with LN-1, fibronectin or RGD peptides resulted in a significant increase of the rate of polar body formation. Moreover, oocytes showed a more compact metaphase II spindle compared with unmodified alginate matrices, clearly indicating that LN-1 play an important role in oocyte maturation.

Accordingly, it is possible that aLN-1 affect LN-1 functions hampering oocyte maturation, which, in turn, may result in a reduction of MII oocyte number. This hypothesis could explain the negative correlation between MII oocyte count and FF-aLN-1 levels demonstrated in our study.

Concerning the relationship between oocyte quality and endometriosis-associated infertility, alterations within oocyte, resulting in embryos with decreased ability to implant has been suggested as a possible cause of reproductive failure (23). In addition, a high incidence of apoptosis, along with enhanced oxidative stress of granulose cells which might affect oocyte quality has been reported (24-25). Finally, a significant reduction of estradiol concentrations in FF, leading to impaired oocyte maturation and oocyte quality has been described in patients with endometriosis undergoing IVF (26).

Our data suggest that aLN-1 presence in follicular environment may alter oocyte maturation, thus providing new insights into further detrimental effect of aLN-1 on reproductive processes.

Since IVF plays an important role in the management of endometriosis-associated infertility, another objective of our study was to investigate the effects of aLN-1 on IVF outcome.

We found no relationship between serum and/or FF-aLN-1 presence and IVF outcome. In fact, the rate of ongoing pregnancy was not significantly different between aLN-1 positive and aLN-1 negative patients, nor was any significant difference in FF-aLN-1 levels found between women with or without ongoing pregnancy. Although these observations lack statistical power and require confirmation on a larger cohort of patients, it could be speculated that if some oocytes reached maturation despite the presence of FF-aLN-1, fertilization and pregnancy may occur. Another possible explanation for the lack of relationship between FF-aLN-1 presence and IVF failure is that the putative effect of aLN-1 on implantation may be overcome by IVF procedures.

On the whole, our results indicate that, although aLN-1 may reduce fertility in women with endometriosis, there is no evidence that the same auto-Abs affect IVF outcome.

On the other hand, also the impact on IVF outcome of other auto-Abs impairing fertility, such as APA, has been questioned and it is not clear whether the presence of APA is associated with implantation failure (27).

We showed that aLN-1 levels detected at T2 were significantly higher in comparison with those found at T0 and T1 and this finding parallels our previous results on APA in infertile women undergoing IVF (15).

Estrogens enhance B cell activation, protect B cells from apoptosis and may increase autoantibody production by allowing auto-reactive B lymphocytes to escape normal tolerance (28-30). Accordingly, it is possible that hyperestrogenism induced by IVF treatment may account for aLN-1 increase.

In this respect, even though we found no significant positive correlation between E_2 evaluated at the hCG day administration and aLN-1 levels detected at T2, a tendency to significance ($P = 0.09$) was demonstrated. In addition, the possibility that each patient may show a different B cell response to hyperestrogenism should be taken into account. On the other hand, only a systematic monitoring of aLN-1 levels over the time could elucidate whether auto-Abs increase is sustained or not and whether this phenomenon has any clinical relevance.

In conclusion, in this study we have shown for the first time that aLN-1 concentrations detected

in FFs inversely correlated with MII oocyte count, suggesting that FF-aLN-1 inhibit oocyte maturation.

However, IVF outcome seems not to be affected by the presence of either serum- or FF-aLN-1, suggesting that: i) IVF procedure may overcome detrimental effect(s) of aLN-1; ii) the presence of aLN-1 is not predictive of IVF failure.

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REFERENCES

1. Colognato H, Yurchenco PD. Form and function: the laminin family of heterotrimers. Dev Dyn 2000; 218: 213-34.
2. Mercurio AM. Laminin receptors: achieving specificity through cooperation. Trends Cell Biol 1995; 5:419-23.
3. Smyth N, Vatansever HS, Murray P, Meyer M, Frie C, Paulsson M, Edgar D. Absence of basement membranes after targeting the LAMC1 gene results in embryonic lethality due to failure of endoderm differentiation. J Cell Biol 1999; 144:151-60.
4. Weeks BS, Klein NW, Kleinman H, Frederickson T, Sackett GP. Laminin immunized monkeys develop sera toxic to cultured rat embryos and fail to reproduce. Teratology 1989; 40:47-57.
5. Foidart JM, Yaar M, Figueroa A, Wilk A, Brown KS, Liotta LA. Abortion in mice induced by intravenous injections of antibodies to type IV collagen or laminin. Am J Pathol 1983; 110:346-57.
6. Matalon ST, Blank M, Matsuura E, Inagaki J, Nomizu M, Levi Y, Koike T, Shere Y, Ornoy A, Shoenfeld Y. Immunization of naïve mice with mouse laminin-1 affected pregnancy outcome in a mouse model. Am J Reprod Immunol 2003; 50:159-65.
7. Inagaki J, Matsuura E, Nomizu M, Sugiura-Ogasawara M, Katano K, Kaihara K, Kobayashi K, Yasuda T, Aoki K. IgG anti-laminin-1 autoantibody and recurrent miscarriages. Am J Repro Immunol 2001; 45:232-8.
8. Gupta S, Goldberg JM, Aziz N, Goldberg E, Krajcir N, Agarwal A. Pathogenic mechanisms in endometriosis-associated infertility. Fertil Steril 2008; 90:247-57.
9. Gleicher N, el-Roeiy A, Confino E, Friberg J. Is endometriosis an autoimmune disease? Obstet Gynecol 1987; 70:115-22.
10. Vinatier D, Dufour P, Oosterlynck D. Immunological aspects of endometriosis. Hum. Reprod Update 1996; 2:371-84.
11. Inagaki J, Sugiura-Ogasawara M, Nomizu M, et al. An association of IgG anti-laminin-1 autoantibodies with endometriosis in infertile patients. Hum Reprod 2003; 18:44-9.
12. Inagaki J, Kondo A, Lopez LR, Shoenfeld Y, Matsuura E. Anti-laminin-1 autoantibodies, pregnancy loss and endometriosis. Clin Dev Immunol 2004; 11:261-6.
13. Inagaki J, Kondo A, Lopez LR, Shoenfeld Y, Matsuura E. Pregnancy loss and endometriosis: pathogenic role of anti-laminin-1 autoantibodies. Ann NY Acad Sci 2005; 1051:174-84.
14. Kreeger PK, Deck JW, Woodruff TK, Shea LD. The in vitro regulation of ovarian follicle development using alginate-extracellular matrix gels. Biomaterials 2000; 27:714-23.
15. Caccavo D, Pellegrino NM, Lorusso F, Capotorto M, Vacca M, Vimercati A, Depalo R. Anticardiolipin antibody levels in women undergoing first *in vitro* fertilization/embryo transfer. Hum Reprod 2007; 22: 2494-500.
16. de Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: pathophysiology and management. Lancet 2010; 376:730-38.
17. Matarese G, De Placido G, Nikas Y, Alviggi C. Pathogenesis of endometriosis: natural immunity dysfunction or autoimmune disease? Trends Mol Med 2003; 9:223-8.
18. Nothnick WB. Treating endometriosis as an autoimmune disease. Fertil Steril 2001; 76:223-31.
19. Mathur S, Peress MR, Williamson HO, Youmans CD, Maney SA, Garvin AJ, Rust PF, Fudenberg HH. Autoimmunity to endometrium and ovary in endometriosis. Clin Exp Immunol 1982; 50:259-66.
20. Taylor PV, Maloney MD, Campbell JM, Skerrow SM, Nip MM, Parmar R, Tate G. Autoreactivity in women with endometriosis. Br J Obstet Gynaecol 1991; 98:680-4.
21. Mathur SP. Autoimmunity in endometriosis:

relevance to infertility. Am J Reprod Immunol 2000; 44:89-95.

22. Klaffky E, Williams R, Yao CC, Ziober B, Kramer R, Sutherland A. rophoblast-specific expression and function of the integrin alpha 7 subunit in the peri-implantation mouse embryo. Dev Biol 2001; 239: 161-75.
23. Simón C, Gutiérrez A, Vidal A, de los Santos MJ, Tarín JJ, Remohí J, Pellicer A. Outcome of patients with endometriosis in assisted reproduction: results from in-vitro fertilization and oocyte donation. Hum Reprod 1994; 9:725-9.
24. Nakahara K, Saito H, Saito T, Ito M, Ohta N, Sakai N, Tezuka N, Hiroi M, Watanabe H. Incidence of apoptotic bodies in membrana granulosa of the patientsparticipating in an in vitro fertilization program. Fertil Steril 1997; 67:302-8.
25. Saito H, Seino T, Kaneko T, Nakahara K, Toya M, Kurachi H. Endometriosis and oocyte quality. Gynecol Obstet Invest 2002; 53(S)1:46-51.
26. Wunder DM, Mueller MD, Birkhäuser MH, Bersinger NA. Steroids and protein markers in the follicular fluid as indicators of oocyte quality in patients with and without endometriosis. J Assist Reprod Genet 2005; 22:257-64.
27. Coulam CB. Antiphospholipid antibody round table report. Am J Reprod Immunol 2002; 48:262-5.
28. Bynoe MS, Grimaldi CM, Diamond B. Estrogen up-regulates Bcl-2 and blocks tolerance induction of naive B cells. Proc Natl Acad Sci USA 2000; 97: 2703-8.
29. Grimaldi CM, Cleary J, Dagtas AS, Moussai D, Diamond B. Estrogen altersthresholds for B cell apoptosis and activation. J Clin Invest 2002; 109: 1625-33.
30. Grimaldi CM, Hill L, Xu X, Peeva E, Diamond B. Hormonal modulation of B cell development and repertoire selection. Mol Immunol 2005; 42:811-20.