

• Title (maximum number of words: 25)

Antinuclear antibody (ANA) of discrete speckled immunofluorescence staining pattern is associated with polypronuclear fertilization

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• Study question (maximum number of words: 25)

The present study was conducted to investigate the influence of immunofluorescence staining pattern and titer of antinuclear antibody (ANA) on maturation and fertilization of human oocyte.

• Summary answer (maximum number of words: 25)

Maturation rates were similar between ANA-negative and positive irrespective of immunofluorescence staining pattern. Polypronuclear fertilization increased significantly in ANA of discrete speckled pattern either by ICSI or IVF.

• What is known already (maximum number of words: 100)

ANA is an autoantibody against nuclear components of eukaryotic cells such as DNA, centromere and histone. Immunofluorescence staining pattern of ANA is commonly applied to identify corresponding antigens. Discrete speckled staining pattern indicates that its corresponding antigen is centromere. The relationship of anti-centromere antibody (ACA) with abnormal oocytes maturation, compromised embryo developmental competence and poor prognosis post pregnancy in human IVF has been reported.

• Study design, size, duration maximum number of words: 75

Total of 1646 cycles of IVF (355 cases with ANA determined) from January 2012 to December 2013 were retrospectively investigated.

• Participants/materials, setting, methods (maximum number of words: 75)

Patients with positive ANA were divided into 4 groups according to their patterns and ANA negative patients were used as control (A: homogeneous, B: speckled, C: nucleolar, and D: discrete speckled pattern and Control). The rates of oocytes maturation and polypronuclear fertilization either by IVF or ICSI were compared among its patterns and also to group A. Patients with discrete speckled pattern were further divided into 5 groups

based on their titer. Correlation between high polypronuclear fertilization rate (over 25%) and antibody titer was also analyzed.

• Main results and the role of chance (maximum number of words: 200)

The rates of maturation, polypronuclear fertilization in IVF and polypronuclear fertilization in ICSI of control were 75.9% (3772/4870), 9.0% (72/798) and 4.6% (137/2974), respectively. Rates of group A were 80.3% (965/1201), 11.7% (33/282) and 4.0% (27/683), respectively. Rates of group B were 82.6% (2037/2465), 11.7% (58/495) and 4.3% (66/1542), respectively. Rates of group C were 94.6% (210/232), 7.4% (8/108) and 4.9% (5/102), respectively. Rates of group D were 84.2% (85/101), 50.0% (27/54) and 51.6% (16/31), respectively. Maturation rates were similar between ANA-negative and positive regardless of staining pattern. The ANA-positive patients with discrete speckled pattern showed significantly higher incidence of polypronuclear fertilization both in IVF and ICSI than control ( $p < 0.01$ ;  $\chi^2$ -test). Percentages of the patients with high polypronuclear fertilization rates in the group of antibody titer 1:80, 1:160, 1:320, 1:640 and 1:1280 were 100.0% (2/2), 100.0% (3/3), 0.0% (0/1), 0.0% (0/1) and 90.9% (10/11), respectively. No correlation was confirmed between high polypronuclear fertilization rate and intensity of antibody titer.

• Limitations, reasons for caution (maximum number of words: 50)

Discrete speckled staining pattern indicates its corresponding antigen is centromere. However, evaluation of staining pattern is not well established.

• Wider implications of the findings (maximum number of words: 50)

Polypronuclear fertilization increased in the patients with discrete speckled pattern of ANA. This result suggests that ACA might cause polypronuclear fertilization and further research is required to clarify the mechanism. Embryos for transfer should be carefully selected for the patients with positive ANA, especially with discrete speckled staining pattern.