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Shortening the time from ovulation trigger to insemination is effective in reducing multiple pronuclei formation in anti-centromere antibody-positive patients.

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### Study question:

Does shortening the time from ovulation trigger to insemination reduce multiple pronuclei formation (MPF) and improve the outcome after fertilization?

### Summary answer:

Shortening the time from ovulation trigger to insemination increased the normal fertilization rate and increased the number of cycles in which good embryos were obtained.

## What is known already:

When anti-centromere antibody (ACA)-positive patients undergo IVF, low oocyte maturation rates and MPF may be observed, which tend to occur when ACA antibody titers are high. In this case, the patient tends to present with poor embryonic development. There is currently no effective treatment for ACA-positive patients. It has been reported that MPF is caused by chromosome dispersion due to significant abnormal spindle deformation. In such cases, it has been reported that the oocyte spindle is often not visualized by polarized light microscopy. The oocyte is possible that the spindle is affected by ACA during the meiosis, but the mechanism is not well understood.

# Study design, size, duration:

A retrospective study was conducted on 764 mature oocytes from 174 cycles in 45 patients who underwent oocyte retrieval and inseminated by intra-cytoplasmic sperm injection (ICSI) with consent from 2009 to 2022. Patients were limited to those who tested positive for ACA by blood antinuclear antibody testing and whose oocytes occurred MPF.

#### Participants/materials, setting, methods:

Oocyte retrieval was performed 36 hours after ovulation trigger. After ICSI, embryos with normal fertilization were cultured until day 3 or blastocyst. The group was divided into two groups: the time reduced from ovulation trigger to ICSI (S group, n=95) and the normal protocol (N group, n=79). The normal fertilization rate, MPF rate, the rate of good embryo (Veeck's classification G3 or higher) on day 3, the percentage of IVF cycles was performed freezing good embryo, and clinical pregnancy rate per transfer were compared. A t-test or chi-square test was used for comparison.

Main results and the role of chance:

The mean time from maturation trigger to insemination was shorter in the S group than in the  $\ensuremath{N}$ 

group (38.2h vs. 41.8h, P<0.01). The normal fertilization rate was significantly increased in the S

group compared to the N group (49.9 vs. 40.7, P<0.01) and the MPF rate was significantly

decreased (39.1 vs. 28.3, P<0.01). The rate of transferable embryo on day 3 tended to increase in

the S group compared to the N group (70.6 vs. 61.9, P=0.09). In addition, the percentage of IVF

cycles was performed freezing good embryo was significantly increased in the S group compared

to the N group (57.4 vs. 28.4, P<0.01). There was no difference in pregnancy rate per embryo

transfer. There was no difference in the age of the wife at the time of oocyte retrieval.

Limitations, reasons for caution:

The data were conducted from a single facility. and the limited number of cases, resulting in a

small sample size. The process of visualizing the oocyte spindle was not performed in this study

because it is time consuming and increases the time from ovulation trigger to insemination.

Wider implications of the findings:

Shortening the time from maturation trigger to insemination was decreased MPF, suggesting that

the oocyte spindle is affected by ACA in a time-dependent manner. It is recommended that ICSI

be performed as soon as possible after oocyte retrieval.

Study funding/competing interest(s):

None

Trial registration number:

None