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REPRODUCTIVE BIOLOGY: SHORT COMMUNICATION

The N501Y Mutation of SARS-CoV-2 Spike Protein Impairs Spindle Assembly in Mouse Oocytes

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Abstract

The COVID-19 pandemic has been continuing for one and a half year and caused a profound effect on human health. Although advanced researches and literatures are gathered, the influences of SARS-CoV-2 on the reproduction systems are largely unknown, especially on the female reproductive functions. The purpose of this study was to investigate the effect of N501Y mutant spike protein of SARS-Cov-2 on oocyte maturation. We demonstrated that the N501Y mutant of SARS-CoV-2 spike protein impaired the mouse oocyte maturation accompanied by abnormal spindle assembly. Furthermore, the mean spindle length and the plate width were significantly increased in the N501Y-treated group compared to the control group. These results indicated the potential impairment of maturation of the oocytes caused by the infection of SARS-CoV-2, albeit current results were derived from mouse oocytes. The present study provided a theoretical basis for the attention of female reproductive health during the COVID-19 pandemic and shed light on the potential risk of SARS-CoV-2 in the successful rate of assisted reproduction.

Keywords SARS-CoV-2 · Spike protein · N501Y variant · Oocyte maturation

Introduction

The coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is known as the global pandemic, of which the spread rate and infection rate are very high. SARS-CoV-2 is composed of four structural proteins, namely, nucleoprotein (NP), spike (S), envelope (E), and membrane (M) proteins. The S protein of SARS-CoV-2 mediates viral entry into host cells by binding to its receptor, angiotensin-converting enzyme 2 (ACE2), through the receptor-binding domain (RBD) [1]. The spread rate of the virus has become faster and cross-species due to variable genetic mutation in the receptor-binding domain. For example, the mutation of asparagine at position 501 to tyrosine (N501Y), which is one of the residues in the RBD-ACE2 contact area, can enhance the binding affinity of SARS-CoV2 spike protein to ACE2 both in human and mouse [2].

While SARS-CoV-2 is known to impair the respiratory system, SARS-CoV2 may affect the hematologic, cardiovascular, renal, gastrointestinal and hepatobiliary, endocrinologic, neurologic, ophthalmologic, and dermatologic systems [3]. Although the scientists in the reproductive community appealed to pay more attention to the potential impairment of fertility caused by SARS-CoV-2, few studies have reported the impact of SARS-CoV-2 on the reproduction system. In male, emerging evidence demonstrated that the SARS-CoV-2 was able to infect the testis, leading to spermatogenic damages and semen impairments [4, 5]. In female, studies have demonstrated the expression of SARS-CoV-2-interacting proteins, ACE2, TMPRSS2, and CD147, in human oocytes and blastocysts [6, 7]. Barragan et al. have detected the viral RNA for gene N of SARS-CoV-2 in the oocytes from two SARS-CoV-2 positive women [8], suggesting that the oocytes of SARS-CoV-2 positive women may be exposed to these viral RNA and protein products. However, whether the spike protein of SARS-CoV-2, as the host entry molecule, has an effect on the oocytes remains elusive.

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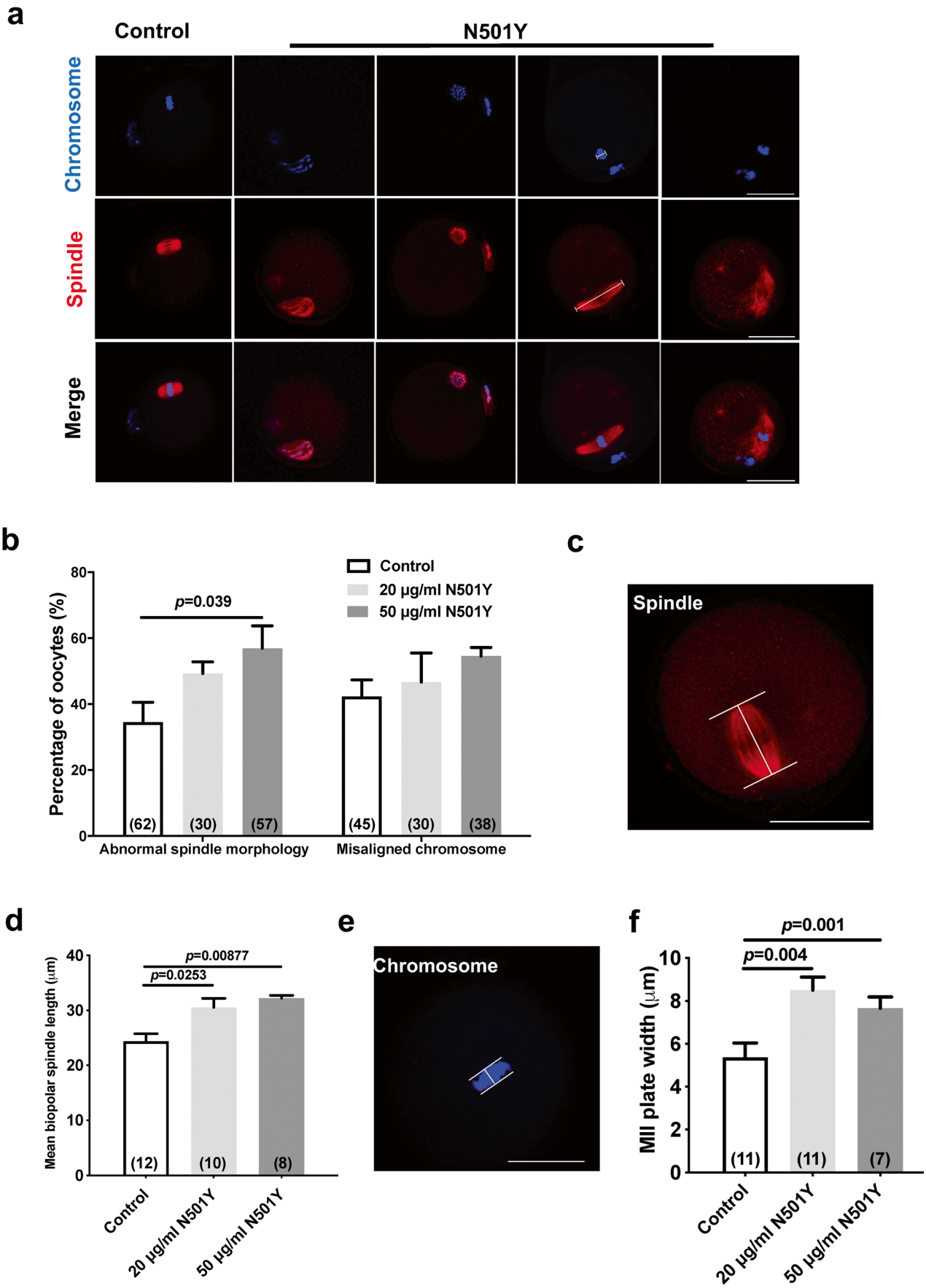
a
Control
N501Y
Chromosome
Spindle
Merge
b
Control
20 μg/ml N501Y
50 μg/ml N501Y
Percentage of oocytes (%)
p=0.039
(62)
(30)
(57)
(45)
(30)
(38)
Abnormal spindle morphology
Misaligned chromosome
c
Spindle
d
Mean biopolar spindle length (μm)
p=0.00877
p=0.0253
(12)
(10)
(8)
Control
20 μg/ml N501Y
50 μg/ml N501Y
e
Chromosome
f
MII plate width (μm)
p=0.001
p=0.004
(11)
(11)
(7)
Control
20 μg/ml N501Y
50 μg/ml N501Y

◂**Fig. 1** Effects of N501Y mutation spike protein on spindle assembly and chromosome aggregation in murine oocytes. **a** Representative photos of meiotic spindles in MII oocytes after labeling with β-tubulin antibody (red) and counterstaining of DNA with DAPI (blue). Bar = 50 μm. **b** Incidence of spindle and chromosome abnormalities in MII oocytes between control group and N501Y groups (control, $n = 62$; 20 μg/ml N501Y, $n = 30$; 50 μg/ml N501Y, $n = 57$). **c** Representative image of spindle length in MII oocyte. Bar = 50 μm. **d** Statistical data of spindle length in control and N501Y-treated oocytes after cultured for 24 h (control, $n = 12$; 20 μg/ml N501Y, $n = 10$; 50 μg/ml N501Y, $n = 8$). **e** Representative image of the plate width in MII oocyte. Bar = 50 μm. **f** Statistical result of MII plate width in control and N501Y-treated oocytes after cultured for 24 h (control, $n = 11$; 20 μg/ml N501Y, $n = 11$; 50 μg/ml N501Y, $n = 7$). The experiment was independently replicated 3 times. The data are presented as the mean ± SEM

The present study aims to evaluate the effects of N501Y variant of the SARS-CoV-2 spike protein on the in vitro maturation of mouse oocytes. We have investigated the nuclear and cytoplasmic maturation by examining the spindle assembly and mitochondrial distribution upon the treatment of N501Y spike protein.

Materials and Methods

Chemicals and Culture Media

All chemicals and culture media components were purchased from Sigma-Aldrich, Inc. (St. Louis. MO, USA) unless otherwise stated.

Ethics Statement

All animal experiments were carried out in accordance with the principles and guidelines for the use of laboratory animals and approved by the Laboratory Animal Center of Nantong University.

N501Y Treatment

One hundred μg N501Y recombinant protein (Sino Biological Inc., Cat: 40,592-V05H, Beijing, China) were dissolved in 400 μl of sterile water. In the present study, the treatment of N501Y was divided into three groups with the final concentration of 0 μg/ml (vehicle control, named control), 20 μg/ml, and 50 μg/ml in the culture medium.

Oocyte Collection and Culture

Germinal vesicle (GV)-intact oocytes were obtained from the ovaries of CD-1® (ICR) female mice (Laboratory Animal Center of Nantong University, Nantong, China) at 6–8 weeks of age. The mice were kept under controlled light (12 h light and 12 h dark) and temperature (22–25 °C) conditions with food and water provided ad libitum. Briefly, 5–6 mice were selected and euthanized by cervical dislocation 44 h after they were primed with 10 IU pregnant mare serum gonadotropin (PMSG). Ovaries were dissociated and immediately transferred to M199 medium with 4 mM hypoxanthine (HX-M199 medium). GV oocytes were isolated by careful manual dissection of ovaries under a microscope (SMZ745T, Nikon, Tokyo, Japan) using a pair of sterilized 26 gauge needles in HX-M199 media. Only cytoplasmic uniform of oocytes surrounded with at least 4 layers of cumulus cells were used to do following experiments. After washing with M199 media, groups of 30 cumulus–oocyte complexes (COCs) selected randomly were cultured in 500 μL in vitro maturation (IVM) medium (M199 medium containing 0.3% bovine serum albumin (BSA), 0.23 mM sodium pyruvate, 2 mM L-Glutamine, 100 unit/mL penicillin, 100 unit/mL streptomycin [9]) with different concentrations of N501Y (0 μg/ml, 20 μg/ml, or 50 μg/ml) at 37 °C, 5% CO_2, and saturated humidity. After cultured for 24 h, oocytes were denuded of cumulus cells using phosphate-buffered saline (PBS) (Sangon Biotech, Shanghai, China) containing 0.1% (w/v) hyaluronidase. The oocytes were then counted using a light microscope (SMZ745T) and classified as mature metaphase II oocytes (MII) with a single polar body in the perivitelline space and germinal vesicle breakdown (GVBD) without a polar body. After collection, MII oocytes were used for immunofluorescence and assessment of mitochondrial distribution pattern.

Immunofluorescence and Confocal Microscopy

Oocytes were fixed with 4% paraformaldehyde fix solution (Sangon Biotech) for at least 30 min at room temperature for immunofluorescent staining. Then, the oocytes were transferred to a membrane permeabilization solution (1% Triton X-100 in PBS) for 1 h at room temperature. After three washes in washing buffer containing 0.1% Tween 20 and 0.02% Triton X-100 in PBS, the oocytes were blocked in blocking buffer (2% BSA in PBS) for 1 h at 37 °C. The oocytes were then incubated for 2 h at 37 °C with primary antibodies (β-tubulin, SC-134230, 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA). After washing three times, oocytes were stained with donkey anti-mouse Rhodamine Red 568 (1:500 dilutions, A10037, Invitrogen Corporation, CA, USA) antibodies at 37 °C for 1 h. Then, the samples were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) for 8 min. In the negative control group, the first antibody was replaced by 2% BSA, with the other steps being the same.

Assessment of Mitochondrial Distribution Pattern

Collected oocytes were cultured with fluorescent probe MitoTracker Red (100 nM) (Invitrogen Corporation, CA, USA) for 30 min at 37 °C. Then oocytes were fixed with 4% paraformaldehyde fix solution (Sangon Biotech) for 30 min at room temperature for immunofluorescent staining. The mitochondrial distribution pattern in oocytes was assessed by using ZEN Imaging software (Zeiss) under a confocal microscope (Axio Imager M2, Carl Zeiss Meditec AG, Jena, Germany) and was classified as homogeneous or clustered distribution [10]. Data for mitochondrial distribution patterns are the percentage of the oocytes with the indicated pattern of the total number examined.

Statistical Analysis

When the data containe percentages greater than 90% or less than 10%, percentage data were subjected to arcsine transformation before statistical analysis. For each treatment, at least 3 biological replicates were performed with data expressed as means ± standard error of measurement (SEM). The measurement of spindle length and MII plate width were tested by Image J software. All analyses were performed using SPSS 17.0 software and GraphPad Prism 8.0 software. One-way ANOVA two-sample *t* test and Mann–Whitney *U* test were used for the analysis of continuous data and non-parametric data, respectively. $p < 0.05$ was considered significant.

Results

After 24-h maturation, the percentage of germinal vesicle breakdown (GVBD) and metaphase II (MII) oocytes were not significant different between control and N501Y treatment (Supplementary Fig. S1), suggesting that N501Y spike protein did not block the nuclear maturation in mice oocyte.

A recent study demonstrated that the morphology of the oocyte meiotic spindle was a predictive marker for embryo development [11]. To further determine whether N501Y spike protein can damage the cytoplasmic maturation of mouse oocytes, we assessed spindle and chromosome assembly of MII oocytes. The immuno-confocal microscopy of spindle and chromosomes revealed that N501Y mutant spike treated oocytes displayed the obvious spindle morphology defects (Fig. 1a, b). However, the chromosome misalignment was comparable between the N501Y-treated groups and control group (Fig. 1b). Interestingly, the mean spindle length and the plate width were significantly increased in the N501Y-treated group compared to the control (Fig. 1c–f), suggesting N501Y mutant spike might impair meiotic spindle assembly in mouse oocytes.

It was reported that mitochondrial distribution was involved in the cytoplasmic maturation of oocytes. During the formation of the first metaphase (MI) spindle, mitochondria translocate to the perinuclear region and subsequently demonstrate a uniform distribution during and after the first polar body extrusion [12]. Homogeneous distribution of mitochondria is considered to be an important indicator of the good quality of the oocyte. MitoTracker staining in MII oocytes revealed a modest increase in clustered distribution of mitochondria in N501Y mutant spike protein-treated group (Fig. S2). However, no statistical significance was observed in this experiment. These results indicate that the N501Y treatment did not significantly alter the mitochondrial distribution of oocytes.

Discussion

The present study has demonstrated the abnormal spindle assembly in mouse oocytes after treatment of N501Y mutant spike protein. Recent studies have demonstrated that spindle morphology, size, and position are important for the oocyte quality and further embryo development [11, 13, 14]. Apart from the abnormal spindle morphology after the N501Y treatment, the present study also found a significant increase of spindle length and plate width after 24 h N501Y treatment in the morphologically normal oocytes (Fig. 1c–f). Unexpectedly, the N501Y mutant spike protein did not alter the nuclear maturation and mitochondrial distribution during oocyte maturation. Of note, the effects were consistent in all oocytes examined. Since the oocytes were randomly isolated from 5 ~ 6 mice in each experiment, we conclude that the observed effects are genuine rather than an artifact from individual variation. Taken together, these results suggest that N501Y mutant spike protein was able to affect the oocyte spindle assembly in mouse oocytes. Since the spindle assembly of oocyte is associated with embryo development, we postulate that the N501Y mutant may further affect the embryo development. In addition to the N501Y mutant, the effect of highly transmissible variants such as the b.1.617 variant (also named as delta variant) and delta plus variant (AY.1 or B.1.617.2.1) on oogenesis or oocyte maturation warrants further investigations.

Collectively, we have demonstrated the adverse effect of SARS-CoV-2 N501Y spike protein on mouse oocytes. Our results have provided a theoretical basis for the attention of female reproductive health during COVID-19 pandemic and shed light on the risk of SARS-CoV-2 in the successful rate of assisted reproduction.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s43032-021-00809-7.

Author Contribution H. C. conceived the project. H. L. performed the experiments and the data analysis. H. L., J. W. S, and H.C. wrote the manuscript. H. C. and K. L. F. revised the manuscript.

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Availability of Data and Materials Data and materials are available on request from the corresponding author.

Code Availability Not applicable.

Declarations

Ethics Approval All animal experiments were carried out in accordance with the principles and guidelines for the use of laboratory animals and approved by the Laboratory Animal Center of Nantong University.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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