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Influence of Phosphatidylcholine on Oolemma Fluidity and Breakage Pattern in Human Oocytes during ICSI

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ABSTRACT

Introduction: *Intracytoplasmic sperm injection (ICSI) is a central procedure in assisted reproductive technologies. Its success depends not only on sperm quality and micromanipulation technique, but also on the structural and functional state of the oocyte. The oolemma is directly exposed to mechanical stress during penetration by the injection pipette, and its breakage pattern reflects membrane fluidity, elasticity, cortical cytoskeleton organization and the ability of the oocyte to tolerate micromanipulation. This pilot study investigated whether phosphatidylcholine, one of the major phospholipids of biological membranes, can modify the mechanical response of the human oocyte oolemma during ICSI-like penetration.*

Materials and Methods: *Human oocytes obtained within assisted reproductive technology programs were assessed at the GV, MI and MII stages. A total of 28 oocytes from four donors were initially evaluated; 24 viable oocytes were included in the final analysis. Oocytes were allocated into control and experimental groups according to maturation stage. Experimental groups were exposed for 30 minutes to L-alpha-phosphatidylcholine from soybean at a final concentration of 100 μM, while control groups were maintained under comparable culture conditions without phosphatidylcholine. Oolemma response was evaluated during standardized ICSI-like micromanipulation using an inverted Leica DMi8 microscope at 37 °C and classified according to the A-E typology of oolemma reaction.*

Results: *Primary penetration showed that immature GV and MI oocytes predominantly demonstrated type A oolemma reaction, whereas mature MII oocytes showed type B reaction. Four of the 28 initially assessed oocytes did not survive primary penetration, resulting in a final survival rate of 85.7%. In control groups, the oolemma reaction type remained unchanged after incubation. In experimental groups, phosphatidylcholine exposure produced a consistent directional shift: GV and MI oocytes changed from type A to type B, while MII oocytes changed from type B to type C. The differences between control and experimental groups were statistically significant for each maturation stage.*

Conclusion: *This study provides preliminary evidence that phosphatidylcholine can modify the mechanical behavior of the human oocyte oolemma during ICSI-like micromanipulation. The oolemma reaction type may serve as a functional marker of oocyte quality, while phosphatidylcholine may be considered a promising factor for further optimization of ICSI conditions and reduction of oocyte degeneration risk.*

Keywords

Oolemma, human oocyte, Phosphatidylcholine, ICSI, Membrane fluidity, Oocyte quality, Micromanipulation.

Introduction

ICSI is one of the most widely used micromanipulation procedures in modern reproductive medicine. During this procedure, the injection pipette sequentially crosses the zona pellucida, the perivitelline space and the oolemma before sperm deposition

into the ooplasm [1-3]. Although ICSI is often discussed as a primarily technical procedure, its outcome is also determined by the biological readiness of the oocyte to tolerate local membrane deformation and rupture [1,2].

The oolemma is not a passive envelope of the oocyte. It is a dynamic membrane-cytoskeletal system composed of a lipid bilayer, membrane proteins, cholesterol-rich domains and an underlying cortical actin network [4-6]. These components together regulate membrane fluidity, microviscosity, deformability and the ability to restore integrity after mechanical injury [5-7]. Therefore, the way in which the oolemma responds to the injection pipette provides valuable functional information about the oocyte [2,6,8].

The problem is particularly relevant for oocytes of different nuclear maturation stages. GV and MI oocytes are characterized by incomplete maturation, while MII oocytes have acquired a more advanced cytoplasmic and membrane-cytoskeletal organization [8,-10]. This difference may be reflected in the mechanical pattern of oolemma penetration. A fragile or instantly rupturing oolemma may increase the risk of cytoplasmic loss and degeneration, whereas controlled invagination before rupture indicates more favorable membrane plasticity [6,11,12].

Phosphatidylcholine is one of the main phospholipids of biological membranes and an important regulator of membrane architecture [6,12]. It contributes to bilayer stability, lipid packing, membrane fluidity and deformation capacity [7,13-15]. Because the lipid composition of the oolemma is directly related to its biophysical behavior, experimental modulation by phosphatidylcholine may represent a relevant approach for improving oocyte tolerance to micromanipulation [13-15].

The aim of this pilot study was to evaluate the influence of phosphatidylcholine on the mechanical properties of the human oocyte oolemma at different stages of nuclear maturation during ICSI-like micromanipulation.

Literature review

The oolemma plays a key role during both natural fertilization and assisted reproduction. During physiological fertilization, it participates in sperm-oocyte recognition, membrane fusion, calcium oscillations, cortical reaction and prevention of polyspermy [4,5]. In ICSI, however, these physiological membrane fusion events are bypassed, and the oolemma is subjected to direct mechanical penetration by the injection pipette [1,2].

Oolemma behavior during ICSI has been described using a typological classification based on the degree of membrane deformation and the manner of rupture [2,11]. Type A is characterized by immediate membrane breakage without marked invagination. Type B involves controlled invagination and breakage after slight aspiration and is generally considered a favorable response [2,8,11]. Type C reflects more pronounced deformation and increased elasticity. Types D and E are associated with repeated penetration or unstable mechanical behavior [12].

The mechanical response of the oolemma depends on lipid composition, cholesterol content, cytoskeletal organization, temperature, osmotic conditions and maturation status [6,8,13,14]. Lipid composition is particularly important because phospholipid saturation, cholesterol distribution and membrane microdomains regulate the balance between stiffness and flexibility [7,15]. Excessive rigidity may complicate penetration, while excessive fragility may lead to uncontrolled rupture [6,12].

Phosphatidylcholine has attracted attention as a potential modulator of membrane properties [7,13,15]. Its molecular structure includes a glycerol backbone, two fatty-acid chains and a polar choline head group. Depending on fatty-acid composition, phosphatidylcholine can influence membrane order, fluidity and capacity for deformation [7,13,15]. Experimental studies on oocytes and other cellular systems suggest that modifying membrane phospholipids may alter cell tolerance to stress, cryopreservation and micromanipulation [13,14].

In this context, oolemma reaction during ICSI-like penetration can be interpreted as an indirect but practical marker of oocyte quality [3,6,8]. Unlike purely morphological evaluation, this approach reflects the functional state of the membrane-cytoskeletal complex at the moment of micromanipulation [6,9].

Materials and Methods

The study was conducted on human oocytes obtained within assisted reproductive technology programs at I.D. Medical Group LLC / I.D. Clinic. Biological material was obtained from four donors with documented informed consent and in accordance with ethical principles for work with human biological material [16,17]. Because human oocytes are a limited biological material, the study was designed as a pilot investigation.

A total of 28 oocytes at the GV, MI and MII stages were initially assessed. After primary oolemma evaluation, 24 viable oocytes were included in the final analysis and distributed into six groups: GV control, GV experimental, MI control, MI experimental, MII control and MII experimental. Each group contained four oocytes. This design allowed comparison of oolemma behavior both between maturation stages and between control and phosphatidylcholine-exposed conditions.

After follicular aspiration, cumulus-oocyte complexes were identified under a stereomicroscope and transferred into handling medium. Standardized denudation was performed using Hyaluronidase 80 U/ml followed by mechanical pipetting with Flexipet denudation tips. After denudation, the oocytes were assessed morphologically, and their nuclear maturation stage was determined according to standard assisted reproductive technology laboratory procedures [16].

For experimental exposure, L-alpha-phosphatidylcholine from soybean was solubilized in DMSO to prepare a 10 mM stock solution. Immediately before exposure, the stock solution was diluted in SAGE 1-Step medium to obtain a final concentration

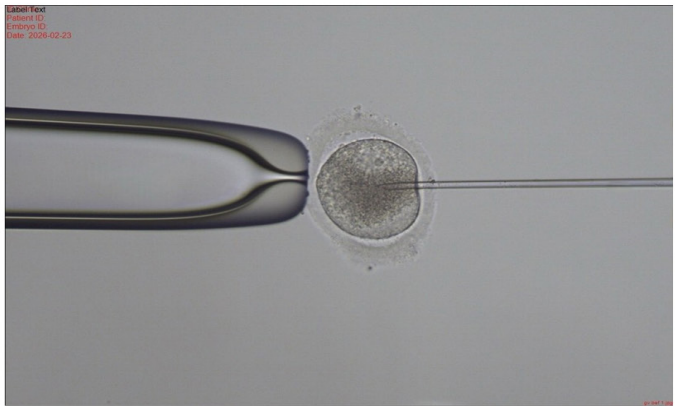

Figure 1: GV-stage oocyte in the control group before phosphatidylcholine exposure (Obj. x20, Oc. x10).

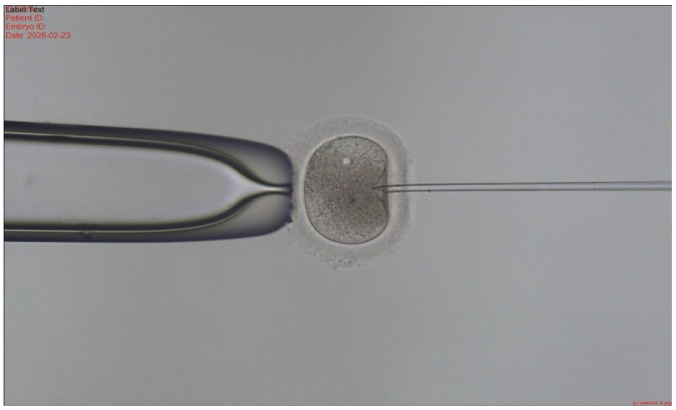

Figure 2: GV-stage oocyte after phosphatidylcholine exposure (Obj. x20, Oc. x10).

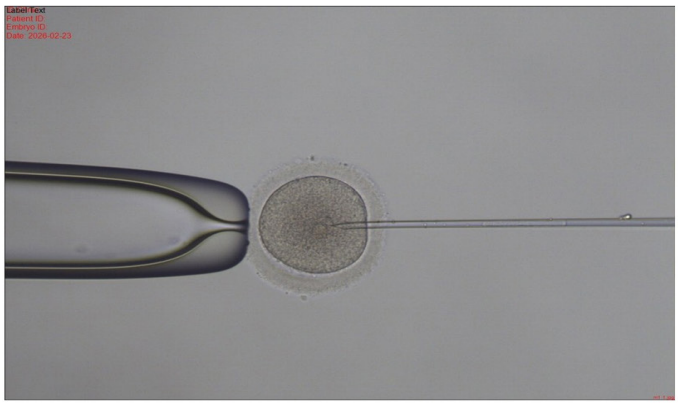

Figure 3: MI-stage oocyte in the control group before phosphatidylcholine exposure (Obj. x20, Oc. x10).

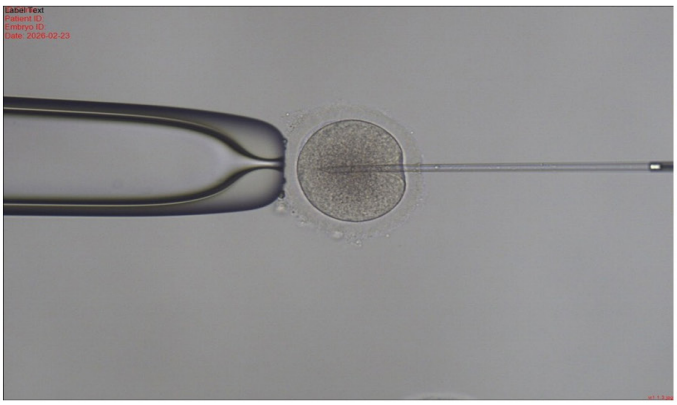

Figure 4: MI-stage oocyte after phosphatidylcholine exposure (Obj. x20, Oc. x10).

of 100 µM. Experimental oocytes were exposed for 30 minutes, while control oocytes were maintained in the same culture medium without phosphatidylcholine.

Oolemma response was evaluated during ICSI-like micromanipulation using a Leica DMi8 coded inverted microscope. Manipulations were performed on a heated stage at 37 °C. Holding pipettes with an outer diameter of approximately 90 µm and injection pipettes with an inner diameter of approximately 4.5 µm were used to standardize the mechanical conditions of penetration. During penetration, the degree of membrane deformation, the need for aspiration and the moment of membrane rupture were recorded and classified according to the A-E reaction typology.

The results were summarized as absolute values and percentages. Comparisons between control and experimental groups were performed using Fisher's exact test. Paired directional changes of oolemma reaction type were additionally assessed using exact McNemar test. P values below 0.05 were considered statistically significant.

Results

Primary assessment showed a stage-dependent pattern of oolemma behavior. GV and MI oocytes predominantly demonstrated type A reaction, which corresponds to immediate oolemma rupture without evident prior invagination. In contrast, MII oocytes showed type B reaction, characterized by controlled membrane invagination and breakage after slight aspiration. This suggests that the mature MII stage is associated with a more stable and functionally competent membrane-cytoskeletal organization [6,8-10].

Four of the 28 initially evaluated oocytes did not survive primary penetration: three GV oocytes and one MI oocyte. Thus, 24 oocytes were included in the final analysis, corresponding to an overall survival rate of 85.7%. Among oocytes demonstrating type A reaction, 16 of 20 remained viable, corresponding to an 80.0% survival rate. These data indicate that instant rupture does not always lead to degeneration, but it is associated with lower mechanical tolerance compared with more controlled responses [2,12,18].

In the control groups, no change in the type of oolemma reaction was observed after incubation. GV and MI control oocytes

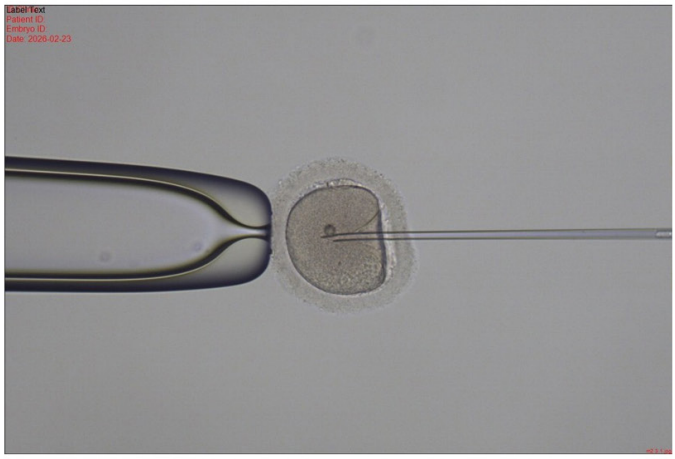

Figure 5: MII-stage oocyte in the control group before phosphatidylcholine exposure (Obj. x20, Oc. x10).

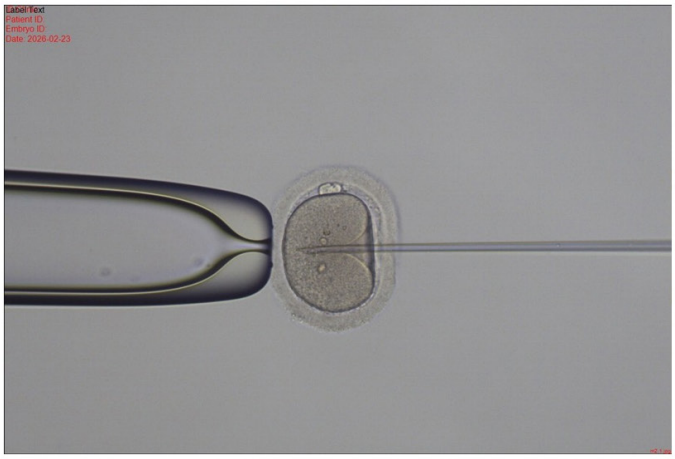

Figure 6: MII-stage oocyte after phosphatidylcholine exposure (Obj. x20, Oc. x10).

Table 1: Distribution of oolemma reaction types in control and experimental groups before and after phosphatidylcholine exposure.

Oocyte stage	Group	n	Primary oolemma reaction	Reaction after incubation / exposure
GV	Control	4	A	A
GV	Experimental	4	A	B
MI	Control	4	A	A
MI	Experimental	4	A	B
MII	Control	4	B	B
MII	Experimental	4	B	C

Note: In control groups, the type of oolemma response did not change after incubation. In experimental groups, phosphatidylcholine exposure induced a directional shift A -> B in GV and MI oocytes and B -> C in MII oocytes.

remained type A, while MII control oocytes remained type B. This indicates that short-term incubation alone did not modify the mechanical response of the oolemma.

In the experimental groups, phosphatidylcholine exposure induced a consistent directional shift in oolemma reaction. GV and MI oocytes changed from type A to type B, whereas MII oocytes changed from type B to type C. The shift from A to B suggests that the oolemma acquired a more controlled deformation pattern before rupture. The B to C shift in MII oocytes may reflect increased membrane elasticity and a higher capacity for deformation.

Fisher's exact test showed statistically significant differences between control and experimental groups for GV, MI and MII oocytes ($P = 0.0286$ for each stage). When GV and MI oocytes were analyzed together, the A to B shift was highly significant ($P = 0.00016$). Paired analysis using exact McNemar test confirmed the directional character of the observed changes ($P = 0.0078$ for separate groups; $P = 0.00003$ for the combined GV + MI group).

Discussion

The results of this pilot study suggest that the mechanical behavior of the human oolemma is closely related to the stage of oocyte maturation. GV and MI oocytes showed a predominance of type A reaction, which may reflect incomplete cytoplasmic maturation, insufficient cortical cytoskeleton organization and lower capacity for controlled membrane deformation. MII oocytes demonstrated type B reaction, supporting the idea that nuclear and cytoplasmic maturation are accompanied by improved membrane-cytoskeletal stability.

The observed effect of phosphatidylcholine is biologically plausible. As a major membrane phospholipid, phosphatidylcholine can influence bilayer organization, lipid packing and microviscosity [7,13,15]. Its incorporation or interaction with the membrane environment may increase the ability of the oolemma to undergo gradual deformation rather than immediate rupture [13,14]. In practical embryology, such a shift may be important because controlled deformation reduces the risk of cytoplasmic loss and mechanical degeneration [2,6,18].

The A to B transition in GV and MI oocytes may indicate improved oolemma plasticity under the influence of phosphatidylcholine. At the same time, the B to C transition in MII oocytes should not be interpreted simply as improvement or deterioration; rather, it may reflect enhanced elasticity of an already mature membrane. Whether this change is beneficial for fertilization and embryo development requires additional evaluation in larger cohorts.

The study has limitations. The sample size was small, which is expected for a pilot study using human oocytes. The work focused on mechanical oolemma response rather than full clinical

outcomes such as fertilization rate, blastocyst development and pregnancy [3,9,10]. In addition, the effect of DMSO as a solvent should be controlled in further experiments. Nevertheless, the consistency of the directional shifts supports further investigation of phosphatidylcholine as a modifier of oocyte membrane properties [13,15].

From a broader perspective, this work supports the concept that oocyte quality should be assessed not only morphologically, but also functionally [6,9]. The oolemma response during ICSI-like penetration provides direct information about the biomechanical competence of the oocyte and may help identify laboratory strategies aimed at reducing degeneration during micromanipulation [8,18].

Conclusion

This pilot study demonstrates that the mechanical response of the human oocyte oolemma during ICSI-like micromanipulation depends on the stage of nuclear maturation and can be modified by short-term exposure to phosphatidylcholine.

GV and MI oocytes were mainly characterized by type A oolemma reaction, while MII oocytes demonstrated type B reaction. After phosphatidylcholine exposure, GV and MI oocytes shifted from type A to type B, and MII oocytes shifted from type B to type C, indicating a change toward more pronounced and controlled membrane deformation.

Oolemma reaction type may be considered an informative functional marker of oocyte quality [6,8]. Phosphatidylcholine may represent a promising experimental factor for optimizing ICSI conditions, improving membrane tolerance to micromanipulation and reducing the risk of oocyte degeneration [2,3,13,18]. Further research with larger sample sizes and assessment of fertilization and embryo development outcomes is required [3,9,10].

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