Size of Perivitelline Space and Incidence of Polyspermy in Mouse Oocytes Treated with Tunicamycin

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Summary

The size of the perivitelline space was measured in mouse oocytes matured in TYH medium containing tunicamycin, an inhibitor of glycoprotein synthesis, and the incidence of polyspermy in those following insemination was observed, in order to examine whether the relationship between the incidence of polyspermy and the size of perivitelline space exists in mouse oocytes.

The size of perivitelline space in oocytes treated with tunicamycin was 4.5 μm, which was significantly smaller than 5.4 μm in control oocytes matured in TYH medium. The rate of fertilization after insemination was similar between oocytes matured in TYH medium containing tunicamycin (91.5 %) and control oocytes matured in TYH medium (92.2 %), while the incidence of polyspermy was significantly higher in oocytes matured in TYH medium containing tunicamycin (79.1 %) than in control oocytes matured in TYH medium (57.6 %).

From these findings, it was clarified that the higher incidence of polyspermy is closely related to the smaller perivitelline space in mouse oocytes.

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Key words : mouse oocyte, size of perivitelline space, incidence of polyspermy

The perivitelline space is the gap between the plasma membrane and the zona pellucida of the oocyte. Although not present in the oocytes in the germinal vesicle stage, the perivitelline space is known to start forming when meiotic maturation resumes and to enlarge in matured oocytes at the metaphase Ⅱ stage (Kaufman *et al*., 1989; Okada *et al*., 1993).

Recently, it has been suggested that there is a relationship between the size of perivitelline space in porcine oocytes and the incidence of polyspermy in those following insemination (Funahashi *et al*., 1994; Wang *et al*., 1998), but it is not clear whether the relationship is present in oocytes of another animals.

It is generally thought that glycoproteins synthesized and secreted by cumulus cells are involved in the enlargement of the perivitelline space (Talbot and Dandekar, 2003). Glycoproteins secreted from cumulus cells accumulate between cumulus cells and in the perivitelline space (Talbot, 1984). Hyaluronan, a major component of the glycoprotein synthesizd and secreted by cumulus cells, in particular has a water absorbing property which results in enlargement of the matrix of cumulus and the perivitelline space (Talbot and Dandekar, 2003). In the cumulus-oocyte complex (COC) with cumulus cells whose glycoprotein synthesis was inhibited, therefore, it is supposed that the perivitelline space in oocytes fails to enlarge and the incidence of polyspermy after insemination increases.

In the present study, the size of the perivitelline space was measured in mouse oocytes treated with tunicamycin, an inhibitor of glycoprotein synthesis, and also the incidence of polyspermy in those following insemination was observed, in order to examine whether the relationship between the incidence of polyspermy and the size of perivitelline space exists in mouse oocytes.

MATERIALS AND METHODS

Animals

Fifty-eight female mature mice of ICR strain were used in the present study. They were housed in autoclaved metal cages and were given a standard chow (MF, Oriental Yeast Co., Tokyo, Japan) and tap water *ad libitum* in an air-conditioned room (24 ℃), under controlled-lighting conditions (14L/10D). They received humane care as outlined in the Guide for the Care and Use of Laboratory Animals (Niigata University Animal Care Committee). These mice were intraperitoneally injected with 5 i.u. of PMSG (Serotropin® , Teikoku Hormone Manufacturing Co. Ltd., Tokyo, Japan).

Collection and culture of oocytes

In the present investigation, mice were sacrificed under anesthesia by diethyl ether to collect oocytes. In order to observe the effect of tunicamycin on the size of perivitelline space, COCs were collected from antral follicles 48 hrs after the PMSG injection and cultured in TYH medium (Toyoda *et al*., 1971) containing 1.0 % (w/v) tunicamycin (Sigma-Aldrich, NJ, USA), 5 % (v/v) fetal bovine serum (FCS, Gibco BRL, NY,

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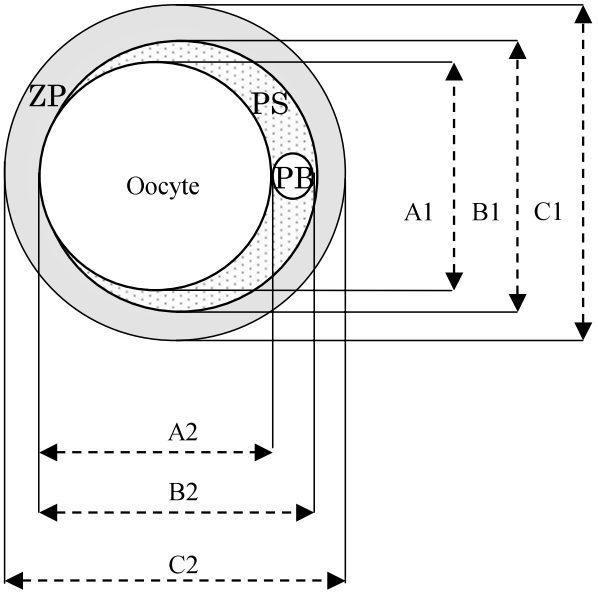

ZP: Zona pellucida, PS: perivitelline space, PB: 1st polar body.

Diameter of oocyte (A) = (A1+A2)/2
Inner diameter of zona pellucida (B) = (B1+B2)/2
Outer diameter of zona pellucida (C) = (C1+C2)/2
Thickness of zona pellucida = (C−B)/2
Size of perivitelline space = (B−A)/2

Fig.1. Calculation method of the size of each part in oocyte

USA) and 10 i.u./ml PMSG at 37 ℃ in a CO_2 incubator (5 % CO_2 in air). COCs cultured in TYH medium containing 5 % FCS and 10 i.u./ml PMSG were used as controls.

Measurement of each part in oocytes

After cultured for 14 hrs in TYH medium with and without tunicamycin, COCs were immersed in PBS (pH 7.4) (Dulbecco and Vogt, 1954) containing 0.1% (w/v) hyaluronidase to disperse their cumulus cells. Then, the each part was measured using a micrometer only for the oocytes with 1st polar body under a microscope, and the size of perivitelline space was calculated according to the method described in **Fig.1**.

Observation of polyspermy

In order to observe the incidence of polyspermy, only oocytes with 1st polar body were inseminated *in vitro*. Sperm suspension was prepared by minutely cutting caudal epididymis of mature males in TYH medium for 1 hr. A small volume of the sperm suspension was introduced into 100 μl droplets of TYH medium, so that the final concentration of spermatozoa was adjusted to 2×10^6/ml. The denuded oocytes were introduced into the droplets of sperm suspension and cultured for 6 hrs at 37 ℃ in a CO_2 incubator. After cultured with spermatozoa, inseminated oocytes were observed under a phase contrast microscope. The oocytes including 2 or more pronuclei in their cytoplasm were judged to be fertilized ones, and those including 3 or more pronuclei were judged to be polyspermic ones.

Statistical analysis

The size of each part including perivitelline space was statistically analyzed by One-way analysis of variance (ANOVA). The rate of nuclear maturation in cultured

Table 1. The rate of maturation and the size of each part in mouse oocytes treated with tunicamycin

Treatment	No. of oocytes cultured	No. and (%) of oocytes matured	Outer diameter of zona pellucida (μm)	Diameter of oocyte (μm)	Thickness of zona pellucida (μm)	Size of perivitelline space (μm)
None	63	55(87.3) [a]	94.6 ± 0.4 [a] *	66.9 ± 0.5 [a]	8.4 ± 0.1 [a]	5.4 ± 0.3 [a]
Tunicamycin	32	31(96.9) [a]	94.2 ± 0.6 [a]	67.6 ± 0.5 [a]	8.8 ± 0.2 [a]	4.5 ± 0.2 [b]

* Mean ± S.E.

Values with different superscripts in the same column are significantly different ($P < 0.05$).

Table 2. The incidence of polyspermy in mouse oocytes treated with tunicamycin

Treatment	No.of oocytes inseminated	No. and (%) of oocytes fertilized	No. and (%) of monospermic oocytes	No. and (%) of polyspermic oocytes
None	128	118(92.2) [a]	50(42.4) [a]	68(57.6) [b]
Tunicamycin	47	43(91.5) [a]	9(20.9) [b]	34(79.1) [a]

Values with different superscripts in the same column are significantly different ($P < 0.05$).

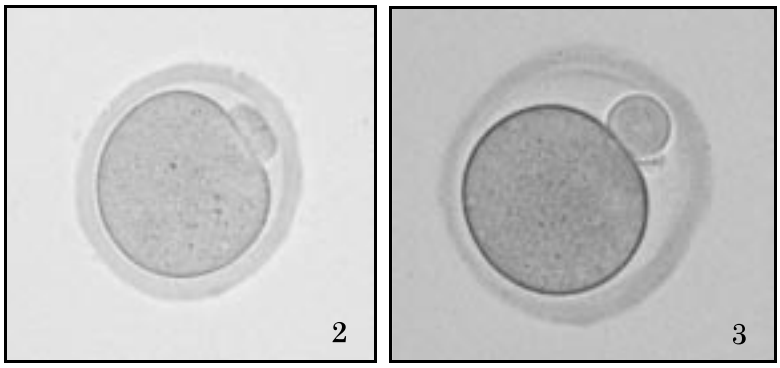

Fig.2. A small perivitelline space of mouse oocyte cultured for 14 hrs in TYH medium containing tunicamycin.
Fig.3. A large perivitelline space of mouse oocyte cultured for 14 hrs in TYH medium.

oocytes, and the rates of fertilization and polyspermy in oocytes following insemination were statistically analyzed by Chi-square test.

RESULTS

Size of each part in cultured mouse oocytes

The size of each part in mouse oocytes cultured for 14 hrs in TYH medium with and without tunicamycin was shown in **Table 1**. Outer diameter of zonae pellucidae, thickness of zonae pellucidae and diameter of oocytes matured in TYH medium containing tunicamycin were 94.2, 8.8 and 67.6 μm, respectively, which were similar to those of control oocytes matured in TYH medium without tunicamycin. The size of perivitelline space was significantly smaller in oocytes matured in TYH medium containing tunicamycin (4.5 μm, **Fig.2**) than in control oocytes matured in TYH medium (5.4 μm, **Fig.3**).

As shown in **Table 1**, the maturation rate of oocytes matured in TYH medium containing tunicamycin was 96.9 %, showing no significant difference from 87.3 % of control oocytes matured in TYH medium.

Incidence of polyspermy in cultured mouse oocytes

The rate of fertilization and the incidence of polyspermy in mouse oocytes matured in TYH medium with and without tunicamycin were shown in **Table 2**. Although no significant difference was observed in the fertilization rate between oocytes matured in TYH medium with tunicamycin (91.5 %) and control oocytes matured in TYH medium without tunicamycin (92.2 %), the incidence of polyspermy was significantly higher in oocytes matured in TYH medium containing tunicamycin (79.1 %) than in control oocytes matured in TYH medium (57.6 %).

DISCUSSION

It has been reported that porcine oocytes matured in Whitten medium with decreased concentration of NaCl (low NaCl-Whitten medium) had significantly larger perivitelline space, compared with that of oocytes matured in Whitten medium (Funahashi *et al*., 1994). It has also been reported that the incidence of polyspermy in porcine oocytes matured in low NaCl-Whitten medium was significantly lower than that in those matured in Whitten medium (Funahashi *et al*., 1994). Furthermore, it has been known that the perivitelline space in porcine oocytes matured *in vivo* was larger and the incidence of polyspermy in those after insemination was lower than those in the oocytes matured *in vitro* (Wang *et al*., 1998). Therefore, it has been suggested that a close relationship between the size of perivitelline space and the incidence of polyspermy is present in porcine oocytes, although it is unclear whether this relationship exists in oocytes of another animals.

In the present study, it was confirmed that the size of perivitelline space in mouse oocytes treated with tunicamycin was smaller and the incidence of polyspermy in those after insemination was higher than those in the control oocytes matured in usual TYH medium.

From these findings, it was clarified in mouse oocytes that the higher incidence of polyspermy is closely related to the smaller perivitelline space.

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ツニカマイシン処置したマウス卵母細胞の囲卵腔の大きさと多精子侵入の頻度

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要　約

糖タンパク質合成の阻害剤であるツニカマイシンを含むTYH液で培養したマウスの卵丘卵母細胞複合体について、成熟後の卵母細胞の囲卵腔の大きさと媒精後の多精子侵入の頻度を観察し、囲卵腔の大きさと多精子侵入との間に関係があるのか否かを検討した。

囲卵腔は、対照のTYH液で培養して成熟させた卵母細胞では5.4μmあったが、ツニカマイシン処置した卵母細胞では有意に小さく、4.5μmであった。一方、媒精後の受精率は、ツニカマイシン処置した卵母細胞では91.5％であり、対照の卵母細胞の92.2％と相違なかったが、多精子侵入率は、ツニカマイシン処置した卵母細胞では79.1％であり、対照の卵母細胞の57.6％に比べて有意に高かった。

以上の結果から、囲卵腔が小さい卵母細胞では媒精後の多精子侵入の頻度が高いことが確かめられ、囲卵腔の大きさと多精子侵入の頻度との間には密接な関係のあることがマウスでも確認された。

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