In Vitro Analysis of Oocyte Cumulus Complex Pickup Rate in the Hamster Mesocricetus auratus

SUSAN HUANG, NATALIE DRIESSEN, MICHAEL KNOLL, AND P. TALBOT*
Department of Biology, University of California, Riverside, California

ABSTRACT In mammals, the oocyte and its surrounding cumulus cells constitute an oocyte cumulus complex (OCC). During ovulation, OCCs are extruded into the peritoneal or bursal cavity, depending on the species, and are then rapidly picked up by the fimbria on the outer surface of the oviductal infundibulum and transported to the ampulla, where fertilization occurs. We developed a method to measure OCC pickup rates quantitatively in vitro, and we used this method to evaluate the effects of viscosity and temperature on pickup rates. Hamster infundibula are placed in a holding pipette in a chamber modified to study OCC pickup. Ciliary beat frequencies (CBF) can be measured in the same preparation. Pickup rates vary depending on the pathway on which the OCC travels over the surface of the infundibulum; however, rates for a given pathway are very consistent. The average pickup rate at room temperature calculated from three different pathways/infundibulum was 55.2 ± 10.6 μm/sec. Both rates between infundibula from the same female and rates among infundibula from different females were in most cases similar. Preparations preincubated in vitro for 2.75 hr produced rates similar to nonpreincubated samples, while longer preincubation resulted in decreased rates. Inclusion of Ficoll in culture medium to increase viscosity caused a concentration-dependent decrease in both OCC pickup rate and CBF. However, a significant decrease in OCC pickup rate was only observed at viscosities higher than those found in bursal fluid. When trials were run at physiological temperature (36.4°C) rather than ambient temperature, rates increased to 136.7 ± 29.9 (SD) μm/sec. Linear regression analysis demonstrated a strong positive correlation (r = 0.94) between OCC pickup rate and temperature. The OCC pickup rate assay can be used experimentally, and should be valuable in evaluating factors that affect rate and in studies dealing with the mechanism of OCC pickup. Mol. Reprod. Dev. 47:312-322, 1997. © 1997 Wiley-Liss, Inc.

Key Words: oocyte cumulus complex; oviduct; gamete transport; cilia; hamster

INTRODUCTION
Ovulation results in expulsion of an oocyte cumulus complex (OCC) from a mature ovarian follicle (Harper, 1994; Talbot, 1991). The OCC is released into either the peritoneal or bursal cavity, depending on the species, and is quickly picked up by the fimbria on the outer surface on the infundibulum of the oviduct (Blandau, 1969). Both the inner and outer surface of the infundibulum are covered with cilia which are considered vital for picking up the OCC (Di Carlantonio et al., 1995; Nakatani et al., 1985). Pickup is followed by transport of the OCC into the ampulla of the oviduct, where fertilization occurs. Transport of the preimplantation embryo (PE) through the remainder of the ampulla and isthmus ultimately results in its delivery to the uterus for implantation. The processes of OCC pickup by the fimbria and transport by the ampulla and isthmus are crucial for normal reproduction; failure of pickup or transport to occur at the proper rate can result in infertility or ectopic implantation (Cummings and Perreault, 1990; Hafez, 1973).

Although oocyte and PE transport through the ampulla and isthmus of the oviduct have been studied extensively using OCCs, PEs, and microspheres as OCC or PE surrogates (Blandau and Verdugo, 1976; Bourdage and Halbert, 1988; Harper, 1994; Moore and Croxatto, 1988), relatively little is known about the process of OCC pickup by the infundibulum. Studies which have been done on pickup are generally descriptive (Blandau and Verdugo, 1976) or are based on a positive/negative evaluation of OCC pickup occurring (Blandau and Verdugo, 1976; Mahi-Brown and Yanagimachi, 1983). It would be useful to have a method to study OCC pickup quantitatively so that evaluations could be made on its rate, its mechanism, and the factors which affect it. The purpose of this study was to develop and evaluate a method for measuring OCC pickup rate in vitro under controlled experimental conditions. The method was then used to measure the effect of viscosity and temperature on OCC pickup rate. Hamster infundibula, which we previously showed were well-suited for in vitro studies (Di Carlantonio et al., 1995), were used to develop this method. Because the assay is performed in a chamber originally devel-
ophe to measure ciliary beat frequency (CBF) (Di Carlantonio et al., 1995), both OCC pickup rate and CBF can be studied in the same preparation.

MATERIALS AND METHODS

Animals

Female golden hamsters (Mesocricetus auratus), purchased from Harlan Sprague Dawley (San Diego, CA), were maintained on a 14:10 hr light:dark cycle (lights on 6 AM–8 PM) in a room at 26°C, as described previously (Magers et al., 1995). To promote cumulus expansion, females with stable estrous cycles were injected with 25 international units of human chorionic gonadotropin (hCG) on the evening of the third day of their estrous cycle. All pickup rate assays were done using infundibula from females on day 1 of estrus.

Media

Earle's balanced salt solution (EBSS) was made fresh daily from a 10× stock solution of inorganic salts. Sodium bicarbonate (26.2 mM) and HEPES (25 mM) were added to a single-strength salt solution to produce EBSS-H. EBSS-H was then enriched with 0.5% bovine serum albumen (BSA, fraction V, tissue culture-tested; Sigma Chemical Co., St. Louis, MO) to produce EBSS-HA, the medium used for dissection and analysis of OCC pickup rates. The pH of EBSS-HA was adjusted to 7.4 with NaOH at the beginning of each experiment and was used at 22–25°C. In some experiments, EBSS-HA was supplemented with 1%, 5%, or 10% Ficoll to increase its viscosity.

Dissection Protocols

About 1 hr before expected ovulation (12 hr after hCG injection), females were sacrificed with CO₂ gas, and the ovaries and oviducts were dissected and transferred to plastic petri dishes (Fisher or Falcon, San Francisco, CA) containing EBSS-HA. Using a Wild M5A dissecting microscope (Heersbrugg, Switzerland) equipped with a heat shield, the infundibulum, the ampulla, and several coils of isthmus were cut free with Vannas fine surgical scissors (Foster City, CA.) and transferred to a petri dish containing EBSS-HA for immediate use in the OCC pickup rate assay. The second oviduct was stored at room temperature in EBSS-HA until used.

After the ovaries were removed from the oviduct, mature follicles were poked with a small insect pin, and expanded OCCs were collected from the surface of follicles by pipette. OCCs were stained in 0.002% methylene blue in EBSS-HA for 3–5 min and then transferred to and stored in fresh EBSS-HA until subsequently used to measure OCC pickup rates. Preliminary trials established that OCC pickup was not affected by this staining protocol.

The Chamber

Organ culture dishes (Falcon #3037), containing a central well (0.8 × 75 mm) which holds about 1 ml of

![Fig. 1. Schematic diagram showing the chamber used to measure OCC pickup rates and CBF. To measure pickup rates, OCCs are placed on the infundibulum adjacent to the holding pipette, and the time required for the OCC to move along a particular pathway to the ostium is measured. The chamber also contains an inlet and outlet pipette (only one shown) for perfusion of media. Incubations are done in EBSS-HA covered with oil.](image-url)
EBSS-HA, were modified for OCC pickup assays (Fig. 1). Chambers were fitted with three glass tubes; one tube served as the holding pipette for the infundibulum, while the other two were used for perfusion of test solutions (Di Carlantonio et al., 1995). The oviduct was cut between the ampulla and isthmus, and the cut end of the ampulla was drawn carefully into the holding pipette so that the ciliated epithelium on the outer surface of the infundibulum remained free in the well filled with EBSS-HA. The EBSS-HA in the central chamber was covered with paraffin oil to prevent evaporation. Each chamber was used only once and then discarded.

**Analysis of OCC Pickup Rates**

After staining in methylene blue, a mouth pipette was used to pick up one OCC and position it on the infundibulum near the edge of the holding pipette. OCCs of similar sizes were used in all trials. OCC pickup rates (µm/sec) were determined by measuring the time required for an OCC to travel over the surface of the fimbria from the base of the infundibulum to the ostium. This distance was measured using a calibrated ocular micrometer. The OCC was recovered at the ostium with a mouth pipette and reused. At least 6 repetitions were done for each infundibulum. All experiments were done at ambient temperature (22–25°C) unless otherwise indicated. In some experiments, 8 ml of a Ficoll solution were perfused through the chamber to evaluate the effect of viscosity on OCC pickup rate. Ficoll was used to increase viscosity because it is a highly soluble, relatively inert polymer that has been used successfully with gamete studies (Suarez et al., 1991).

The viscosity of bursal fluid was determined by comparing the rate at which Lycopodium spores traveled through a column of bursal fluid to rates obtained in Ficoll solutions of known viscosity. Bursal fluid was collected from females on day 1 of their estrous cycle using a capillary pipette. About 25–50 µl could be collected from each bursal cavity. The capillary pipette was mounted vertically and viewed with a dissecting microscope, and the rate at which Lycopodium spores dropped through the fluid was determined and compared to rates obtained in fluids of known viscosity.

**Analysis of Ciliary Beat Frequency**

The modified organ culture chambers were also used to measure CBF in some experiments. Infundibula were positioned in the holding pipette as described above. CBFs were determined using a Nikon Diaphot 200 microscope.
inverted microscope (Melville, N.Y.) interfaced with the brightness vs. time program in Image 1 (Universal Imaging, West Chester, PA) as described previously (Di Carlantonio et al., 1995).

Statistical Analyses

In experiments involving the comparison of two groups, Student's t-test was used. In experiments with more than two groups, data were analyzed by a one-factor analysis of variance (ANOVA) followed by Dunnett's post hoc test. All data were examined to verify that they satisfied the assumptions of ANOVA. Analysis of OCC pickup rates for data from different females (Fig. 3) was done using the Kruskal-Wallis nonparametric test followed by Dunn's post hoc test, as these data did not satisfy the test for homogeneity of variances. Analyses were done using either Statistica (StatSoft, Tulsa, OK) or DOS Instat (GraphPad, San Diego, CA). Means were considered significantly different when \( P < 0.05 \).

Results

OCCs travel along particular pathways on the surface of the infundibulum, depending on where they are placed initially during the assay. The pathways are well-defined and may be used repeatedly. In the first experiment, OCC pickup rates were compared on three different pathways over a 14-min interval. Figure 2 shows OCC pickup rates on three pathways in a representative experiment. Rates did not change on a particular pathway over the 14-min interval; however, rates did vary among pathways. Average rates for paths in Figure 2 are \( A = 40.9 \mu m/sec (\pm 1.4 \text{ SD}) \), \( B = 69.1 \mu m/sec (\pm 2.8 \text{ SD}) \), \( C = 54.3 \mu m/sec (\pm 4.4 \text{ SD}) \). Other infundibula (7 in all) showed similar differences in OCC pickup rates among pathways; however, there was no consistent correlation between rate and position of pathway.

To determine if variation exists in OCC pickup rate between oviducts from the same female, average rates based on 18 rate measurements (3 pathways and 6 measurements/pathway) were obtained for each infundibulum from 8 females (Fig. 3). Pickup rates from the two infundibula within a female were in most cases not significantly different (\( P > 0.05 \)), females 2 (\( P < 0.0001 \)) and 5 (\( P = 0.0001 \)) being exceptions (Fig. 3). Moreover, average pickup rates were similar between females, except for females 1 and 2 which had rates significantly lower (\( P < 0.05 \)) than the other 6 females. The overall average pickup rate based on all data in Figure 3 is \( 55.2 \pm 10.6 \mu m/sec \).

To determine if infundibula can continue to successfully pick up OCCs after prolonged incubation in vitro, rates were taken after 0.75–1.75, 2.0–2.75, or 3.0–4.0 hr of preincubation in EBSS-HA (Fig. 4). Rates collected after 1.75 or 2.75 hr of preincubation were not signifi-
significantly different from each other \((P = 0.12)\). However, after 3.0–4.0 hr of preincubation, rates were significantly slower than after 1.75 hr of preincubation \((P = 0.006)\), and in several cases pickup did not occur. In all subsequent experiments, rates were measured within the first 2.75 hr of in vitro incubation.

To determine if using different cumuli of the same size on the same path affects the OCC pickup rate, four cumuli of similar (typical) size were run on a single infundibulum, on the same pathway. The mean OCC pickup rates (based on \(N = 24\) for each OCC) were as follows: 47.6 \(\mu\)m/sec \((\pm 3.2\ SD)\), 46.8 \(\mu\)m/sec \((\pm 3.0\ SD)\), 46.1 \(\mu\)m/sec \((\pm 2.5\ SD)\), and 46.2 \(\mu\)m/sec \((\pm 3.0\ SD)\). These means are not significantly different from each other \((P = 0.32)\), indicating that different OCCs may be used to measure rates on a particular pathway.

To determine the optimal number of trials needed to obtain reliable OCC pickup rates in EBSS-HA, average rates were measured for different numbers of trials \((6, 8, 10, 14, 16, 18, 20,\) and \(22)\) on the same pathway (Fig. 5). Average rates were not different, as the number of trials used to calculate rate increased. These results show that determining OCC pickup rate by averaging 6 trials for a particular pathway gives a reliable estimate of OCC pickup rate for that pathway in control medium (EBSS-HA), and that taking additional measurements does not improve the accuracy of the measurement.

The above observations indicated that the in vitro procedure could be used to assay OCC pickup rates, provided control and experimental data are collected on the same pathway. To evaluate the assay's performance experimentally, the effect of viscosity on OCC pickup rate was examined by using Ficoll to increase the viscosity of EBSS-HA. Infundibula were incubated first in EBSS-HA, followed by sequential incubation in increasing concentrations of Ficoll. In each experimental setting, OCC pickup rates were measured over a 10–14-min interval. Figure 6 shows a representative experiment. Increasing concentrations of Ficoll caused an immediate and sustained decrease in OCC pickup rates. When Ficoll was washed out with EBSS-HA, pickup rates returned to control levels immediately. Fourteen experiments similar to that shown in Figure 6 were performed, and comparison of the average data from the 10-min time point shows that rates are significantly decreased by both 5% and 10% Ficoll when compared to the control (Fig. 7). The combined data also show a return to control values when 10% Ficoll is
replaced with EBSS-HA. To determine if the decrease in pickup rate produced by Ficoll correlated with an effect on the cilia, CBF was measured in EBSS-HA and in EBSS-HA containing 10% Ficoll (Fig. 8). CBF, like OCC pickup rate, was significantly (P < 0.0001) decreased by 10% Ficoll. The viscosity of hamster bursal fluid was measured and found to be equivalent to a 4% Ficoll solution.

All the above experiments were done at ambient temperature (22–25°C). Since CBF, which influences OCC pickup rate, is affected by temperature (Holloway et al., 1988), an experiment was done to determine OCC pickup rate at physiological temperature (Fig. 9). The rate was first determined at 20.9°C for a particular pathway. Temperature, when elevated to 36.4°C, produced a significant increase in pickup rate on the same pathway. The rate returned to control values when temperature was lowered to 22.4°C. Linear regression analysis of the temperature data gave a very strong correlation between temperature and OCC pickup rate (r = 0.94) (Fig. 9).

**DISCUSSION**

The oviduct is critical for very early mammalian life, including that of humans (Puikkinen, 1995). The primary functions of the oviduct are gamete pickup and transport, and provision of a suitable milieu for fertilization and preimplantation development. It is becoming increasingly clear that interactions between the oviduct and gametes are incompletely understood (Nancarrow and Hill, 1995), in part because these processes have been difficult to study in vitro.

We previously described a method for measuring CBF in oviductal explants from hamsters (Di Carlantonio et al., 1995). We now report that it is feasible to measure OCC pickup rates in vitro using the perfusion chamber originally developed for CBF measurements. The pickup rate assay gives a more direct measurement of the normal biological functioning of the oviductal infundibulum, and it can be coupled to CBF measurements in the same preparation.

In developing the OCC pickup rate assay, we learned that the OCC travels along a particular pathway depending on where it is placed on the surface of the infundibulum. This is probably due to the fact that the OCC adheres to the tips of the cilia during pickup (Mahi-Brown and Yanagimachi, 1983; Norwood and Anderson, 1980); thus, once it touches down on the infundibular surface, it is committed to travel along a certain pathway of cilia. It is important in performing the pickup rate assay to use the same pathway when comparing control and experimental conditions, as rates may vary among pathways. However, it is easy to select and reuse a pathway when OCCs are positioned on the infundibulum using a capillary tube.

The assay should be performed with OCCs of the same size. Partial OCCs or fragments of OCCs may travel at different rates than whole, intact OCCs and...
should be avoided if comparisons of data are to be made. We found that removing OCCs from mature follicles of hCG-primed females consistently gives OCCs of uniform size. Since explants do show signs of aging after 3.0–4.0 hr of in vitro incubation, we collect data within the first 2.5 hr of in vitro culture.

Although a large number of trials can be performed rapidly on a single infundibulum incubated in EBSS-HA, statistical analyses have shown that means generated from 6 trials per pathway provide a reliable estimate of OCC pickup rate on that pathway. Solutions can readily be perfused through the chamber, making it easy to compare different experimental conditions. The chamber used for the assay is inexpensive, is easily made by hand, and is used only once and then discarded.

The OCC pickup rate assay specifically measures the role of ciliary beating and adhesion of the OCC to the tips of the cilia in pickup. These two factors are known to be important in successful transfer of the OCC from the ovary to the oviduct (Halbert et al., 1976; Norwood and Anderson, 1980; Norwood et al., 1978). Norwood et al. (1978) were the first to show that polycationic compounds could block OCC pickup without affecting CBF; they presumed that the polycations interfered with cilia-mediated pickup by blocking the formation of transient adhesive bonds between the tips of the cilia and elements of the OCC. Mahi-Brown and Yanagimachi (1983) showed that cumulus-free oocytes are not efficiently picked up by the infundibulum in hamsters, apparently because they do not adhere well to the cilia without the presence of cumulus cells or cumulus matrix. Likewise, in humans, peritoneal fluid from women with endometriosis contains a factor, thought to be a macromolecule >100,000 kD, which in in vitro experiments has been shown to coat the surface of hamster cilia on the fimbria, thereby blocking adhesion of the OCC to the cilia and consequently preventing OCC pickup (Suginami and Yano, 1988; Suginami et al., 1986). Data in our study show that when adhesion is normal, alterations in CBF correlate with increased or decreased rates of OCC pickup. While contraction of the oviductal smooth muscles may play a role in gamete transport in the ampulla and isthmus (Harper, 1994), muscle contraction is probably not important in OCC pickup (Harper, 1994). In rats, movement of surrogate ova across the fimbria was smooth, in the direction of the ostium, and of constant rate in the presence or absence of smooth muscle cell contraction (Halbert et al., 1989). In our experiments, muscles in the infundibulum

**Fig. 6.** Representative experiment showing OCC pickup rate plotted over time at different concentrations of Ficoll. Infundibula were first incubated in control medium (EBSS-HA), and pickup rates were determined over a 14-min interval. The chamber was then flushed with 8 ml of 1% Ficoll, and rates were again measured. This was repeated with 5% and 10% Ficoll and finally with EBSS-HA. Rate decreases as Ficoll concentration increases. When 10% Ficoll is washed out of the culture dish and replaced with EBSS-HA, pickup rate returns to control values.
Lum exhibited variable degrees of contraction, which did not appear to influence movement of the OCC across the fimbriae.

Slight variations in OCC pickup rate were observed within and between female hamsters. This probably represents normal biological variation. For any given path on a particular oviduct, the rates were highly reproducible.

The effects of viscosity on OCC pickup rate are of interest, since viscosity is known to alter CBF in other tissues such as the respiratory system (Wilson, 1988) and could therefore indirectly alter pickup rates. In vivo, OCC pickup in hamsters occurs in the bursal fluid, which we found to be more viscous than EBSS-HA. However, since bursal fluid has a viscosity equivalent of about 4% Ficoll in EBSS-HA, and since OCC pickup rate was not affected significantly until 5% Ficoll was used, we routinely ran our assays in EBSS-HA without a Ficoll addition. Four percent Ficoll could, however, be included to produce a viscosity similar to that in bursal fluid. The decrease in OCC pickup rate observed in solutions containing 5% and 10% Ficoll was apparently due to a decrease in CBF in these higher-viscosity solutions. Increases in viscosity in the respiratory system are likewise known to decrease CBF (Wilson, 1988). We did not see any evidence that adhesion of the OCC to the ciliary tips was affected by 5% or 10% Ficoll.

Most of our experiments were performed at ambient temperature. CBF, which influences OCC pickup rates, is very sensitive to temperature and increases dramatically at 37°C (Holloway et al., 1988). In our temperature experiment, the average rate for OCC pickup increased significantly at 36.4°C to 136.7 µm/sec. Our value for OCC pickup rate in hamsters at physiological temperature is similar to the rate of transport (110–120 µm/sec) through the rabbit ampulla in the presence or absence of muscle contractions (Halbert et al., 1976); however, the hamster OCC pickup rate is considerably faster than the rate obtained for OCC transport through the rat ampulla (30–40 µm/sec) (Halbert et al., 1989). These comparisons, however, must be interpreted cautiously, since the hamster rates are based only on pickup, i.e., movement of the OCC over the surface of the fimbria, while the rabbit and rat rates include transport of the OCC within the lumen of the ampulla. In all species, nevertheless, pickup proceeds rapidly and efficiently, thereby assuring delivery of the OCC to the ampulla for fertilization.

The strong positive linear correlation observed for temperature and pickup rate is probably due to an acceleration of CBF at higher
**Fig. 8.** Effect of 10% Ficoll on CBF. CBF was measured first in control medium and then in 10% Ficoll. Each bar is the mean ± SD of seven experiments. Ficoll causes a significant decrease in CBF ($P < 0.0001$).

**Fig. 9.** Effect of temperature on OCC pickup rate. Infundibula were first incubated at 20.9°C, then raised to 36.4°C, and then returned to 22.4°C. OCC pickup rate increased significantly at 36.4°C and returned to starting values when temperature was decreased. Each bar is the mean ± SD of six experiments.
temperatures. Similar correlations have been reported by others to exist for CBF and temperature (Holloway et al., 1988).

We previously showed that both mainstream and sidestream smoke solutions decrease CBF of hamster oviducts in vitro (Knoll et al., 1995). We are currently using the OCC pickup rate assay to determine if these smoke solutions result in a similar decrease in pickup rate in vitro and if the effects of smoke can be reversed (Knoll and Talbot, 1996). The OCC pickup rate assay may have other applications in toxicity testing and in studies directed at understanding the mechanisms of OCC pickup in mammals.

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REFERENCES


Fig. 10. Relationship between OCC pickup rate and temperature. Linear regression analysis of the temperature data shows a strong positive correlation (r = 0.94) between temperature and OCC pickup rate.