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Assay and Importance of Adhesive Interaction Between Hamster (*Mesocricetus auratus*) Oocyte-Cumulus Complexes and the Oviductal Epithelium[1]

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ABSTRACT

Adhesion between the oocyte-cumulus complex and infundibulum plays an important, but poorly understood, role in oocyte pick-up. The purposes of this study were to determine which components of the oocyte-cumulus complex and oviductal epithelium function in adhesion, to measure adhesion under physiological conditions, and to examine the effect of modulation of adhesion on oocyte-cumulus complex pick-up rate. Oocyte-cumulus complexes containing an expanded matrix were readily transported into the oviduct, while unexpanded complexes lacking an extracellular matrix were not picked up, indicating that the matrix is necessary for pick-up. Transmission electron microscopy revealed that during pick-up, adhesion occurred specifically between the ciliary crowns of the oviduct and the granules and filaments of the cumulus matrix. An assay was developed using vacuum from a low-flow peristaltic precision pump, modified for bi-directional flow, to measure the strength of adhesion between the oocyte-cumulus complex and the oviductal epithelium, and adhesion was measured during physiological conditions. The lectin wheat germ agglutinin and the polycation poly-L-lysine were then used to modulate adhesion, and the effects of increasing or decreasing adhesion on oocyte pick-up rate and ciliary beat frequency were examined. The data show that 1) the matrix of the oocyte-cumulus complex and the ciliary crowns of the oviduct function in adhesion during pick-up and that adhesion is necessary for pick-up, 2) adhesion can be assayed quantitatively and is very uniform among control infundibula, and 3) decreasing or increasing adhesion decreases oocyte pick-up rate and in some cases prevents pick-up without affecting ciliary beat frequency.

INTRODUCTION

Mammalian oocyte-cumulus complexes (OCCs) contain an oocyte surrounded by the zona pellucida, corona radiata cells, cumulus cells, and an extensive extracellular matrix [1]. OCCs are ovulated into the peritoneal cavity of humans and into the bursal cavity of rodents [1–3]. Once contact is made between an OCC and the infundibulum of the oviduct, the ciliated cells of the oviductal epithelium transfer the OCCs to the ampulla, where fertilization occurs [3–5]. Pick-up of OCCs by the oviductal infundibulum is the first in an important series of gamete transport events that assure entry of embryos into the uterus at the proper time. In women, disruption of pick-up and transport of the OCC can produce infertility and ectopic pregnancies [3].

Oocyte pick-up is a complex process involving both ciliary beating and adhesion between the oviductal epithelium and the OCC [2, 6–10]. Cilia provide the motive force that moves OCCs over the surface of the infundibulum and into the ostium of the oviduct. Factors that affect ciliary beat frequency are known to alter OCC pick-up rate. For example, in vitro experiments have shown that increasing the viscosity of the culture medium decreases both beat frequency and pick-up rate, while increasing the temperature increases beat frequency and pick-up rate [5]. In addition, cyanide, which is present in cigarette smoke, can inhibit ciliary beating and prevent OCC pick-up [11].

Adhesion between the OCC and infundibular epithelium plays an essential role in OCC pick-up [8, 10, 12]. Fluid currents established by ciliary beating can move small objects, such as *Lycopodium* spores, over the infundibular surface [7, 10], but the large mass of the OCC precludes its movement in these currents. Adhesion is necessary to transiently anchor the OCC to the oviductal epithelium, thus enabling the beating cilia to pass the OCC over the infundibular surface without ever losing contact with the OCC. This mechanism of pick-up may have evolved to enable relatively small motors (the cilia) to move a large object (the OCC) in a vectorial manner. Adhesion between the OCC and oviduct is specific. OCCs do not stick well to other surfaces, and other surfaces do not stick well to oviductal cilia. Removal of the OCC matrix with hyaluronidase prevents oocyte pick-up, apparently because the zona pellucida per se is not able to adhere to the oviductal epithelium [8]. QuickTime movies showing adhesive interaction during OCC pick-up by hamster oviducts are available for viewing on the Internet [10].

In spite of the importance of adhesion in oocyte pick-up, little is known about the structural components of the OCC and the oviductal epithelium that participate in adhesion, the strength of adhesion, or the ways in which factors that alter adhesion affect OCC pick-up. The adhesive partner on the infundibular surface could be the secretory cell plasma membranes, the entire ciliary membrane, or a restricted area of the ciliary membrane, such as the ciliary crown. Within the OCC, either the cells or the matrix or both could adhere to the oviductal epithelium, and if the matrix is involved, adhesion could occur via either hyaluronic acid [13] or matrix proteins [14–16]. Prior experiments suggest that altering adhesion affects OCC pick-up [7, 8], but no assay has existed to quantitatively measure adhesion between the OCC and infundibulum, nor have quantitative studies been done to show how altering adhesion changes pick-up. The purposes of this study were 1) to determine which components of the OCC and the oviductal epithelium function in adhesion during OCC pick-up, 2) to develop an assay to measure adhesion between the OCC and infundibulum, and 3) to determine how experimental modulation of adhesion alters OCC pick-up.

MATERIALS AND METHODS

Animals

Female golden hamsters (*Mesocricetus auratus*), purchased from Harlan Sprague Dawley (San Diego, CA) at

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9–12 wk of age, were maintained on a 14L:10D cycle in a room at 26°C. No more than four females were grouped in a cage. Purina rodent chow and water were provided ad libitum. Females were evaluated in the morning for the presence of a vaginal discharge (Day 1 of estrus) and grouped according to the day of their four-day estrous cycle [17]. In the first experiment, females received injections of 25 IU of eCG on the morning of Day 1 of the estrous cycle, and unexpanded OCCs were harvested from follicles on Day 4 of the cycle [18]. For pick-up rate assays, females received injections of 25 IU of hCG on the evening of Day 3 of the estrous cycle to induce maturation of ovarian follicles. The following day, oviducts and OCCs were harvested for pick-up rate assays. All OCCs used in pick-up rate assays were collected from mature ovarian follicles.

Media, Chemicals, and Supplies

OCC pick-up rate assays, adhesion assays, and ciliary beat frequency assays were done in Earle's balanced salt solution (EBSS-H) [19] containing 26.2 mM sodium bicarbonate and 25 mM HEPES. Culture medium was prepared daily from a 10-strength stock of salts and supplemented with 0.1% BSA (EBSS-HA) [11]. The pH of all solutions was adjusted to 7.4 with NaOH, and pH remained stable throughout the period of the experiments (3–4 h). Methylene blue was diluted from a 60-strength stock solution and filtered immediately before use. All experiments were done at ambient temperature to facilitate performance of the beat frequency and pick-up rate assays. The influence of temperature on these assays has been described previously [5,20].

Methylene blue was purchased from Eastman Kodak (Rochester, NY). Glutaraldehyde, osmium tetroxide, cacodylate, lead nitrate, uranyl acetate, and Spurr's plastic were obtained from Electron Microscopy Supplies (Fort Washington, PA). Chemicals and supplies used for scanning electron microscopy (SEM) were obtained from Ted Pella Inc. (Tustin, CA). Poly-L-lysine, eCG, and hCG were obtained from Sigma Chemical Company (St. Louis, MO). Wheat germ agglutinin (WGA) was purchased from Vector Laboratories (Burlingame, CA).

Light and Electron Microscopy

Unexpanded and expanded OCCs were videotaped using a system described previously [10]. For electron microscopy, infundibula with OCCs attached to their outer surfaces were fixed in cacodylate-buffered 1% osmium tetroxide (pH 7.4) containing 0.5% ruthenium red to stabilize the cumulus matrix for 30–60 min, and then washed three times in 0.1 M cacodylate buffer. Samples were fixed for 1.5 h in 3% glutaraldehyde (pH 7.4) containing 0.5% ruthenium red, washed three times in deionized water, dehydrated in an ethanol series, and further processed for either SEM or transmission electron microscopy (TEM). For SEM, samples were dried from liquid CO_2 in a Samdri PVT-3 critical point drier (Tousimis Research Corp., Rockville, MD), mounted on aluminum pedestals, coated with gold/palladium in a Technics (Alexandria, VA) Hummer II or an EMscope (International Electron Optics, Inc., Houston, TX) coater, and examined in a Philips (Eindhoven, The Netherlands) FG30 SEM. Video prints were made using a Sony (Carson, CA) printer, and digital images were stored on ZIP disks. For TEM, tissue was infiltrated and embedded in Spurr's plastic and then cut on a Sorval MT2B ultramicrotome using glass or diamond knives. Thin sections were either left unstained or were poststained with uranyl acetate and lead citrate, then examined in a Hitachi (Tokyo, Japan) H-500 TEM.

Measurement of OCC Adhesion to the Oviductal Cilia

To measure the adhesion of OCCs to oviductal cilia on the outer surface of the infundibulum, an assay was developed using a vacuum from a low-flow peristaltic pump (model P720) modified for bi-directional flow (Instech Laboratories Inc., Plymouth Meeting, PA). The pump was calibrated before use according to the manufacturer's recommendations. Polyethylene (0.157-cm i.d.) was attached to BS-062S-BS connectors (Instech) on the pump and was adapted to hold a 50-μl Microcap pipette (Drummond Scientific Company, Broomall, PA) used for placement and retrieval of the OCCs. The adhesion assay was run in conjunction with the OCC pick-up rate assay. OCCs of comparable size and appearance were selected for each adhesion test. The infundibulum was mounted in the same manner as described for the OCC pick-up rate assay [21]. For each assay, an OCC was gently placed on an infundibulum using the pump set at a flow rate of 0.023 ml/min; then the OCC was allowed to begin pick-up. Once the OCC had traveled a distance of 0.4 mm, adhesion measurements were taken in the following manner. The micropipette attached to the pump was placed flush with the OCC in a position perpendicular to the infundibular surface to which an OCC was attached. This positioning allowed the OCC to be retrieved with minimal contact and prevented further pick-up. The pump, preset to a flow rate of 0.009 ml/min or lower, was turned on, and suction was gradually increased by turning the dial speed until the OCC was completely suctioned off of the infundibulum. The flow rate at which the OCC was removed was recorded and used as a method of quantifying adhesion. For each assay, six measurements were made using the same OCC.

OCC Pick-Up Rates

OCC pick-up rate was assayed in vitro using a procedure described in detail previously [5]. OCCs were recovered from mature ovarian follicles, stained with methylene blue, washed thoroughly in 3 ml of EBSS-HA, and used to assay OCC pick-up rate at room temperature. In some experiments, OCCs were used without methylene blue staining. Infundibula were mounted in modified culture chambers (Falcon #3037; Fisher Scientific, Pittsburgh, PA), and within 10 min, control readings of pick-up rate were made using fresh OCCs, which were placed on the surface of the infundibulum using a 50-μl Microcap pipette and Instech pump as described above.

Ciliary Beat Frequency Assays

Ciliary beat frequency was assayed using a method described in detail previously [20, 21]. In brief, a Nikon (Melville, NY) Diaphot inverted microscope was used to image cilia. A video image of beating cilia was sent to a computer, and brightness-over-time data were collected using Image 1 (Universal Imaging, Fort Washington, PA). Data were filtered and subjected to Fourier transformation, and the highest-amplitude peak was interpreted to be the dominant beat frequency. For each infundibulum, 4–6 measurements were made and the average beat frequency was computed.

Effect of Modulation of Adhesion on OCC Pick-Up Rate

In some experiments, OCCs or infundibula were pretreated with the lectin WGA or the polycation poly-L-lysine

to modulate their adhesiveness, and the effects of modulation were then measured using the adhesion and OCC pick-up rate assays. Changes in adhesion were correlated with OCC pick-up rate and ciliary beat frequency in the same experiment. In the first experiment, OCCs, infundibula, or both OCCs and infundibula were preincubated in 0.1 mg/ml of WGA in EBSS-HA for 15 min, thoroughly washed in EBSS-HA, and used to measure adhesion, oocyte pick-up rate, and ciliary beat frequency. In the second experiment, OCCs were preincubated in either 0.001% or 0.01% poly-L-lysine and infundibula were preincubated in 0.01% poly-L-lysine for 15 min; then they were washed thoroughly in EBSS-HA and used to assay adhesion, oocyte pick-up rate, and ciliary beat frequency.

Statistical Analysis of Data

To compare means for control adhesion measurements on eight different infundibula, data were analyzed by the Kruskal-Wallis nonparametric test followed by Dunn's post hoc test. The effects of WGA and poly-L-lysine on adhesion were analyzed in a similar manner except that Dunn's post hoc test comparisons were made only to the control group. All data were examined to verify that they satisfied the assumptions of ANOVA. Analyses were done using either Statistica (StatSoft, Tulsa, OK) or Instat (GraphPad, San Diego, CA). Means were considered significantly different when $P < 0.05$.

RESULTS

What Parts of the OCC Adhere to the Oviductal Epithelium?

Both cells and extracellular matrix are present at the periphery of OCCs, and either could potentially bind to the oviductal epithelium. To determine whether cumulus cells adhere to the oviductal epithelium, unexpanded OCCs were collected from ovarian follicles before secretion of the cumulus matrix. When placed on infundibula using our in vitro set-up for assaying OCC pick-up rate, unexpanded OCCs failed to adhere to the oviduct and were not transported to the ostium. They either sat stationary on the surface of the infundibulum or rolled off onto the bottom of the culture dish. Unexpanded OCCs appeared to be too large to be transported by ciliary currents in the absence of adhesion. Some unexpanded OCCs were placed directly in the ostium and allowed to incubate for 60 min. Even though ciliary beating was normal, these OCCs did not enter the ostium (Fig. 1, A–C).

When expanded OCCs were used in the same experiment, OCCs attached to the surface of the infundibulum, and pick-up occurred normally (Fig. 1, D–F). Movement of the OCC over the infundibular surface usually required about 10 sec. Expanded OCCs were able to pass through the ostium into the lumen of the infundibulum in 3–5 min (not shown). These experiments indicated that the matrix, but not the cells, of the OCC adhere to the oviduct and that adhesion of the matrix to the epithelium is necessary for successful pick-up and transport through the ostium.

Extracellular Matrix of the OCC and its Interaction with the Oviductal Epithelium

The oviductal epithelium is composed of ciliated and secretory cells. The matrix of the OCC is composed of hyaluronic acid and proteins that appear, respectively, as filaments and granules when fixed for electron microscopy in the presence of ruthenium red [1, 22]. To determine

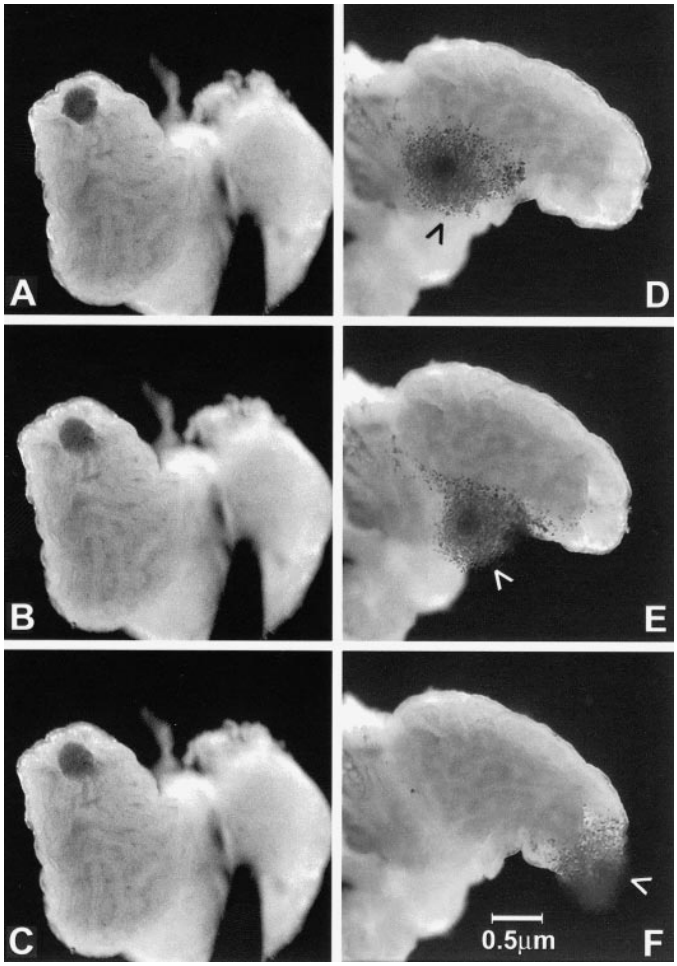


FIG. 1. Video images of unexpanded (**A–C**) and expanded (**D–F**) OCCs interacting with infundibula. The unexpanded OCC shown in **A** did not adhere to the surface of the infundibulum and did not undergo normal pick-up. In **A–C,** an unexpanded OCC is shown 0, 3, and 6 min after being placed in the ostium. The position of this unexpanded OCC did not change in **A–C** even after an additional 55 min of incubation. In contrast, the presence of the cumulus matrix enabled the expanded OCC in **D–F** to adhere to the infundibular surface and move over its surface in about 10 sec. Several additional minutes were required for the expanded OCC to become compacted and completely enter the oviduct.

which components of the oviductal epithelium and OCC function in adhesion, infundibula with attached OCCs were fixed for electron microscopy in the presence of ruthenium red. The extracellular matrix of the OCC constituted the most peripheral structure of the OCC; all cells at the periphery were covered by at least a thin layer of matrix. Thin sections through OCCs attached to the outer surface of the infundibulum revealed that adhesion occurred between the extracellular matrix of the OCC and the tips of the cilia (Fig. 2A). The tips of the cilia possess a ''crown'' characterized by a thick electron-dense plasma membrane and a robust glycocalyx (Fig. 2B). Adhesion specifically involved the ciliary crowns and both the granules and filaments of the extracellular matrix present between cumulus cells (Fig. 2, C and D). Adhesion was not limited exclusively to the crown, as occasionally elements of the matrix attached along the lateral surface of cilia just below the crown (Fig. 2D). Nevertheless, the matrix, in general, did not penetrate deeply between cilia, and the greatest interaction was observed in the region of the crown.

Some infundibula were fixed for SEM after OCCs had

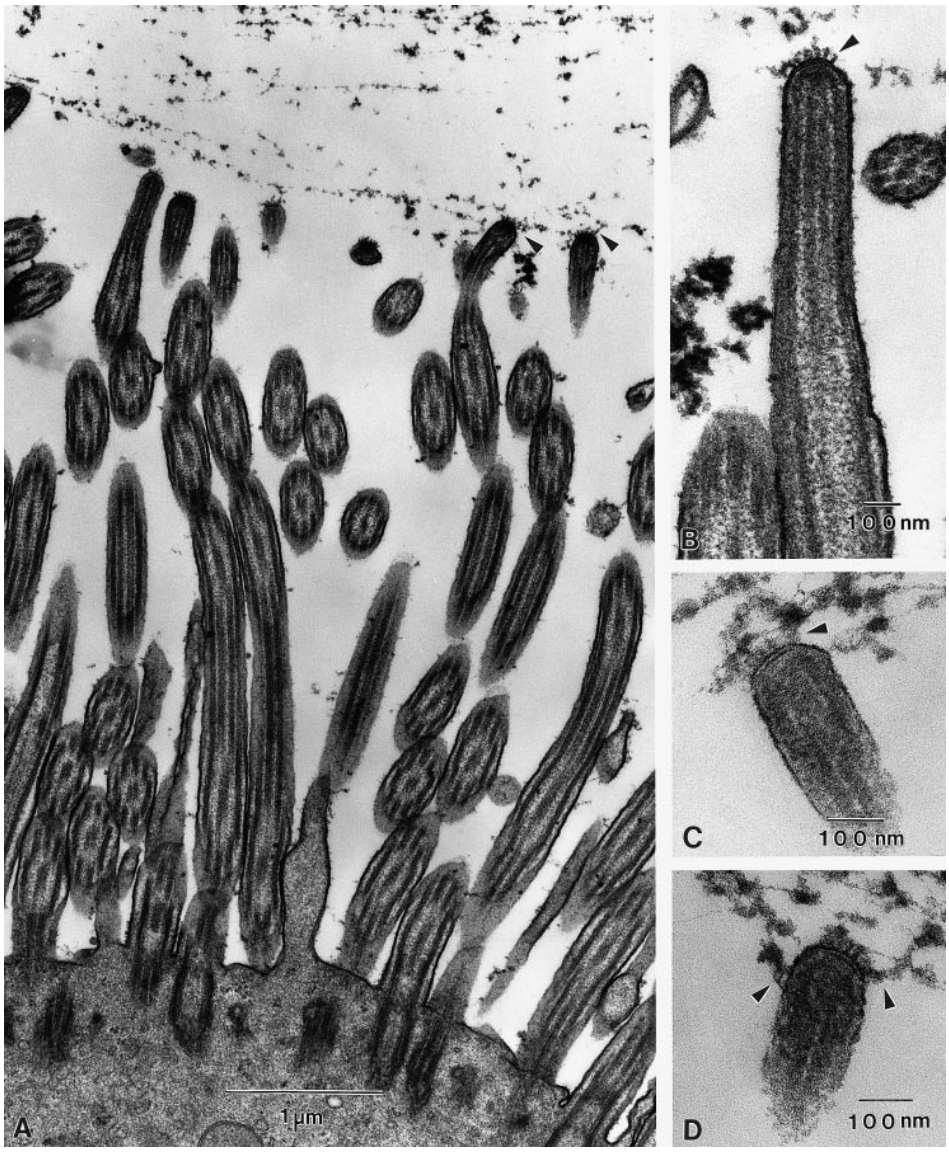


FIG. 2. Transmission electron micrographs showing interaction of the infundibular cilia and OCC extracellular matrix. **A**) The periphery of an OCC showing extracellular matrix that has attached to the tips of cilia (arrowheads); matrix is not present between cilia. **B**) Higher-magnification micrograph showing structure of the ciliary crown. The arrowhead points to the glycocalyx of the crown. **C**) Micrograph showing granules and filaments interacting with the ciliary crown (arrowhead). **D**) Micrograph showing both granules and filaments interacting with the ciliary crown. The arrowhead on the left indicates a granule attached just lateral to the crown.

traveled over their surfaces (Fig. 3). Remnants of the OCC matrix remained attached to the tips of cilia after pick-up had occurred and could be seen along the pathway taken by the OCC on the outer surface of infundibula. Both granules and filaments were present in these matrix remnants. SEM also showed that interaction between the matrix and cilia occurred at the tips of the cilia where the crown is located. Control infundibula not exposed to OCCs lacked matrix on the tips of their cilia (not shown).

Measurement of Adhesion Between the OCC Matrix and Cilia

An assay was developed using an Instech P720 pump to measure adhesion between the OCC matrix and the tips of the cilia. All OCCs used to develop this assay were harvested from mature follicles and had fully expanded matrices. Pumps were first calibrated, and flow rates were found to be linear over the range of the pump speeds on the course and fine dials (Fig. 4, A and B). To measure adhesion, an OCC was placed on the surface of an infundibulum and allowed to begin movement toward the ostium. A glass pipette connected to the pump was then placed flush to the surface of the OCC, and pump speed was increased until the OCC released from the surface of the infundibulum. The pump speed that produced release was converted to flow rate (ml/min) and used as a relative measure of adhesion. The flow rates shown in Figure 4, A and B, provided a sufficient range to measure adhesion during control and most experimental situations.

The reproducibility of the adhesion assay was next examined. A fresh control OCC was placed on an infundibulum, and six consecutive adhesion measurements were made at the same place on the infundibulum (Fig. 5A). OCCs were always positioned on the infundibular surface away from the ostium but not touching the cut surface of the bursal membrane. Adhesion remained remarkably consistent among readings, varying only from 0.021 to 0.027 ml/min in this experiment (Fig. 5A). These data show that six consecutive control readings can be made from the same location on an infundibulum with the same OCC without any significant effect on the measurement. When 10 or more readings were made, a decrease in adhesion was sometimes observed (not shown), probably because transfer of matrix to the tips of the cilia eventually precluded normal binding of the OCC to the cilia.

To compare adhesion among infundibula, the experiment

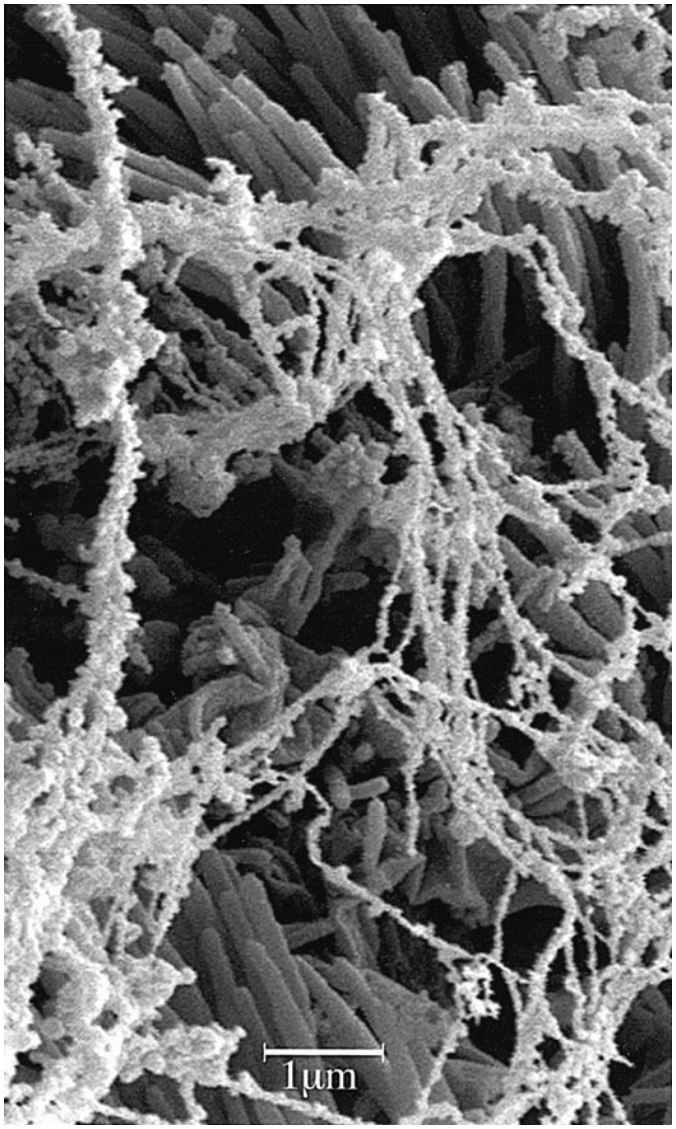


FIG. 3. Scanning electron micrograph showing granules and filaments of the OCC extracellular matrix left on the outer surface of an infundibulum after OCC pick-up. Matrix consisting of granules and filaments adhered to the tips of the cilia.

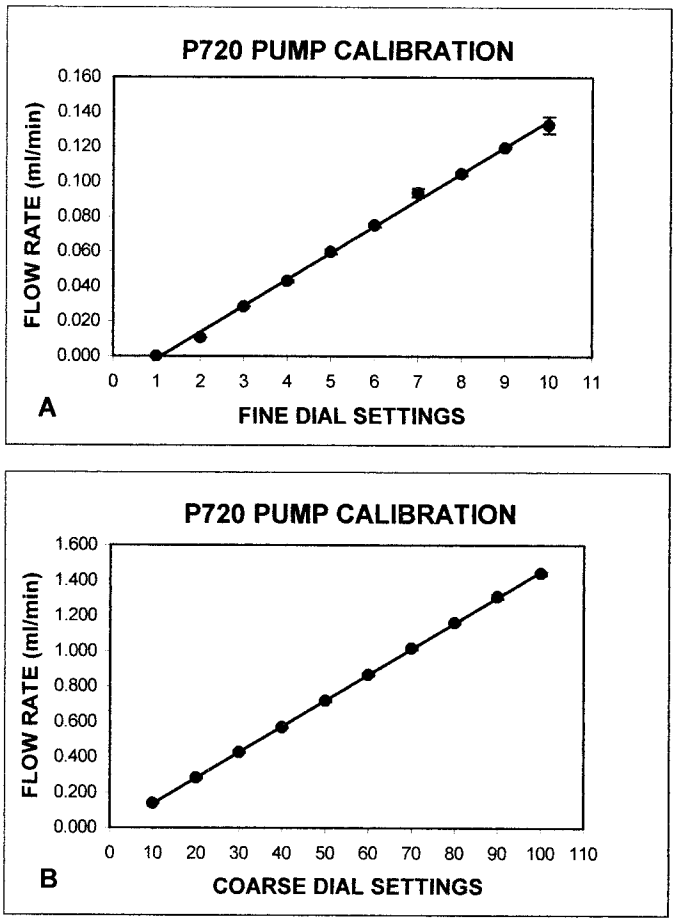


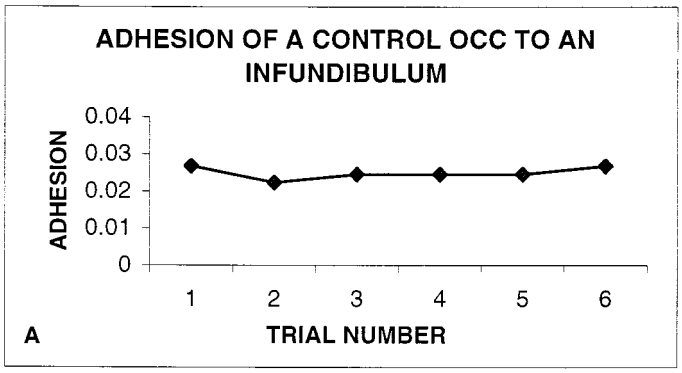
FIG. 4. Calibration curves used in the in vitro assay to measure adhesion between the OCC and oviductal cilia. **A, B**) Calibration of the pump revealed that flow rates were linear over the entire range of both the fine and course dial. Most adhesion measurements were made using readings on the fine dial. Each point is the mean of three measurements. Standard deviations were so small they show only for the highest reading on the fine dial.

shown in Figure 5A was repeated using eight different infundibula. Means were computed for each experiment, and comparison of these is shown in Figure 5B (columns 1–8). In each experiment, similar results were obtained. Adhesion did not differ significantly among infundibula/OCCs in these eight experiments using control (nontreated) OCCs and control (nontreated) infundibula ($P < 0.01$). The grand mean obtained by combining means from all eight experiments (column #9, Fig. 5B) was 0.026 ± 0.003 (SD) ml/min.

To determine whether adhesion varied at different locations on the infundibulum, adhesion was also measured as the OCC entered the ostium. Because the OCC is wider than the diameter of the ostium, it normally takes several minutes for an OCC to churn in the ostium and become sufficiently compacted to pass through the ostium and enter the oviduct [10]. In these experiments, adhesion was measured at 30-sec intervals after placing an OCC in the ostium. Adhesion of OCCs to the ostium was always 10–40 times greater than adhesion of OCCs to the infundibular surface. As consecutive measurements were made at 30-sec intervals, adhesion increased until it became so great that the OCCs could not be removed from the ostium, even with the maximum flow rate (1.5 ml/min) provided by the P720 pump. After compaction of the matrix through churning of the OCC in the ostium, adhesion decreased, and OCCs could be withdrawn relatively easily from the lumen of the oviduct. OCCs with compacted matrices did not adhere well to the surface of the infundibulum, and adhesion was usually lower than could be measured with the P720 pump.

Effect of Modulating Adhesion on OCC Pick-Up Rate

The purpose of the next experiments was to determine whether decreasing or increasing adhesion between the OCC and infundibulum altered OCC pick-up rate. To increase adhesion, OCCs, infundibula, or both were pretreated with the lectin WGA. Fluorescein isothiocyanate-conjugated WGA binds to the matrix of hamster OCCs and to the tips of oviductal cilia (unpublished results). WGA, being multivalent, was predicted to cross-link its receptors in the matrix and crown, thereby increasing adhesion. Figure 6A shows six consecutive control adhesion readings taken before WGA treatment and six readings taken after treatment of the OCC, the infundibulum, or both. The control readings were uniform and were as expected on the basis



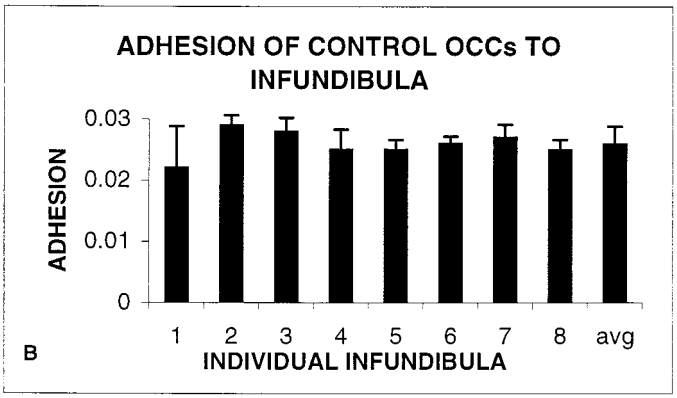
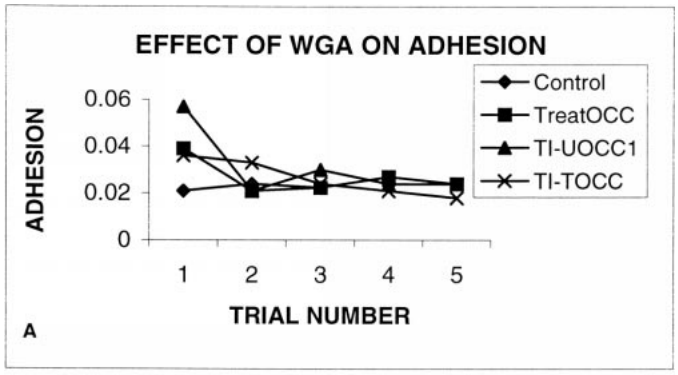


FIG. 5. Adhesion of control OCCs to control infundibula. Adhesion is measured in flow rate (ml/min) **A**) Adhesion of an OCC to the same spot on an infundibulum in six consecutive trials. Adhesion is similar in all trials. **B**) Mean adhesion values for eight experiments similar to that shown in **A.** Different OCCs and different infundibula are represented by each number (infundibula #1–8) on the x axis and do not differ significantly. Columns 1–8 are each means ± SD of six adhesion measurements. The last column (avg) is the mean ± SD of the eight individual experiments (columns 1–8).



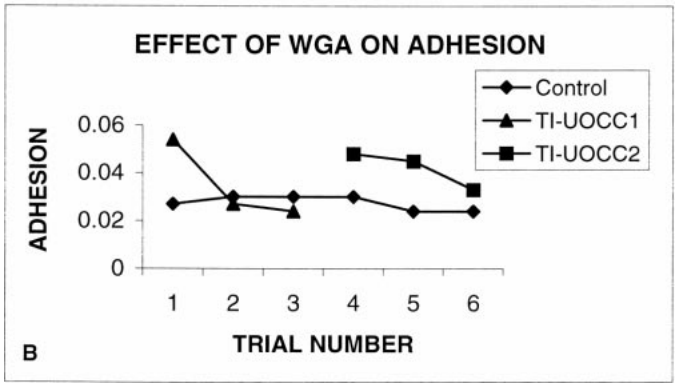
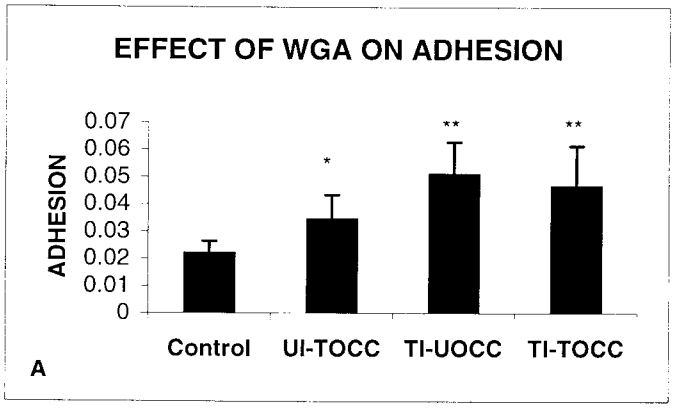


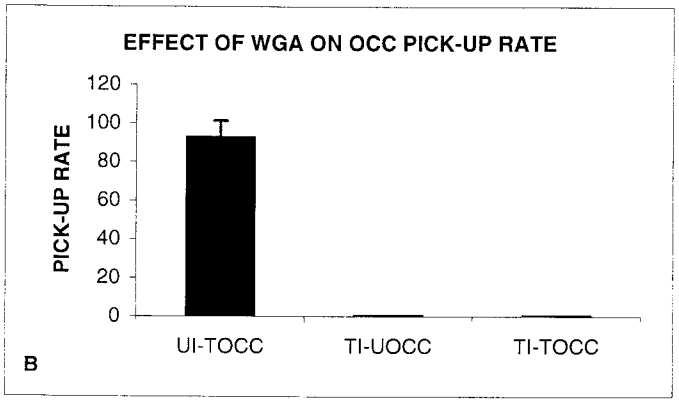
FIG. 6. The effect of WGA on adhesion (measured as flow rate in ml/min). **A**) Pretreatment of OCCs (TreatOCC), infundibula (TI-UOCC1), or both (TI-TOCC) with 0.1% WGA caused adhesion to increase above control values in the initial trial. However, adhesion was similar to that of controls in trials 2–5 when the same OCC was again retested on the same site. **B**) An experiment showing adhesion of an untreated OCC on two different sites on a treated infundibulum. Control measurements of adhesion made before treatment were normal. When the untreated OCC was placed on the first site on the treated infundibulum, adhesion increased in trial 1, then decreased in trials 2 and 3 (TI-UOCC1). Adhesion increased again when the same OCC was placed on a new site on the same infundibulum (trial 4, TI-UOCC2), then decreased slightly in trials 5 and 6 done at the same site.

of previous experiments (Fig. 5). In contrast, adhesion increased significantly in each WGA treatment group, in particular in the treated infundibulum/untreated OCC group. In all treatment groups, the first readings were higher than the control, while subsequent adhesion measurements decreased. Cumulus cells and matrix were sometimes seen adhering to the infundibulum after the first trial, suggesting that residual matrix left after the first or second reading may have prevented subsequent strong adhesion. To test this idea, adhesion was measured three times on one spot (Fig. 6B, triangles); then the OCC was placed in a different location not previously exposed to an OCC, and adhesion was measured again (Fig. 6B, squares). Control measurements (untreated OCC and untreated infundibulum) were normal (Fig. 6B, diamonds). When the infundibulum was pretreated with WGA, adhesion was elevated above control levels in the first trial and decreased to control levels in trials two and three, as seen previously. When the same OCC was placed at a new spot on the same pretreated infundibulum (trial four), adhesion again increased well above control values.

Eleven experiments similar to the one shown in Figure 6A were done, and the data were combined (Fig. 7A). Adhesion was significantly increased in each treatment group, especially in groups having pretreated infundibula. To test the effect of increased adhesion on OCC pick-up rate, control pick-up rates were measured; then an OCC was incubated in 0.1% WGA and placed on an untreated infundibulum, and pick-up rate was measured again. The infundibulum was then incubated in 0.1% WGA, and an untreated OCC was tested. Finally, both a treated infundibulum and a treated OCC were tested. The pick-up rates in the treatment groups are shown in Figure 7B as a percentage of the control rate. There was a modest decrease in pick-up rate when just the OCC was pretreated. However, treatment of the infundibulum or the infundibulum and OCC caused pick-up to cease; these treatment conditions had been shown (Figure 7A) to increase adhesion significantly. To verify that WGA treatment was not inhibiting OCC pick-up by cross-linking the cilia, beat frequency was measured for the treated infundibula in Figure 7A. In no case did WGA treatment inhibit ciliary beat frequency (Fig. 7C), indicating that the cessation of pick-up by WGA was not due to cross-linking of the cilia by WGA.

Poly-L-lysine was found to both increase and decrease adhesion, depending on the type of pretreatment used (Fig. 8A). Pretreatment of the OCC with 0.01% poly-L-lysine completely abolished adhesion. In contrast, pretreatment of the infundibulum with 0.01% poly-L-lysine caused adhesion to increase. When both the infundibulum and the OCC were pretreated with 0.01% poly-L-lysine, adhesion was significantly less than the that of the control. OCC pick-up





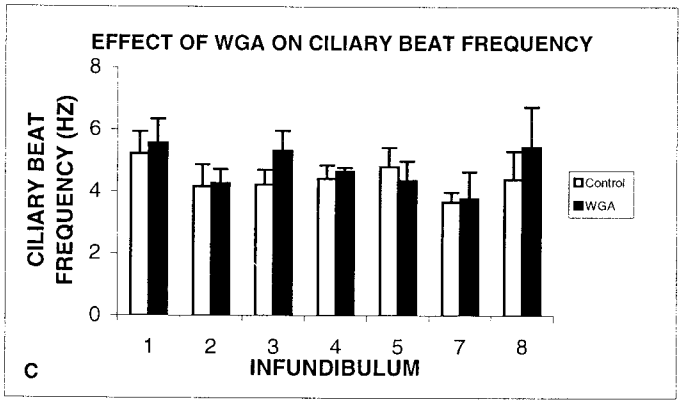
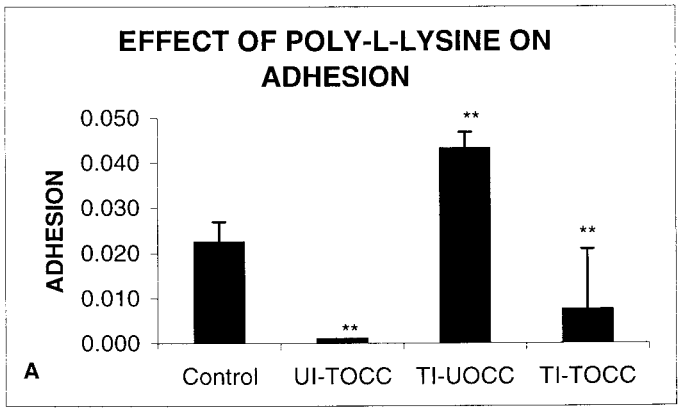
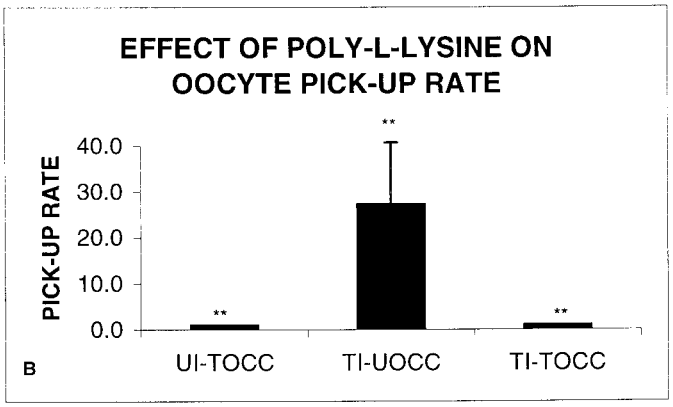


FIG. 7. Effect of increasing adhesion (ml/min) with WGA on OCC pick-up rate (percentage of control) and on ciliary beat frequency (Hz). **A**) Eleven adhesion experiments similar to that shown in Figure 6A were performed, and the data from the first trial in each experiment were combined. The means of all treatment groups were significantly higher than the control mean. Treating just the infundibulum (TI-UOCC) or treating the infundibulum and OCC (TI-TOCC) produced a greater increase in adhesion than treating just the OCC (UI-TOCC). **B**) Effect of increasing adhesion on OCC pick-up rate. OCC pick-up rate was plotted as a percentage of the control rate for the three treatment groups. When OCCs only (UI-TOCC) were pretreated with WGA, pick-up rate decreased by about 10% when compared to that of controls. When the infundibulum (TI-UOCC) or infundibulum plus OCC (TI-TOCC) were pretreated, pick-up rate decreased to zero. N = 11. **C**) Effect of WGA on ciliary beat frequency (Hz). Ciliary beat frequency was not significantly altered by WGA. Beat frequencies are compared before (control) and after WGA treatment for eight different infundibula. Each beat frequency is the mean ± SD of 4–6 measurements. $*P < 0.05$; $**P < 0.01$.





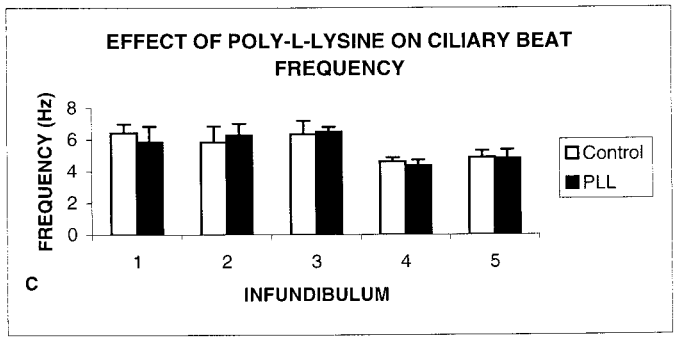


FIG. 8. Effect of poly-L-lysine pretreatment on adhesion (ml/min), OCC pick-up rate (percentage of control), and ciliary beat frequency (Hz). **A**) Pretreatment of the OCC and infundibulum with poly-L-lysine affected adhesion differently. Pretreatment of the OCC (UI-TOCC) with 0.01% poly-L-lysine decreased adhesion to zero, but adhesion increased significantly when 0.01% poly-L-lysine was used to pretreat the infundibulum (TI-UOCC). Pretreatment of both the OCC and infundibulum (TI-TOCC) decreased adhesion compared to that of the control group. Statistical comparisons were made between the control and each treatment group. **B**) OCC pick-up rate was plotted as a percentage of the control rate for each treatment group. OCC pick-up rate decreased when adhesion was either increased or decreased by poly-L-lysine pretreatment. For **A** and **B,** each bar represents the mean ± SD of four experiments. Statistical comparisons were made between the control and each treatment group. **C**) Poly-L-lysine (PLL) pretreatment did not alter ciliary beat frequency in five different infundibula. Control and PLL pairs were compared by *t*-tests. Each bar is the mean ± SD of 4–6 measurements. $**P < 0.01$.

rate was measured in the same experiments and is shown as a percentage of the control in Figure 8B. For all treatment groups in which adhesion was affected (either increased or decreased), pick-up rate decreased significantly. To verify that the pretreatment was not altering ciliary function, beat frequency was measured before and after treating five infundibula and was not significantly affected by poly-L-lysine (Fig. 8C).

DISCUSSION

Pick-up of an OCC by the infundibulum of the oviduct is an essential event in mammalian development. While

adhesion plays a role in this process, little is known about the factors mediating adhesion, the strength of adhesion, or the ways alterations in adhesion affect OCC pick-up. In this study, it was shown microscopically that both the granules and filaments in the cumulus matrix adhere to the ciliary crown of the oviductal epithelium. An assay was developed for quantitatively measuring adhesion between the OCC matrix and the ciliary crowns on the surface of infundibula. This assay was used to show that adhesion measurements were very similar for OCCs and infundibula from different females. When adhesion was either decreased or increased experimentally, OCC pick-up rate decreased or pick-up failed to occur at all. Measurements further revealed that a twofold increase in adhesion was sufficient to completely inhibit OCC pick-up.

Adhesion during OCC pick-up occurred specifically between the granules and filaments of the cumulus matrix and the crowns at the tips of the infundibular cilia. In electron micrographs, both of these elements appeared to bind to the ciliary membrane. The granules and filaments have previously been shown to be sensitive to trypsin and hyaluronidase, respectively [22]. Several glycoproteins, which are potential ligands for the ciliary crown, have been identified in cumulus matrix and include inter-α-trypsin inhibitor [14], a dermatan sulfate proteoglycan, and a 46-kDa protein [15]. In addition, immunohistochemical data suggest that proteins known to influence adhesion, such as fibronectin, tenascin-C, and laminin, are located in the extracellular matrix of human OCCs [16]. The oviductal ciliary crown has not yet been characterized biochemically, but pretreatment of cilia with neuraminidase abolishes pick-up, apparently by altering the crown; this suggests that sialic acid residues in the crown participate in adhesion [7, 8]. The binding of hyaluronic acid filaments to cilia suggests that the crown may also contain hyaladherins or receptors for hyaluronic acid [23].

Although adhesion between the oviductal epithelium and the OCC has been recognized previously [7, 8, 10], it has never before been measured quantitatively. With the assay described in this study, adhesion was measured in the flow rate (ml/min) needed to remove an OCC from the infundibulum. Although this assay does not give a physical measurement of adhesion, it is straightforward to perform, and it allows adhesion to be studied experimentally. The adhesion assay can be used in conjunction with the OCC pick-up rate and ciliary beat frequency assays, making it particularly useful for studying the mechanism of OCC pick-up. This assay could also easily be adapted to other mammalian systems.

The adhesion assay clearly showed that adhesion between the OCC and the exterior surface of the infundibulum was remarkably constant in control incubations. No significant difference was detected in adhesion between OCCs and infundibula in eight different control experiments. When OCCs moved into the ostium, adhesion increased very significantly, because more matrix around the circumference of the OCC was able to bind cilia. The increase in adhesion that occurred in the ostium held the OCC in place and allowed churning to occur. There was no net forward movement of the OCC during this time. After OCCs had undergone churning and become compacted, the matrix lost much of its adhesiveness, as shown qualitatively previously [10]. These observations support the idea that churning is necessary to decrease the diameter of the OCC so it can pass through the ostium and make forward progress into the ampulla.

The experiments with WGA and poly-L-lysine further showed that the proper amount of adhesion was critical for OCC pick-up. When adhesion was decreased, as occurred after compaction of the OCC matrix by treatment with 0.01% poly-L-lysine or by churning, pick-up was inhibited. Pick-up also ceased when adhesion increased by as little as a factor of two through WGA or poly-L-lysine pretreatment of the infundibulum. It can be concluded from these experiments that slight modulations in adhesion above or below control values prevent pick-up, even when cilia are beating normally.

The mechanism by which WGA increased adhesion can be deduced from its known properties. WGA is a multivalent lectin that binds strongly to *N*-acetylglucosamine and sialic acid residues [24]. It is likely that WGA increased adhesion by binding to sialic acid in the ciliary crown and to sialic acid and/or *N*-acetylglucosamine in the OCC matrix. Sialic acid is thought to be the adhesive partner in ciliary crowns because pretreatment of infundibula with succinylated WGA, which binds *N*-acetylglucosamine but not sialic acid, does not alter adhesion or pick-up rate significantly (unpublished data). Neither WGA nor succinylated WGA pretreatment of OCCs altered adhesion sufficiently to allow conclusions about the residue(s) in the OCC that binds this lectin (unpublished data). When infundibula were pretreated with WGA, the bonds formed by multivalent molecules of WGA cross-linking the crowns and matrix added to the adhesive interactions that are normally present in control incubations, and ciliary beating was not powerful enough to overcome this increase in adhesion and move OCCs on treated infundibula. It is also possible that adhesion between the crowns and matrix is normally reversible and that WGA established irreversible adhesion that the beating cilia were not able to overcome.

WGA-induced increases in adhesion were always greater when the infundibulum rather than the OCC was pretreated. This paradox could be explained if the multivalent WGA complexes bound differently to the crowns and matrix. Pretreatment of the infundibulum with WGA may have allowed WGA to bind to sialic acid in the ciliary crowns without saturation of all the WGA binding sites. This would have left unoccupied sites on the WGA molecules available to cross-link to sialic acid or *N*-acetylglucosamine in the OCC matrix. Cross-linking the OCC and infundibulum by WGA augmented adhesion, thereby stopping OCC pick-up. In contrast, pretreating the OCCs with a similar concentration of WGA produced a modest increase in adhesion and little decrease in pick-up rate. This could be explained if WGA cross-linked its receptors within the OCC matrix, leaving few unoccupied WGA sites on the OCC surface for cross-linking to the ciliary crowns. This condition could increase adhesion somewhat but not enough to stop OCC pick-up.

Poly-L-lysine has been reported previously to alter OCC pick-up, although its effects on adhesion were not directly measured [7, 8]. Our data show that poly-L-lysine may either decrease or increase adhesion depending on the pretreatment used. The conformation of the OCC matrix is important for adhesion as shown by pretreatment of OCCs with 0.01% poly-L-lysine, which caused the matrix to become compacted and less adhesive, similar to what is seen during the normal process of churning. Compaction of the matrix by poly-L-lysine may have altered presentation of matrix ligands to the crowns. These data further suggest that failure of the matrix to expand properly would probably impair adhesion and OCC pick-up. Pretreatment of

infundibula with 0.01% poly-L-lysine significantly increased adhesion, probably by loading the crowns with a high positive charge that was able to electrostatically crosslink to the negatively charged molecules in the OCC matrix. This additional increase in adhesion provided by poly-L-lysine, like the increase produced with WGA pretreatment, was sufficient to stop OCC pick-up.

Numerous functions have been proposed for the expanded cumulus and its matrix. The expanded matrix surrounds a relatively small oocyte within a large antrum of a tertiary follicle and facilitates extrusion of the oocyte from the antrum during ovulation [1, 25]. The present study showed that OCC pick-up failed to occur when matrix was not present between cumulus cells, indicating that the OCC matrix, by enabling adhesion to the cilia, is necessary for successful transfer of the oocyte from the tertiary follicle to the ampulla of the oviduct. These observations suggest that the cumulus and its matrix have evolved to facilitate evacuation of the oocyte from the large mammalian antrum during ovulation and to assure rapid pick-up of the ovulated OCCs and transfer of the oocyte into the ampulla for fertilization. Once the OCC enters the ampulla, its matrix facilitates occurrence of acrosome reactions in sperm penetrating the cumulus layer [26–30]. The OCC may in addition stimulate sperm motility [31–33], function in sperm attraction [34], and participate in sperm selection [35, 36].

In summary, these data further our general understanding of the mechanism by which the OCC is picked up by the infundibulum of the oviduct. Adhesion between the matrix and the crowns of the cilia is essential for holding the OCC to the oviduct. The control and modulation experiments on adhesion showed that for successful OCC pick-up, adhesion between the cilia and matrix must be transitory and of the proper strength. The main functions of the cilia are to provide the motive force for breaking the adhesive interactions between the OCC and infundibulum, and to direct movement of the OCC towards the ostium. This is done in a manner such that the OCC is never completely released, and therefore does not fall off the infundibulum. The experiments with WGA and poly-L-lysine demonstrate that a delicate balance exists between the adhesive interactions occurring during OCC pick-up and show that factors capable of altering adhesion prevent pick-up. Complications in OCC-to-infundibular adhesion in humans could lead to infertility or ectopic pregnancy.

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REFERENCES

1. Talbot P, DiCarlantonio G. The oocyte-cumulus complex: ultrastructure of the extracellular components in hamsters and mice. Gamete Res 1984; 10:127–142.
2. Blandau R. Gamete transport—comparative aspects. In: Hafez ESE, Blandau R (eds.), The Mammalian Oviduct. Chicago: University of Chicago Press; 1969: 129–162.
3. Harper JK. Gamete and Zygote Transport. In: Knobil E, Neill JD (eds.), The Physiology of Reproduction. Second ed. New York: Raven Press; 1994: 123–185.
4. Halbert SA, Tam PY, Blandau RJ. Egg transport in the rabbit oviduct: the roles of cilia and muscle. Science 1976; 191:1052–1053.
5. Huang S, Driessen N, Knoll M, Talbot P. In vitro analysis of oocyte cumulus complex pick-up rate in the hamster *Mesocricetus auratus.* Mol Reprod Dev 1997; 47:312–322.
6. Blandau R, Verdugo P. An overview of gamete transport—comparative aspects. In: Harper MJK, J PC, Adams CE, Coutinho EM, Paton DM (eds.), Ovum Transport and Fertility Regulation. Copenhagen: Scriptor; 1976: 138–146.
7. Norwood JT, Hein CE, Halbert SA, Anderson RGW. Polycationic macromolecules inhibit cilia-mediated ovum transport. Proc Natl Acad Sci USA 1978; 75:4413–4416.
8. Mahi-Brown CA, Yanagimachi R. Parameters influencing ovum pick-up by oviductal fimbria in the golden hamster. Gamete Res 1983; 8: 1–10.
9. Gordts S, Campo R, Romauts L, Brosen I. Endoscopic visualization of the process of fimbrial ovum retrieval in the human. Hum Reprod 1998; 13:1425–1428.
10. Talbot P, Gieseke C, Knoll M. Oocyte pick-up by the mammalian oviduct. Mol Biol Cell 1999; 10:5–8.
11. Talbot P, DiCarlantonio G, Knoll M. Identification of cigarette smoke components that alter functioning of hamster (*Mesocricetus auratus*) oviducts in vitro. Biol Reprod 1998; 58:1047–1053.
12. Norwood JT, Anderson RGW. Evidence that adhesive sites on the tips of oviduct cilia membranes are required for ovum pickup in situ. Biol Reprod 1980; 23:788–791.
13. Salustri A, Yanagashita M, Hascall V. Synthesis and accumulation of hyaluronic acid and proteoglycans in the mouse oocyte cumulus complex during follicle stimulating hormone induced mucification. J Biol Chem 1989; 264:13840–13847.
14. Chen L, Zhang H, Powers RW, Russell PT, Larsen WJ. Covalent linkage between proteins of the inter-α-inhibitor family and hyaluronic acid is mediated by a factor produced by granulosa cells. J Biol Chem 1996; 27:19409–19414.
15. Camaioni A, Salustri A, Yanagishita M, Hascall V. Proteoglycans and proteins in the extracellular matrix of mouse cumulus cell-oocyte complexes. Arch Biochem Biophys 1996; 325:190–198.
16. Familiari G, Verlengia C, Nottola SA, Renda T, Micara G, Aragona C. Heterogeneous distribution of fibronectin, tenascin-C, and laminin immunoreactive material in the cumulus-corona cells surrounding mature human oocytes from IVF-ET protocols—evidence that they are composed of different subpopulations: an immunohistochemical study using scanning laser confocal and fluorescence microscopy. Mol Reprod Dev 1996; 43:392–402.
17. Orsini MW. The external vaginal phenomena characterizing the stages of the estrous cycle, pregnancy, pseudopregnancy, lactation, and the anestrus hamster, *Mesocricetus auratus.* Proc Anim Care Panel 1961; 11:193–206.
18. Greenwald GS. Analysis of superovulation in the adult hamster. Endocrinology 1962; 71:378–389.
19. Morgan J, Morton H, Parker R. Nutrition of animal cells in tissue culture. I. Initial studies on a synthetic medium. Proc Soc Exp Biol Med 1950; 73:1–8.
20. DiCarlantonio G, Shaoulian R, Knoll M, Magers T, Talbot P. Analysis of ciliary beat frequencies in hamster oviductal explants. J Exp Zool 1995; 272:142–152.
21. Knoll M, Talbot P. Cigarette smoke components inhibit oocyte cumulus complex pick-up by the oviduct independent of ciliary beat frequency. Mol Biol Cell 1996; 7:481 (abstract).
22. Talbot P, DiCarlantonio G. Ultrastructure of opossum oocyte investing coats and their sensitivity to trypsin and hyaluronidase. Dev Biol 1984; 103:159–167.
23. Toole B. Hyaluronan and its binding proteins, the hyaladherins. Curr Opin Cell Biol 1990; 2:839–844.
24. Sharon N, Lis H. Legume lectins—a large family of homologous proteins. FASEB J 1990; 4:3198–3208.
25. Talbot P. Intrafollicular pressure promotes partial evacuation of the antrum during hamster ovulation in vitro. J Exp Zool 1983; 226:129–135.
26. Parrish JJ, Susko-Parrish JL, Handrow RR, Ax RL, First NL. Effect of sulfated glycoconjugates on capacitation and the acrosome reaction of bovine and hamster spermatozoa. Gamete Res 1989; 24:403–413.
27. Ball GD, Bellin ME, Ax RL, First NL. Glycosaminoglycans in bovine cumulus-oocyte complexes: morphology and chemistry. Mol Cell Endocrinol 1982; 28:113–122.
28. Lenz RW, Ball GD, Lohse JK, First NL, Ax RL. Chondroitin sulfate facilitates an acrosome reaction in bovine spermatozoa as evidenced by light microscopy, electron microscopy and in vitro fertilization. Biol Reprod 1983; 28:683–690.
29. Meizel S, Turner KO. Glycosaminoglycans stimulate the acrosome reaction of previously capacitated hamster sperm. J Exp Zool 1986; 237:137–139.

30. Siiteri JE, Dandekar P, Meizel S. Human sperm acrosome reaction-initiating activity associated with the human cumulus oophorus and mural granulosa cells. J Exp Zool 1988; 246:71–80.
31. Tesarik J, Mendoza Oltras C, Testart J. Effect of the human cumulus oophorus on movement characteristics of human capacitated spermatozoa. J Reprod Fertil 1990; 88:665–675.
32. Westphal LM, el Dansasouri I, Shimizu S, Tadir Y, Berns MW. Exposure of human spermatozoa to the cumulus oophorus results in increased relative force as measured by a 760 nm laser optical trap. Hum Reprod 1993; 8:1083–1086.
33. Fetterolf PM, Jurisicova A, Tyson JE, Casper RF. Conditioned medium from human cumulus oophorus cells stimulates human sperm velocity. Biol Reprod 1994; 51:184–192.
34. Hunter RHF. Modulation of gamete and embryonic microenvironments by oviduct glycoproteins. Mol Reprod Dev 1994; 39:176–181.
35. Bedford JM, Kim HH. Cumulus oophorus as a sperm sequestering device, in vivo. J Exp Zool 1993; 265:321–328.
36. Carrell DT, Middleton RG, Peterson CM, Jones KP, Urry RL. Role of the cumulus in the selection of morphologically normal sperm and induction of the acrosome reaction during human in vitro fertilization. Arch Androl 1993; 31:133–137.