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Ciliary Transport, Gamete Interaction, and Effects of the Early Embryo in the Oviduct: Ex Vivo Analyses Using a New Digital Videomicroscopic System in the Cow[1]

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ABSTRACT

Using a digital videomicroscopic analysis system in the bovine, we showed that the mechanisms of transport caused by ciliary beating are distinctly different in ampulla and isthmus of the oviduct. The average particle transport speed (PTS) in the oviduct (mean, 133 μm/sec) does not differ in the cycle (metestrus) and during pregnancy after implantation, but it is locally modulated at the site of the embryo. Using videomicroscopy, we were able to document that after entering the ampulla, the cumulus-oocyte complex (COC) is not transported by ciliary beating down the oviduct, but firmly attaches to the ampullar epithelium. This attachment is mediated by the cumulus cells. However, when a COC is degenerated, it is floating in the oviductal lumen. As soon as a vital COC is in the ampulla, the sperm bound in the sperm reservoir of the ampullar isthmic junction leave the reservoir and hurry to the oocyte. When a sperm has penetrated the zona pellucida, the COC detaches and continues its migration. Quantitative measurements showed that the early embryo is able to locally downregulate PTS during its migration down the oviduct. It locally changes the pattern of vascularization and induces the formation of secretory cells. Our studies imply that the oviductal epithelium is able to select vital oocytes. The early embryo is able to induce the formation of secretory cells, modify vascularization, and downregulate speed of transport, thus creating the prerequisite for the first embryo-maternal communication in the oviduct.

ciliary transport, early embryo, fertilization, oviduct, sperm motility and transport

INTRODUCTION

In the oviduct, pickup and transport of the ovum, transport of sperm, fertilization, and development and transport of the early embryo take place [1, 2]. Fertilization only occurs when the oocyte and the sperm meet in the ampulla in time [3]. After fertilization, a precise timing of the transport is the essential prerequisite for the embryo to obtain the capacity of implantation [4]. Thus, Akira et al. [5] were able to show that in rats, superovulation treatment induces a distinct acceleration of embryonic transport, resulting in a significant reduction of implantation rates. The transport of the oocyte and embryo in the oviduct is achieved by 1) ciliary beating of the oviductal epithelial cells and 2) contraction of the oviductal smooth muscle [6–8]. Until now, mainly the modulators of smooth muscle contraction have been investigated. It is known that the stimulation of α-adrenoreceptors promotes the contraction of smooth muscles, whereas the activation of β-adrenoreceptors inhibits the contraction [9, 10]. When these receptors are blocked, neither embryonic transport nor fertility is reduced [11], implying that the transport of oocytes and embryos is predominantly modulated by 1) endocrine and 2) autocrine/paracrine signals, which are locally produced by the oocyte and the embryo. Whereas estrogens and prostaglandins, such as prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), increase the contractility of the oviductal smooth muscles, resulting in increased speed of embryonic transport [12–14], progesterone leads to relaxation of the smooth muscles and reduces the speed of transport in the oviduct [15].

Besides smooth muscle contraction, the regulation and modulation of ciliary beating of the oviductal epithelium are involved in the transport of the oocyte and the embryo [16]. If a part of the ampulla is excised and reimplanted in the reverse direction, pregnancy sporadically occurs [17]. Women who have Kartagener syndrome, which is characterized by primary ciliary dyskinesia, may or may not be fertile [18]. These findings raise the question of the role and importance of ciliary beating versus myosalpinx contractile activity for oocyte/embryo movement. At the same time, it points to the necessity to precisely define the role of ciliary beating in oocyte/embryo transport. Until now, the modulators of ciliary beating have rarely been investigated. The only known endocrine modulator is progesterone, which is able to reduce the frequency of ciliary beating [19]. Thus, the exact mechanisms of transport in the oviduct and the site-specific mechanisms of gamete transport in infundibulum, ampulla, isthmus, and uterotubal junction of the oviduct are largely unknown. However, the basic events of fertilization and early embryonic development have extensively been investigated and are similar in all mammals. At coitus, millions of sperm are ejaculated, but only a few hundred to thousand enter the oviduct and form a sperm reservoir at the uterotubal junction [20–22]. When ovulation occurs, the cumulus-oocyte complex (COC) is picked up by adhesion at the cilia of the infundibulum and slides over the surface of the infundibulum in direction of the ostium [23]. As the time of ovulation approaches, sperm become capacitated and hyper-

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activated, leave the reservoir, and migrate to the COC in the tubal ampulla [20]. After fertilization, the cumulus cells get lost by the effects of tubal and sperm enzymes, and the presumptive zygote migrates down the oviduct [8]. However, until now the underlying mechanisms of the interaction of the gametes and the early embryo with the oviductal epithelium have not yet been elucidated. This is mainly because of the fact that 1) the oviduct is localized in the mesosalpinx and integrated in the bursa ovarica, so that it is difficult to investigate in vivo, and 2) that oviductal cells rapidly degenerate in cell culture and loose function in vitro [24, 25]. Recently, it has been shown in vivo that distinct alterations in oviductal gene expression occur as a result of sperm and oocyte arrival in the oviduct, leading to distinct changes in the composition of the oviductal fluid [26]. This finding strengthens the need to more exactly clarify the interaction between gametes and oviductal epithelium. Therefore, we established a new digital videomicroscopic system that makes it possible to analyze gamete interaction with the oviductal epithelium, fertilization, embryonic development, and ciliary transport under near-in vivo conditions. With the help of this system, we were able to document the behavior of COCs, sperm, and embryos in the oviduct, to analyze the basic mechanisms of ciliary transport, and to quantify the average particle transport speed in the oviduct. These results were correlated to morphological changes of the oviductal epithelium before and after fertilization and during the migration of the embryo down the oviduct by scanning electron microscopy.

MATERIALS AND METHODS

Cows

Cows aged 3–10 yr (breeds: Holstein-Friesian, Red Pied Cows, Angler, and Deutsches Fleckvieh) were included in the study. The cows were kept in the Clinic for Obstetrics, Gynecology and Andrology of Large and Small Animals of the Justus-Liebig-University. All procedures described within were reviewed and approved by the University of Giessen Institutional Animal Care and Use Committee and were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals.

For investigating cows from Day 2.5 to Day 4.5 of pregnancy seven cows were estrous synchronized and treated as follows: Day 1: 5 ml of buserelin as analog of gonadotropin-releasing hormone (induction of ovulation; Receptal; Intervet GmbH, Unterschleissheim, Germany) intravenously; Day 7: in the morning, 3.5 ml of $PGF_{2\alpha}$ (Tiaprost; Iliren; Intervet) intravenously, and in the evening, 3.5 ml of Tiaprost intravenously (induction of luteolysis); Day 9: 5 ml of buserelin (induction of ovulation) intravenously; Day 10: in the morning and evening, artificial insemination with frozen-thawed sperm of a bull with proven fertility. Time of ovulation was monitored every 8 h by ultrasonography with a 7.5-MHz linear array transducer (Pie Medical Scanner 100 LCVERT; Pie Medical, Maastricht, the Netherlands). In another group, eight cows without estrous synchronization were monitored by ultrasonography and inseminated during natural estrus. All cows were slaughtered at a definite time point on Days 2.5, 3.5, and 4.5 of pregnancy. Additionally, two cows on Day 18 (day of implantation) were investigated. Regularly, both oviducts were removed and vascularization, form, and color of the oviducts were documented by digital photography.

For investigating cows during the cycle (after ovulation: metestrus) and in the course of pregnancy after implantation, the oviducts were obtained from cows from the slaughterhouse immediately after slaughter. The length of gestation was determined by measuring the crown-rump length of the embryos and fetuses. In total, six cyclic cows (metestrus) and 22 cows pregnant from 21 days to 120 days were investigated.

Cumulus-Oocyte Complexes

Bovine COCs were obtained from ovaries of cows collected from a local slaughterhouse. The ovaries were transported to the laboratory in PBS at 30°C. The COCs were aspirated from follicles using a syringe and a 20-gauge needle. Cumulus-oocyte complexes obtained from 2- to 10-mm follicles were immature COCs. Cumulus-oocyte complexes obtained from dominant follicles larger than 15 mm in size and showing signs of maturation were regarded as matured in vivo and were investigated separately. Cumulus-oocyte complexes with few layers of cumulus cells and oocytes with spotted cytoplasm were regarded as degenerated. Only oocytes with a multilayered, compact cumulus oophorus and a dark, evenly granulated cytoplasm were selected for further maturation.

Each of the videomicroscopic experiments was conducted three times. In each experiment, 20–25 immature COCs (in total, 69), three to four mature COCs (in total, 11), and 10–15 degenerated COCs (in total, 36) were investigated. A total of 33 videos were made to document the interaction between immature COCs and oviductal epithelium. A total of 15 videos were taken to demonstrate the attachment of the mature COCs, and 27 movies of the behavior of degenerated COCs in the oviduct were made.

In Vitro Maturation of Oocytes

For studying the differences of in vitro-matured and in vivo-matured COCs by videomicroscopy, in vitro maturation was performed in tissue culture medium 199 (TCM 199; Seromed, Berlin, Germany) supplemented with 2 mM sodium pyruvate, 2.92 mM calcium lactate, 0.01 units of bovine follicle-stimulating hormone (Sioux Biochemicals, Sioux Center, IA), 0.01 units of bovine luteinizing hormone (Sioux Biochemicals), and 60 mg/ml gentamicin (Sigma, St. Louis, MO). Maturation was performed in a humidified atmosphere of 5% CO_2 in air at 39°C for 24 h.

Videomicroscopy was performed in three different experiments using five to eight in vitro-matured COCs (in total, 22). A total of 16 movies were taken for documentation.

Denudation of COCs and Removal of Zona Pellucida

The COCs were mechanically denuded by gently pipetting them through glass pipettes with lumina of the size of the oocyte (150 μm). Videomicroscopy was performed twice using eight denuded oocytes in each experiment.

The zona pellucida (ZP) was removed by putting denuded oocytes in prewarmed PBS, pH 2, for about 8 sec. Under microscopic control, oocytes were put in PBS, pH 7.4, as soon as most of the ZP had disappeared. Videomicroscopy was done twice using seven to nine oocytes without ZP in each experiment. In total, 16 oocytes were investigated, and 14 movies were made.

Sperm

For the videomicroscopic studies, both native and frozen semen was used. Native semen was diluted in Triladyl (Minitüb, Tiefenbach, Germany), and frozen semen was diluted in Bioxcell (IMV Technologies, L'Aigle, France). When applying frozen semen, each straw was thawed in a water bath at 37°C for 10 sec. In frozen semen, the behavior of sperm in the ampulla was compared before and after swim-up according to Parrish et al. [27]. To select motile spermatozoa, swim-up was performed in a Tyrode lactate solution supplemented with 6 mg/ml bovine serum albumin fraction V (Sigma), 0.1 mg/ml sodium pyruvate (Sigma), and 50 μl/ml gentamicin (Sigma). After washing and centrifugation, spermatozoa were resuspended to a final concentration of 10^6 sperm/ml. In 10 different experiments, the behavior of sperm of four bulls of different fertility was analyzed by videomicroscopy. In total, 35 movies were taken.

Videomicroscopy

Immediately after slaughter, both oviducts were removed. The oviduct on the side where the oviduct carried the dominant follicle during estrus, the corpus luteum during metestrus, and the corpus luteum graviditatis during pregnancy was called *ipsilateral*. The other oviduct was the *contralateral* oviduct. The comparison between ipsilateral and contralateral oviducts provides valuable indications for local changes in the ipsilateral oviduct induced by the COC and the early embryo, respectively. One-centimeter-long pieces of the transition of the first to the second third of the ampulla and of the isthmus of the ipsilateral and contralateral oviducts were cut. In total, 40 oviducts were investigated (Days 2.5–4.5, n = 20; Days 18–120, n = 10; cycle, n = 12). Opened specimens were transferred to a Delta T culture dish (Bioptechs, Butler, PA), whose glass bottom was covered with a thin layer of Sylgard polymer (Dow Corning, Wiesbaden, Germany) and the dish filled with 2 ml of cold Hepes-Ringer solution. The preparation was gently rinsed with Hepes-Ringer solution, followed by exchange with 1.5 ml of fresh, warm buffer, submerging the oviduct. The culture dish was transferred to the Delta T Stage holder 2–3 h after the animal's death and was held constant at 36°C. Before each experiment, normal ciliary beating was assessed with an UMPLFL 100 × W/0.70 water immersion objective (Olympus, Hamburg, Germany). Imaging was done with a TillVision imaging system (Till Photonics, Graefelfing, Germany) based on a BX50 WI fixed-stage upright microscope (Olympus) equipped with an Imago CCD camera with a 1280 × 960 pixel CCD chip (Till Photonics).

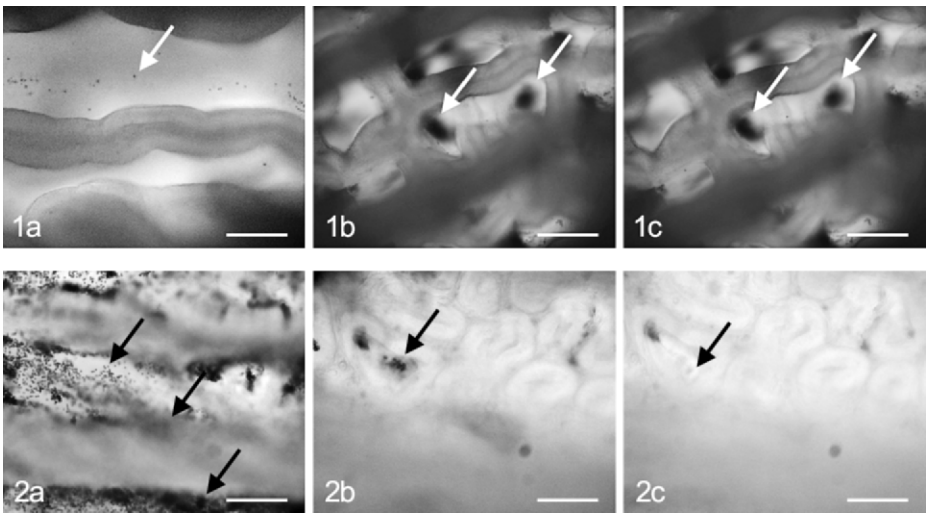

PLATE I. Figures 1 and 2.
FIG. 1. Videomicroscopy of particle transport in the ampulla. See Supplemental Movie 1. **a**) Transport takes place in the depth between the folds (arrow). **b**) A large proportion of the particles settles between the folds (position of particles at 0 sec; arrows). **c**) The particles remain between the folds (position of particles 20 sec later; arrows). Bar = 100 μm.
FIG. 2. Videomicroscopy of particle transport in the isthmus. See Supplemental Movies 2a and 2b. **a**) Particles are rapidly transported to the apical ridges of the folds (arrows). **b**) Some particles settle between the folds (position of particles at 0 sec; arrow). **c**) Particles settled between the folds are quickly removed by ciliary beating (20 sec later; arrow). Bar = 100 μm.

For investigating the basic mechanisms of ciliary transport in the ampulla and in the isthmus, 12 cyclic and 10 pregnant (Days 18–120) cows were examined. In each cow, we included two different pieces of the oviduct in the study. In total, 48 videos documenting particle transport in ampulla and isthmus were taken.

For investigating the interaction of the oviductal epithelium with oocytes and cumulus cells, immature COCs, in vivo-matured COCs, in vitro-matured COCS, denuded COCs, and oocytes lacking ZP were put in the oviduct. For analyzing the behavior of sperm in the oviduct and for investigating oocyte-sperm interaction, about 100 000 sperm were added to the ampulla (with and without COCs) and the isthmus of cows in late estrus and metestrus.

For analyzing mechanisms of transport in the ampulla and isthmus, polystyrene beads of a diameter of 4.5 μm (Dynabeads; Dynal Biotech GmbH, Hamburg, Germany) were added to the buffer solution. Then, the epithelial surface of the oviduct was imaged in bright field mode using an UMPLFL 10 × W/0.30 (COCs) or an UMPLFL 20 × W/0.50 (sperm) water-immersion objective (Olympus), respectively.

Measurement of Particle Transport Speed

For measuring the particle transport speed (PTS), approximately 600 000 polysterene beads (diameter 2.8 μm; Dynabeads) labeled with Cy3 (CyDye Post-Labeling Reactive Dye Pack; Amersham Biosciences) were added to the buffer solution. Then, the epithelial surface of the oviduct was imaged in epifluorescence mode using an UMPLFL 20 × W/0.5 water-immersion objective (Olympus) and an appropriate filter set for Cy3. For each time point, 200 images (640 × 480 pixels, 2 × 2 binning, 12-bit) were taken with an exposure time of 20 msec at a frame rate of 11.76 images/sec. The original films were converted from 12-bit to 8-bit grayscale and were used to track the Dynabeads by an automatic tracking procedure using the TillVision software (Till Photonics). Only tracks that were measured over a length of at least 10 frames and did not differ more than 15% from the direct connection between start and end point were included in further calculations.

The PTS was compared in the oviducts of the following groups: 1) cycle (metestrus, n = 8; this stage of the cycle was chosen to compare the PTS in the first days after ovulation without fertilization with that of the first days after ovulation with fertilization); 2) Pregnancy Days 2.5–3.5 at the site of the embryo (n = 5; the embryo is in the ampulla until Day 2, then it enters the isthmus and reaches the uterus on Day 3.5 of pregnancy); 3) Pregnancy Days 2.5–4.5 at the oviductal site without the embryo; and 4) pregnancy after implantation (n = 8; Days 18–120). In each group, ipsilateral ampulla, ipsilateral isthmus, contralateral ampulla, and contralateral isthmus were measured. For each piece of the oviduct, eight measurements in time intervals of 3 min were carried out, and mean and median values were calculated.

In a pilot experiment, the impact of the postmortem interval on oviductal PTS was determined. The PTS was well maintained within 4 h postmortem. Consequently, all following experiments were performed within this time span.

Statistical Analysis of PTS

Multiple groups were compared with the nonparametric Kruskal-Wallis test. If the resulting value was $P < 0.05$, pairs of groups were compared with the Mann-Whitney test. The results were rated significant if $P < 0.05$. Analyses were done using the SPSS software (SPSS Inc.).

Scanning Electron Microscopy

For investigating the effects of the early embryo on the oviductal epithelium, ipsilateral oviducts from three cows were removed on Days 2.5–3.5 of pregnancy and cut into 1-cm-long pieces from the beginning of the ampulla to the uterotubal junction. All 10–12 specimens of one oviduct were numbered and investigated in sequence. Additionally, the interaction of the sperm with the oviductal epithelium was investigated by incubation (2 min) of the oviducts with 2 μl of frozen-thawed sperm (concentration, 100 000 sperm per microliter of semen diluent Bioxcell).

For scanning electron microscopy, oviducts were freed from the surrounding mesosalpinx, were opened using scissors for iridectomy (FST, Heidelberg, Germany), placed on a cork plate, and fixed with insect needles. Oviducts were then fixed in 1% glutaraldehyde in Soerensen buffer (pH 7.4; 1:5 solution of 0.07 M KH_2PO_4 and 0.07 M Na_2HPO_4-$2H_2O$) at 4°C for 24 h. After further washes in Soerensen buffer, specimens were dehydrated in an ascending series of acetone (10%, 20%, 30%, 40%, 50%, and 60%, twice, 5 min each; 70%, 80%, and 90%, 1 h each; 100%, 12 h). The oviducts were dried in a Union Point Dryer CPD 030 (Bal-Tec, Walluf, Germany) using liquid CO_2 as transitional fluid. After drying, specimens were coated with 12 nm gold-palladium by a Union SCD 040 sputtering device (Bal-Tec). Analyses were performed with the Zeiss scanning electron microscope DSM 950 at magnifications from 50× to 10 000×.

Proportion of Secretory and Ciliated Cells

The number of secretory and ciliated cells in the first part, mid part, and end part of the ipsilateral oviduct on Day 3 of pregnancy was counted in scanning electron microscopic photographs. For each part of the oviduct, six photographs in magnifications of 1800× to 2000× were analyzed. The total size of measured area in each part of the oviduct was 11 000 μm^2. Statistical analysis of the proportion of secretory to ciliated cells in each part of the oviduct was performed using the Pearson chi-square test. The results were rated significant if $P < 0.05$. Analyses were done using the SPSS software.

RESULTS

Mechanisms of Ciliary Transport

The videomicroscopic studies showed that there are distinctly different mechanisms of transport in the ampulla and in the isthmus. In the ampulla, transport took place in the depth between the folds (Fig. 1a and Supplemental Movie 1, see arrows [all Supplemental Movies are available online at www.biolreprod.org]). During their way through the ampulla, a great part of the Dynabeads settled down in between the folds and stayed there (Fig. 1b, time 0 sec, arrows, Fig. 1c, time 20 sec later, arrows, and Supplemental Movie 1). In contrast, in the isthmus particles were rapidly transported to the apical

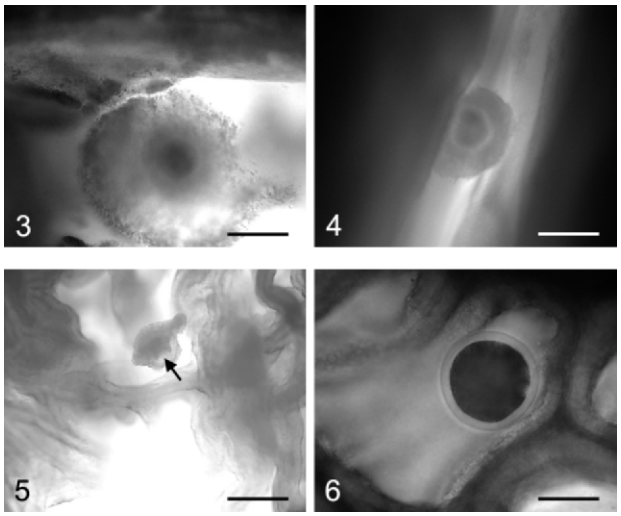

PLATE II. Figures 3, 4, 5, and 6.
FIG. 3. As soon as the COC enters the ampulla, the mature COC attaches to the epithelium. Bar = 200 μm. See Supplemental Movie 3.
FIG. 4. The immature COC characterized by few layers of densely packed cumulus cells also attaches to the epithelium. Bar = 200 μm. See Supplemental Movies 4a and 4b.
FIG. 5. A degenerated COC characterized by the irregular form of the cytoplasm (arrow) floating in the oviductal lumen. Bar = 200 μm. See Supplemental Movie 5.
FIG. 6. A denuded oocyte does not attach; it turns and moves around slightly because of ciliary beating. Bar = 100 μm. See Supplemental Movie 6.

ridges of the folds (Fig. 2a and Supplemental Movie 2a, see arrows). If some particles settled down in the depth between the folds, they were quickly removed by ciliary beating and guided back to the stream of the other Dynabeads (Fig. 2b, time 0 sec, arrows, Fig. 2c, time 20 sec later, arrows, and Supplemental Movie 2a). As seen by quantitative measurements using fluorescent Dynabeads (Supplemental Movie 2b), the average PTS did not differ in the contralateral and ipsilateral ampulla and isthmus in the cycle (metestrus; mean, 133 μm/sec) and during pregnancy after implantation (mean, 123 μm/sec; see Fig. 10).

Gamete Interaction with the Oviductal Epithelium

The videomicroscopic studies showed that as soon as the mature COC entered the ampulla, it immediately firmly attached to the oviductal epithelium (Fig. 3 and Supplemental Movie 3). The attachment of the COC was so strong that it could not be resolved by applying strong currents with a pipette

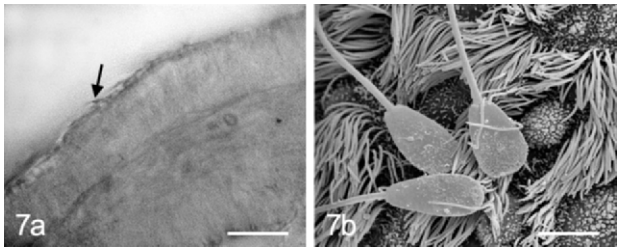

FIG. 7. Videomicroscopy and scanning electron microscopy of sperm. See Supplemental Movie 7. **a**) As soon as the sperm reach the oviduct, they form a sperm reservoir in the isthmus (arrow). Bar = 50 μm. **b**) In the reservoir, the sperm bind with their heads in a tangential angle to ciliated cells. Bar = 6 μm.

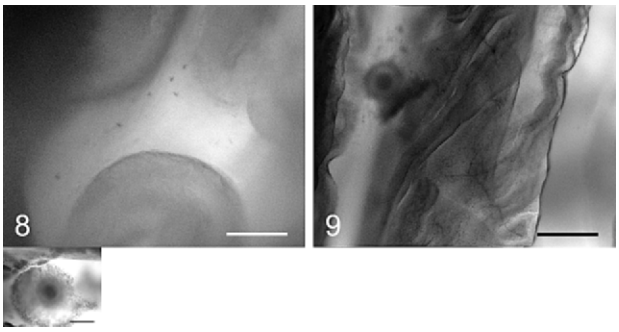

PLATE III. Figures 8 and 9.
FIG. 8. As soon as a vital COC is in the oviduct, the sperm leave the reservoir in the isthmus and hurry to the oocyte. A photo of the COC in the ampulla has been added to clarify the position of the COC, which is in the ampulla. Bar = 200 μm. See Supplemental Movie 8.
FIG. 9. After one sperm has penetrated the zona pellucida, the oocyte continues its migration down the oviduct. Bar = 200 μm. See Supplemental Movie 9.

(Supplemental Movie 3, star). The COC could only be resolved by destroying the cumulus cells. When an immature COC was put into the oviduct (Fig. 4 and Supplemental Movie 4a), it also attached to the oviductal epithelium, pointing to the fact that the adhesion of the COC to the oviductal epithelium is not dependent on maturation. The adhesion of the COC occurred regardless of whether the COC had been matured in vivo or in vitro. The adhesion always occurred in the presence of the strong physiological ciliary beating (see Supplemental Movie 4b: arrow marks cilia, star marks the body of a ciliated cell). However, a degenerated COC characterized by irregular form (arrow in Fig. 5 and Supplemental Movie 5) and spotted color of the cytoplasm of the oocyte was floating in the oviductal lumen (Fig. 5 and Supplemental Movie 5). When a denuded

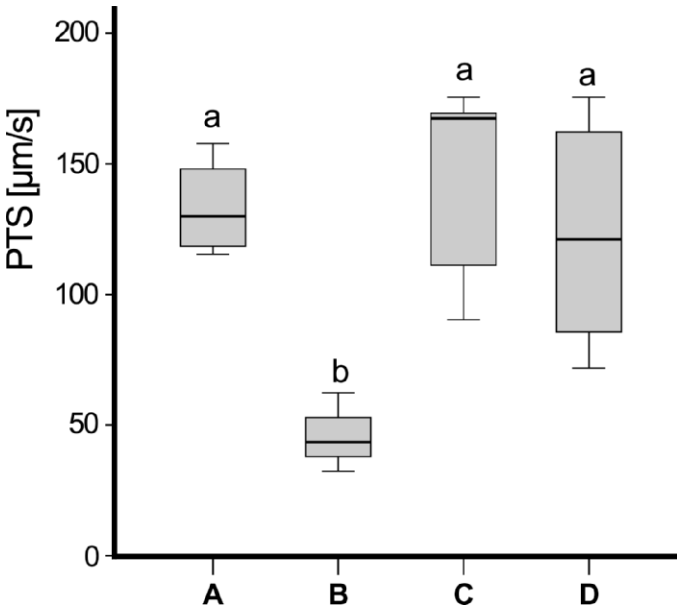

FIG. 10. Particle transport speed (PTS) in the oviduct. A: cycle (metestrus); B: Days 2.5 and 3.5 of pregnancy: site of the embryo in the oviduct; C: Days 2.5, 3.5, and 4.5 of pregnancy: ampulla and isthmus without embryo; D: Days 18–120 of pregnancy: early pregnancy after implantation. Statistical analysis was carried out by the Kruskal-Wallis test, followed by the Mann-Whitney test. $P < 0.05$ was rated significant. Significant differences are marked by different lowercase letters.

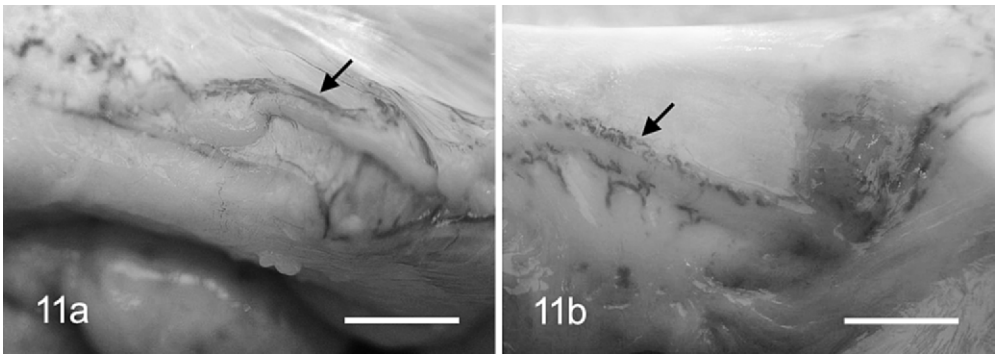

FIG. 11. Macroscopic view on the contralateral and ipsilateral oviducts on Day 3 of pregnancy. Bar = 15 mm. **a**) In the contralateral oviduct, the arteria tubae uterinae runs straight and parallel to the oviduct (arrow). **b**) When the embryo is in the oviduct, the arteria tubae uterinae exhibits a strongly winded pattern. At the same time, the ipsilateral oviduct appears to be thicker, more edematous, and more transparent than the contralateral oviduct (arrow).

oocyte was put into the oviduct, it moved to the depth in between the folds. It did not attach, but was turning and slightly moving around because of the ciliary beating (Fig. 6 and Supplemental Movie 6, star). Thus, the cumulus cells and their intercellular matrix are essential for the attachment of the COC to the oviductal epithelium. This was confirmed by the fact that when the oviductal cells started to degenerate, the COC was not able to attach anymore. If in addition to the cumulus cells the ZP was removed, the oocyte moved into the depth of the oviductal folds and moved around, indicating that the ZP is not involved in the attachment of the COC to the oviductal epithelium.

As soon as the sperm reached the oviduct, they formed a sperm reservoir in the isthmus (Fig. 7a and Supplemental Movie 7, arrows). When forming the reservoir, the sperm bound with their heads in a tangential angle exclusively to ciliated cells (Fig. 7b). When comparing the binding capacity of native and frozen semen to the oviductal epithelium, it became obvious that the density of sperm binding was much higher in native semen. After applying the swim-up procedure in frozen-thawed semen, binding density was clearly improved. However, distinct interindividual differences in binding density were observed in the experiments.

Fertilization

As soon as a vital COC was in the ampulla, the sperm bound in the sperm reservoir were hyperactivated, left the reservoir, and hurried to the oocyte in the ampulla (Fig. 8 and Supplemental Movie 8). When the sperm left the reservoir, they first moved into the mid oviductal lumen. Although the current of the oviductal fluid was stronger in the mid tube, it was easier to move forward there because near the oviductal epithelium the presence of mucus or—if there was locally less mucus—strong whirls inhibited straight sperm movement. When observing the movement of the sperm, it became obvious that contractions of the oviductal smooth muscles were an essential support for the sperm to successfully move against the strong current caused by ciliary beating.

As soon as a sperm had penetrated the ZP, the presumptive zygote detached and continued its migration (Fig. 9 and Supplemental Movie 9).

PTS Before and after Fertilization

Before fertilization, the PTS did not significantly differ in ipsilateral and contralateral ampulla and isthmus, respectively. However, after fertilization the speed of transport was significantly lowered at the site of the embryo (mean, 46 μm/sec; Fig. 10). Thus, in the course of its migration down the oviduct (i.e., at Days 1–3.5 of pregnancy), the embryo was able to locally downregulate the speed of transport. As soon as the embryo entered the uterus, the speed of transport in the oviduct caused by ciliary beating was upregulated. After implantation and in the further course of pregnancy (Days 18–120), there were no significant differences in PTS compared with that of the cycle (metestrus; Fig. 10).

Local Effects of the Early Embryo

When comparing the ipsilateral and contralateral oviducts, it became obvious that the embryo was able to locally induce changes in vascularization. Thus, in the contralateral ampulla and isthmus, the arteria tubae uterinae was running straight and parallel to the oviduct (Fig. 11a). During the stay of the embryo in the oviduct (Days 1–3 after fertilization), the arteria tubae uterinae was running in a strongly winded way, especially at the site of the embryo (Fig. 11b). The wall of the ipsilateral oviduct was thicker, more edematous, and more transparent than the contralateral oviduct (Fig. 11b). As demonstrated by scanning electron microscopy analysis of the whole oviduct, the early embryo was able to induce the formation of secretory cells. Figure 12 shows the morphology of the oviductal epithelium in the ipsilateral oviduct 3 days after fertilization. In the first part of the oviduct (ampulla), which the embryo had passed 1.5 days earlier, there were secretory and ciliated cells in the oviductal epithelium (Fig. 12a). The active secretory cells were characterized by a round form and the lack of surface modifications (Fig. 12a). In the beginning of the isthmus, where the embryo had been 1 to 0.5 days before, the formation of secretory cells out of ciliary cells was seen (Fig. 12b). In this area, the secretory cells showed kinocilia of different lengths (Fig. 12b). At the end part of the isthmus, where the embryo was found (Fig. 12c, inset), mainly ciliated cells were present in the oviductal epithelium (Fig. 12c). Counting of secretory and ciliated cells in an area of 11 000 μm^2 showed that in the first part of the ampulla, 68% of all cells were secretory cells, and in the beginning of the isthmus, 62% of all cells were secretory cells. However, in the end part of the isthmus, merely 19% of the cells were secretory cells, which were hidden in the depths in between the ciliated cells. Statistical analysis using Pearson chi-square test revealed that the proportion of secretory to ciliated cells was significantly higher in the ampulla and the beginning of the isthmus compared with the end part of the isthmus (Fig. 13).

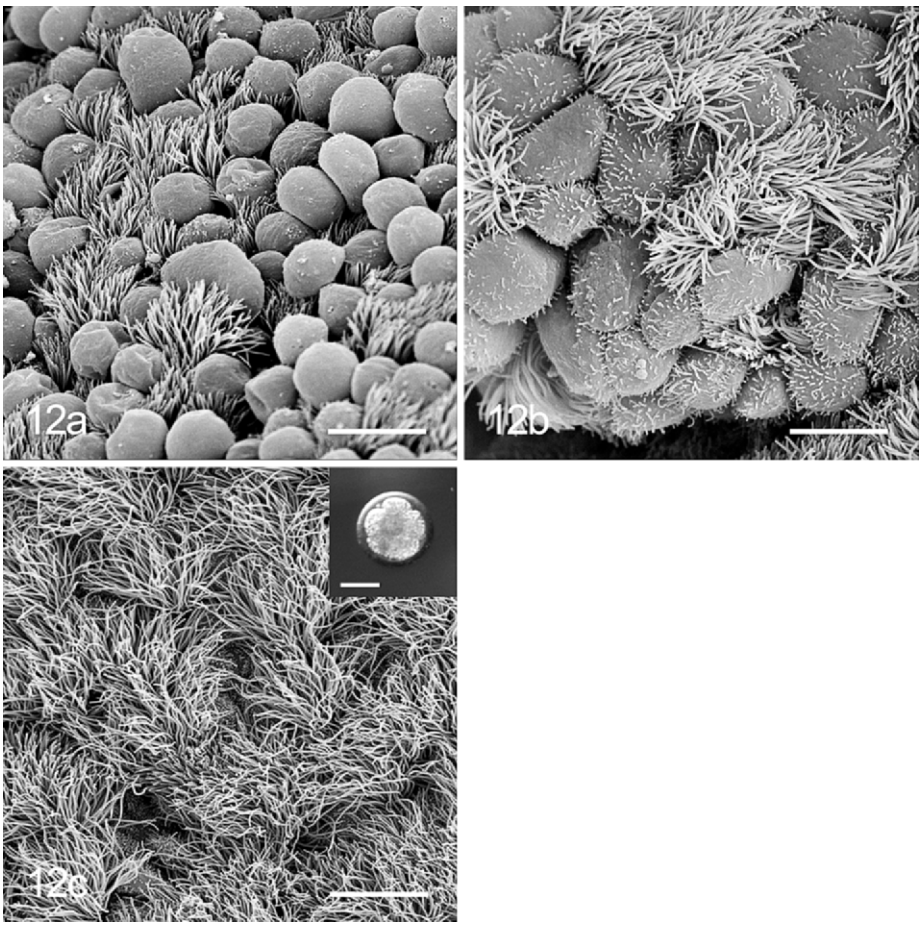

FIG. 12. Scanning electron microscopy of an ipsilateral oviduct on Day 3 of pregnancy. **a**) In the ampulla, where the embryo has been 1.5 days before, there are secretory and ciliated cells in the epithelium. Bar = 9 μm. **b**) In the entry of the isthmus, where the embryo passed 1 to 0.5 days previously, the formation of secretory cells from ciliary cells is seen. Bar = 7 μm. **c**) In the end portion of the isthmus, where the embryo (eight-cell stage, inset) is found, only ciliated cells are present in the oviductal epithelium. Bars = 9 μm (**a–c**) and 75 μm (insert in **c**).

DISCUSSION

In the course of our studies, we were able to establish a digital videomicroscopic analysis system that allows us to analyze and document the basic mechanisms of gamete transport, fertilization, and early embryonic development in the oviduct at near in vivo conditions. With this system, we were able to show for the first time that the mechanisms of transport are completely different in ampulla and isthmus. Thus, in the ampulla, ciliary-driven transport takes place in the depths between the folds. Similarly to the Dynabead particles, the COC tends to move into the deep folds and settle down. For the COC, this is the first time of contact with the oviductal epithelium, thus creating the prerequisite for the first communication between COC and maternal genital tract. Contrary to the ampulla, the isthmus exclusively serves to provide rapid transport of the presumptive zygote to the uterus. This is shown by observations of the transport of the COC after fertilization, and it is confirmed by the fact that in the isthmus, settled particles are quickly removed and guided back to the apical ridges of the oviductal folds. As shown by quantitative measurements, the average PTS does not differ in the ipsilateral ampulla, contralateral ampulla, ipsilateral isthmus, and contralateral isthmus in the cycle and during pregnancy after implantation. Thus, in the oviduct, ciliary beating is relatively constant, ensuring mucociliar clearance of the tube. The constantly high PTS in the oviduct is managed in spite of the fact that the proportions of ciliated and secretory cells significantly differ in the cycle and during pregnancy [28, 29]. This implies that in the oviduct, even a smaller percentage of ciliated cells is able to ensure constant PTS and sufficient mucociliar clearance.

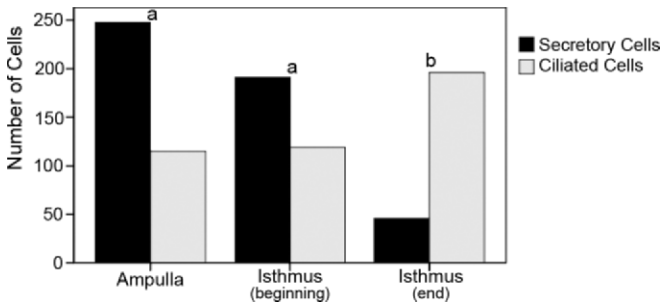

FIG. 13. Number of ciliated and secretory cells in the ipsilateral oviduct on Day 3 of pregnancy (average size of measured area, 11 000 μm^2). In the ampulla and in the beginning of the isthmus, where the embryo has been 1.5 days previously (1 and 0.5 days ago, respectively), the proportion of secretory cells to ciliated cells is significantly higher compared with the end part of the isthmus, where the embryo is located (Pearson chi-square test, $P < 0.001$). Significant differences are marked by different lowercase letters.

Our study is the first to show that as soon as the COC enters the ampulla, it attaches to the oviductal epithelium. In the hamster, it has been documented that in the infundibulum, the oocytes pickup is based on first a slight adhesion of the COC to the tips of the oviductal cilia, and subsequent sliding of the COC over the surface of the infundibulum [23]. The adhesion occurs between the cilia and the cumulus matrix [3, 30]. Similarly, we could show that the adhesion of the COC to the ampullar epithelium is mediated by cumulus cells and their matrix. Both mature and immature oocytes are able to attach to

the oviductal epithelium, pointing to the fact that maturation is not necessarily essential for attachment. However, as soon as the oocyte is degenerated, it is floating in the oviductal lumen. This implies that the oviduct is able to select vital oocytes. With this knowledge, new and more sensitive methods of quality assessment of oocytes used for in vitro fertilization can be established.

Intense research has focused on the formation of the sperm reservoir in the last years. By binding to the oviductal epithelium, the fertility of the spermatozoa is maintained until ovulation [21, 31]. In the bovine, fucose is a key component of the ligand for spermatozoa on the epithelium [32, 33]. Our videomicroscopic studies showed that the binding of sperm to the oviductal epithelium, which is mediated by the bovine seminal plasma protein BSP A1/A2 [34, 35], is distinctly better in native semen compared with frozen-thawed semen. Additionally, clear individual differences in sperm binding were seen, indicating that binding capacity may be correlated with fertility. Similar observations were made by Petrunkina et al. [36], who found that sperm binding to oviductal explants in vitro was individually different in boars [36]. Our studies showed that as soon as a vital COC is in the ampulla, the sperm become hyperactivated and are released from the epithelium. In in vitro studies, it has already been shown that capacitation significantly reduces sperm binding [37], providing evidence that hyperactivation is essential for the sperm to leave the reservoir [38]. In diseases such as endometriosis, sperm binding is altered so that merely a few sperm can leave the reservoir [39]. As soon as the vital COC is in the oviduct, the sperm released from the reservoir hurry to the oocyte. This straight and direct movement in the oviduct is in strong contrast to the fertilization in vitro, during which sperm move in an aimless way and meet the COC by accident. These observations indicate that specifically the interaction between cumulus cells and oviduct may result in a signaling cascade that directs the sperm to the COC.

Although it has been shown that viability of sperm, oocytes, and embryos is enhanced in the presence of oviductal cells [40], the basic underlying mechanisms are unknown. In our studies, we were able for the first time to elucidate the local effects of the embryo during its stay in the oviduct. Thus, the early embryo modifies vascularization and induces the formation of secretory cells, ensuring optimal microenvironment and nutrition during the first days of the embryo's life. These effects already occur 24–48 h after fertilization, implying that signal transduction cascades are rapidly initiated by local signals of the embryo. As shown by quantitative measurements, the early embryo is additionally able to downregulate the speed of transport caused by ciliary beating in the oviduct. In the ampulla, the early embryo settles down in the depths between the folds and gets in close contact with the oviductal epithelium, and the first embryo-maternal communication can take place.

In summary, we established a new videomicroscopic system in the oviduct that allowed us to elucidate basic mechanisms of ciliary transport, fertilization, and the interaction between gametes/embryo and oviductal epithelium under near in vivo conditions. This knowledge is essential to improving the techniques of assisted reproduction and to finding new causal therapies for clinical subfertility, infertility, and insufficient pregnancy rates.

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