Sperm-Induced Modification of the Oviductal Gene Expression Profile After Natural Insemination in Mice

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ABSTRACT

In mammals, the physiological interaction between spermatozoa and oviductal epithelia involves intimate and specific contact between the two cell types. Spermatozoa may undergo stringent selection processes within the female reproductive tract before they meet and fertilize oocytes. The physiological basis of the sperm selection process is largely unknown. Here we tested the hypothesis that the oviduct has a recognition system for spermatozoa that can detect the arrival of spermatozoa in the oviduct after insemination, resulting in alterations of the oviductal transcriptome. We initially performed a global screening of the oviductal transcriptome in mice 1) at the time of estrus (mating) and 2) 6 h after mating. Transcriptional alterations in the oviduct after mating were attributed to the presence of spermatozoa in the oviduct after mating and also to changes in the hormonal environment as female mice underwent the transition from estrus to diestrus. To distinguish these possibilities, female mice were then mated with T145H mutant mice, which because of spermatogenic arrest, produce seminal plasma but no spermatozoa. Focusing on two molecules that in the first experiment were upregulated after mating, it was found that adrenomedullin and prostaglandin endoperoxidase synthase 2 transcripts were upregulated in the oviducts of mice only after mating with fertile males; those mated with T145H infertile males showed significantly less response. These results indicate that it is the arrival of spermatozoa in the oviduct that activates one or more signal transduction pathways and leads to changes in the oviductal transcriptome profiles.

female reproductive tract; oviduct; sperm; sperm motility and transport

INTRODUCTION

The mammalian oviduct is the venue of important events leading to the establishment of pregnancy. These events include final maturation and transport of the female and male gametes, fertilization, early cleavage-stage embryonic development, and transport of the embryo to the uterus. In mammals, the physiological interaction between gametes, embryos, and oviductal epithelia involves intimate and specific contact between the two cell types [1–4].

Much work has been performed to characterize the effects of the oviduct on spermatozoa, oocytes, and embryos; however, less is known about the converse effect, that of gametes and embryos on the oviduct. Embryos are known to affect the transcriptomic profile of the mouse oviduct; this has been demonstrated by comparing gene expression in mouse oviducts containing early embryos with that of oviducts containing unfertilized oocytes [5]. Oocytes also seem to induce oviductal gene expression; recently, Bauer- Sachs et al. [6] reported changes in oviduct epithelial cell (OEC) gene transcription activity by comparing gene expression profiles of ipsilateral and contralateral oviductal epithelia of individual cows after ovulation. These changes are likely to have resulted from the direct contact of oviductal epithelial cells with ovulated cumulus-oocyte complexes.

In birds, spermatozoa seem to induce gene expression in the female reproductive tract. Long et al. [7] have demonstrated that in turkeys, the gene expression profile of sperm storage tubules inseminated with spermatozoa differs from that of tubules inseminated only with extender. It is unclear whether entry of spermatozoa into the mammalian oviduct after insemination in vivo can be recognized by the epithelium, which then initiate a similar response to that of oocytes and embryos.

Spermatozoa are believed to undergo stringent selection processes within the female reproductive tract before they meet and fertilize oocytes [8, 9]. This selection process is especially evident when spermatozoa from more than one male are present simultaneously [10]. Because the female reproductive tract skews sperm selection [11, 12], this results in a process known as cryptic female choice [8, 9]. The physiological basis of the sperm selection process is largely unknown, but whereas sperm-dependent characteristics such as cell shape and vigor of movement have been implicated [13], it is plausible that spermatozoa may elicit a favorable environment for themselves by ensuring that their cell surface characteristics are also compatible with those of the female reproductive tract. If the female reproductive tract can interpret sperm surface proteins in terms of male genetic quality and fitness, the sperm-oviduct dialogue would offer a means both to provide information and to act on it.

Unpublished in vitro observations of porcine OECs and boar spermatozoa performed in our laboratories first alerted...
us to the possibility that spermatozoa could elicit changes in gene expression in cultured cells. For those experiments we exposed growing OECs to washed spermatozoa, and found that 3-h exposure was sufficient to provoke enhancement and depression of gene expression, as detected using differential display methodology. Unfortunately, this approach produces a number of flawed results, mainly false positives, and prompted us to seek an alternative way of testing the hypothesis that when spermatozoa bind to oviductal cells they initiate a cell-cell dialogue that leads to altered gene expression in the OEC. The current investigation of this hypothesis is therefore based on in vivo observations in female mice. We have compared the oviductal gene expression profiles of mouse oviducts that were obtained 6 h after natural mating with those of controls at the same stage of the estrous cycle. To discriminate the effects of spermatozoa from those induced by copulation, or by the effects of seminal plasma, or both, we controlled the study by use of T145H mice that both copulate and ejectulate, but, because they exhibit spermatogenic arrest, their ejaculates do not contain spermatozoa.

**MATERIALS AND METHODS**

**Oviduct Collection and RNA Isolation**

Outbred TO female mice (Harlan, Oxon, U.K.) were used in these experiments. All procedures relating to the care and use of animals were performed according to United Kingdom Home Office regulations, Animals (Scientific Procedures) Act 1986. Oviducts were collected after the animals were killed from 1) two groups of mice at estrus, 2) three groups of mice 6 h after mating with fertile TO mice, and 3) two groups of mice 6 h after mating with T145H infertile mice (Harlan). Each group, representing technical replicates in which variability due to individual should be minimized, comprised a minimum of 15 mice. Estrus was detected using vaginal swabs that were stained and examined for keratinized epithelial cells. For timed matings, male mice were kept for 2 h with cycling females to allow mating to occur. Thereafter, mice were separated, and 5 h later, females were checked for the occurrence of insemination plugs. Those with insemination plugs were regarded as having been mated approximately 6 h earlier, and, after being killed, their oviducts were collected. Total RNA was isolated from oviducts using Trizol Reagent (Invitrogen, Paisley, U.K.). Total RNA samples were further purified using an RNaseasy kit (Qiagen, Crawley, U.K.).

**Microarray Hybridization**

For initial global screening of the postmating oviductal transcriptome, cRNA were prepared from total RNA obtained from oviducts of two groups of mice at estrus and from three groups of mice 6 h after mating with fertile mice as described in the Affymetrix GeneChip Expression Analysis Technical Manual (http://www.affymetrix.com). Probe prepared from each group was hybridized to a high-density Affymetrix murine U74A2V2 Genechip oligonucleotide microarray (five microarrays in total; two groups at estrus, and three groups 6 h after mating with fertile mice) on a GeneChip System (Affymetrix) according to the manufacturer’s instructions. Scanned chip images were analyzed using the Microarray Suite software version 5.0 (Affymetrix) to obtain absence/presence calls and fold ratio values for different probes. The results were exported into Excel (Microsoft, Redmond, WA) for further analysis.

**Microarray Data Analysis**

The transcriptome profile obtained for each group of mice in estrus was compared with that of each group of mice 6 h after mating in six independent comparisons. The ‘signal log ratios’ were converted to fold changes. Only transcripts showing consistent increasing or decreasing changes in at least four of six comparisons with an average of more than 2-fold change were included for further analyses. The annotation tool provided by Dragon Database [14] was used to query the Swiss-Prot database [15] database for functional and structural keywords. Dragon View [16] was used to classify into different Swiss-Prot keyword families those genes differentially expressed after mating. The comparative analysis of expression data using the Gene Ontology [17] structured vocabulary was performed using the GoSurfer analysis tool (www.biostat.harvard.edu/com-plab/gosurfer) [18].

**Real-Time Reverse Transcriptase-Polymerase Chain Reaction**

Total RNA from two groups of mice at estrus, two groups of mice 6 h after mating with fertile mice, and two groups of mice 6 h after mating with T145H infertile mice was used in the experiments. Total RNA was treated with DNase I (DNA-free Kit; Ambion, Huntingdon, U.K.) to remove genomic DNA contamination from samples. First-strand cDNA synthesis was performed using the Superscript II reverse transcriptase system (Invitrogen). Real-time polymerase chain reaction (PCR) was performed using a LightCycler rapid thermal cycle system (Roche Diagnostics, Lewes, U.K.) and Lightcycler-Faststart DNA master SYBR green I (Roche) reaction mix. The quantification data were analyzed with LightCycler analysis software. For detection of adenornedullin (Adn), the forward primer 5'-GCA GTT CCG AAA GAA GTG GAA-3' and reverse primer 5'-TTC GCT CTT ATT GCT GCC TT-3' were used. Prostaglandin endoperoxidase synthase 2 (Pges2) was detected using the forward primer 5'-GTG CTG ACA TCC AGA TCA TAT TGG-3' and reverse primer 5'-TCC TTA TTT CCC TTC ACA CCC A-3'. Finally, for the detection of β-actin, forward primer 5'-AGC CTT CCT TCT TCT GGA TGG AAT-3' and reverse primer 5'-GGA GCA ATG ATC TTC ATC-3' were used. The amplification products of Adn, Pges2, and β-actin were 178, 164, and 211 base pairs, respectively.

Four measurements were carried out (duplicate measurements for each group of samples), and the results were presented as mean ± SEM arbitrary gene expression values, normalized on the basis of β-actin expression. Analysis of variance was used to test the overall significance of difference of Adn and Pges2 expression values between different groups, and least square difference analysis was used to evaluate specific contrasts between individual groups. Statistical significance was regarded as P ≤ 0.05.

**RESULTS**

Expression levels of 11 263 transcripts (93% of total transcripts present on the oligonucleotide array) consistently increased, decreased, or remained unchanged (in four or more comparisons out of six) when the oviductal transcriptome of mice in estrus was compared with that of mice 6 h after mating. Expression levels of 214 genes (2% of transcripts presented on the oligonucleotide array) consistently changed 2-fold or more at 6 h after mating, compared with mice in estrus. Fifty-eight transcripts were upregulated, and 156 were downregulated. The complete list of altered oviductal transcripts is available at the following Internet address: http://www.sheffield.ac.uk/alirezafazeli/file1.xls. A subset of these genes involved in signal transduction, transcription regulation, and cell adhesion is presented in Table 1.

Many of the genes identified as undergoing change after mating were annotated by the Swiss-Prot database [15]. Those that were annotated were classified into various functional and structural categories. A summary presentation depicting some of the Swiss-Prot keyword categories is provided in Figure 1. A comprehensive list of these genes is available at this address: http://www.sheffield.ac.uk/alirezafazeli/file2.xls. We merged the set of genes undergoing upregulation in the oviduct with the set undergoing downregulation 6 h after mating in order to view the biological processes being modulated. The combined merged set was mapped onto the gene ontology [17] hierarchy for biological processes. The resulting map was displayed as a tree that showed only those nodes (biological process terms) represented by genes upregulated, downregulated, or both in the oviduct 6 h after mating. Figure 2A shows this tree at low resolution, depicting only those biological processes involving genes undergoing upregulation, downregulation, or both 6 h post-mating (gray structures with dots representing nodes). The
most general processes are represented at the top (examples are marked by numbers); the most specialized processes are shown at the bottom. Nodes matching at least one upregulated gene (red dot) and those matching at least one downregulated gene (green dots) (many match both; yellow dots) are superimposed on this tree. A comparable tree was also produced for molecular functions with sets of upregulated or downregulated (or both) postmating regulated genes superimposed on it (Fig. 2B).

To determine whether the mating-induced changes in gene expression were caused mainly by spermatozoa or whether alternative causes could also be involved, we compared situations in which copulation occurs with and without the insemination of spermatozoa. For this purpose we repeated the experiments, but in some cases we used T145H males instead of normal TO males. These males produce ejaculates that contain seminal plasma but lack spermatozoa. We used real-time PCR analysis, a technique different from microarray analysis and not based on in situ hybridization, to verify the results obtained in our initial global analysis. We checked expression values of Adm and Ptgs2 in the mouse oviduct during estrus, 6 h after mating with fertile mice, and 6 h after mating with T145H mice (Fig. 3, A and B, respectively). Both Adm and Ptgs2 expression values were significantly increased after mating with fertile mice compared with those of mice in estrus or those mated with T145H mice.

**DISCUSSION**

Two previous in vitro studies [19, 20] investigated the possibility that spermatozoa can induce de novo protein synthesis. Novel protein synthesis was apparently detected in cultured oviduct cells to which spermatozoa were added. However, the proteins could not be defined. The results of the present study strongly support the concept that spermatozoa elicit such de novo protein synthesis in vivo.

In mammals, there is little likelihood that seminal plasma reaches the oviduct after mating [21, 22], the utero-tubal junction acts as an effective filter, and thus the evidence presented here supports the hypothesis that when spermatozoa enter the oviduct they elicit changes in gene expression. T145H mice are infertile because spermatogenesis is arrested during the first meiotic division but otherwise show normal mating behavior, and no difference has been reported in their seminal plasma composition compared to that of fertile mice [23]. The lack of postmating Adm and Ptgs2 upregulation after mating with these mutants therefore indicates that these two genes are responding specifically to the arrival of spermatozoa.

Adm [24] is a hypertensive peptide found in human pheochromocytoma; it consists of 52 amino acids and shows a slight homology with the calcitonin gene-related peptide. It may function as a hormone in circulation control because it is found in blood in a considerable concentration [24]. The significance of Adm upregulation after arrival of sperm in the oviduct remains unclear to us. Adm is, interestingly, localized to the apical surface of the oviductal epithelium [25], which is the precise site of sperm-OEC interaction.

The relevance of Ptgs2 (also known as cyclooxygenase-2, Cox-2) in uterine function, to blastocyst implantation in particular, has previously been demonstrated [26]. It could be argued that the arrival of a blastocyst into the uterus represents another situation in which cells from two distinct individuals meet, but function cooperatively. Infections that represent a similar but undesirable interaction are known to induce 10-fold to 20-fold increases in Ptgs2 (Cox-2) expression. It is therefore not surprising that OECs would

**TABLE 1. A subset of genes involved in signal transduction, transcription regulation, and cell adhesion.***

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*Altered oviductal transcripts in mouse 6 h postmating compared to oviduct in estrus.
recognize and respond to spermatozoa as they would to the presence of pathogenic bacteria.

In mice, the duration of the estrous cycle is 4–5 days and is divided into four stages: proestrus, estrus, metestrus, and diestrus. Diestrus is characterized by the maintenance of basal estrogen concentrations, atrophic endometrium, and sexual inactivity. During proestrus, uterine edema and proliferation of endometrial cells occur. Ovulation and sexual receptivity are diagnostic characteristics of estrus when the uterus becomes trophic under a high level of estrogen and other ovarian steroids. In mice, the duration of estrus is approximately 12 h [27]. Female mice maintained on a constant light:dark cycle tend to ovulate 3–5 h after the onset of estrus. Males maintained under the same condition will copulate with females in estrus at about the midpoint of estrus, approximately 1–2 h after ovulation [28]. Thus oviducts in both groups of mice in our study (mice in estrus and 6 h postmating) were under the influence of estrogen. Changes observed in global gene expression after mating in our study are probably due to the arrival of spermatozoa in the oviduct and the effects (including stress) of the act of mating itself. Whereas oviductal reactions to dynamic changes in the local concentrations of estrogen and other reproductive hormones cannot be excluded, the experiment with T145H mice would support the view that these effects were, at least with regard to Adm and Ptg2 genes, of secondary importance.

When we initially compared gene expression values from 1) unmated outbred female mice (TO) in estrus and 2) TO female mice that had mated naturally with fertile TO males, the results indicated a 2% change in oviductal gene expression values of these genes between mice mated with fertile normal male mice and those mated with T145H infertile male mice or mice at estrus.

FIG. 2. Global comparison of biological processes (A) and molecular functions (B) associated with genes undergoing upregulation, downregulation, or both in oviduct 6 h postmating. The tree (dots and connecting paths) represents those categories (dots) in the gene ontology [17] hierarchy for biological processes (A) and molecular functions (B) that undergo upregulation, downregulation, or both in oviduct 6 h after mating. Superimposed on the tree are the biological processes that matched one or more genes upregulated (red dots), downregulated (green dots), or both (yellow dots) in oviduct 6 h postmating. A) The most general biological processes are represented at the top, and selected examples are marked by the following numbers: 1, behavior; 2, cell communication; 3, cell growth, maintenance, or both; 4, death; 5, development; 6, physiological processes. The most specialized processes are shown at the bottom of the tree. Nearly all general biological processes underwent upregulation or downregulation 6 h after mating. Except for genes involved in behavior, which were only downregulated (B), the most general molecular function categories are represented at the top, and selected examples are marked by the following numbers: 1, defense/immunity protein; 2, enzyme; 3, enzyme regulator; 4, motor; 5, signal transducer; 6, transcription regulator; 7, apoptosis regulator; 8, binding; 9, cell adhesion molecule. The most specialized categories of molecular functions are shown at the bottom of the tree. Genes involved in defense and immunity were upregulated, and genes involved in motor and apoptosis regulation were downregulated.

FIG. 3. Adm (A) and Ptg2 (B) expression values (normalized based on β-actin expression values) in oviduct in mice at estrous, 6 h after mating with fertile mice, and 6 h after mating with T145H infertile mice. There was a significant (P < 0.05) difference in oviductual gene expression values of these genes between mice mated with fertile normal male mice and those mated with T145H infertile male mice or mice at estrus.
responses involving cytokines and the inflammatory response are focused within a surprisingly small group of transcripts. Nearly all general biological processes presented in the oligonucleotide array have gene members that undergo solely upregulation and downregulation after mating (Fig. 2A). Genes involved in behavior were the only group to have been downregulated. Oviductal genes modulated solely by mating involved a small proportion of the molecular functions presented on the oligonucleotide array (Fig. 2B).

The mammalian oviduct appears to be an important participant in the sperm selection process; it selectively sequesters and stores spermatozoa, gradually releasing them around the time of ovulation. This intimate association between two cell types that originate from different individuals in which both cells continue to fulfill their natural function presents an unusual and nonpathogenic combination. In other physiological situations in which such intercellular interactions occur, modulation of gene expression in one or both of the participants, such as the interaction of lymphocytes and pathogens, de novo expression is well known [29]. One might speculate that sperm from different individuals might elicit different responses in the oviduct. There is both theoretical and experimental support for the belief [30] that the female reproductive tract preferentially selects spermatozoa from males with so-called “good genes” [31] or rejects genetically incompatible spermatozoa [32]. Several authors have shown that spermatozoa are able to move through the utero-tubal junction into the oviduct only if their flagellar activity is correctly regulated [33]. This example provides a genetically based, biochemical explanation for transmission ratio distortion in mice heterozygous for the t haplotype of chromosome 17 [34], in which spermatozoa with defective flagellar action caused by inappropriate smok kinase activity fail to reach the oviduct. The female reproductive tract thus has an important role in genetic selection that is especially likely to exert its influence in species in which sperm competition is the norm [35]. Support for this idea was provided by Wyckoff et al. [36], who showed that unusually rapid (>10-fold faster) evolution of spermatogenesis-associated genes is probably a consequence of sperm selection-based processes. The present results suggest that spermatozoa also act on the oviduct by providing signals and eliciting feedback that may be significant in the sperm selection process.

In conclusion, our findings demonstrate for the first time the presence of an in vivo sperm-recognition mechanism in the mammalian oviduct in which arrival of spermatozoa in the oviduct triggers a signal transduction pathway leading to the modulation of gene expression. The existence of such a system profoundly influences our views of oviductal function in relation to sperm transport and the selection of spermatozoa. This recognition system may be used to differentiate between spermatozoa from different males, thus allowing fertilization success to be biased in the favor of the more biologically attractive male.

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REFERENCES


