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Temporal Surge of Glycosyltransferase Activities in the Genital Tract of the Hamster during the Estrous Cycle[1]

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ABSTRACT

Mammalian spermatozoa must undergo maturational changes between the events of mating and fertilization. These biochemical and functional alterations, collectively termed capacitation, take place as spermatozoa traverse the female reproductive tract. The preparatory biochemical changes include removal, modification, and reorganization of sperm surface molecules. Although details of all the changes are not known, lectin binding studies have provided evidence suggesting that carbohydrate moieties of sperm surface glycoproteins are modified during capacitation. In an attempt to gain insight into the potential modifications of sperm plasma membrane glycoproteins, we quantified glycoprotein-modifying enzyme activities in the uterine and oviductal fluid of the hamster during the 4 days of the estrous cycle. These enzymes are known to modify existing glycoproteins, either by adding sugar residues (glycosyltransferases) or by removing terminal sugar residues (glycosidases). Data from these studies showed that 1) levels of all glycosyltransferase activities assayed (sialyltransferase, fucosyltransferase, galactosyltransferase, and *N*-acetylglucosaminyltransferase) were negligible in the uterine fluid at the onset of ovulation (Day 1) but sharply increased preceding ovulation (Day 4); 2) levels of the four glycosyltransferase activities assayed were higher in the oviductal fluid at the onset of ovulation (Day 1) and then gradually decreased through the remainder of the estrous cycle (Day 2 to Day 4); 3) levels of all glycohydrolase activities (acidic α-D-mannosidase, β-D-galactosidase, β-D-glucuronidase, β-D-glucosaminidase, and α-L-fucosidase) and protein in the uterine and oviductal fluids did not vary widely during the 4 days of the cycle. These results demonstrate a temporal surge of glycosyltransferase activities in the genital tract fluids of the hamster. The temporal changes in the glycoprotein-modifying enzymes may have an effect on the glycosylation of sperm plasma membrane and zona pellucida glycoproteins at the site of fertilization or may alter the surface glycoproteins of the fertilized egg in the uterus prior to implantation.

INTRODUCTION

The fertilization process in mammals takes place in a complex microenvironment within the female genital tract [1]. The ejaculated spermatozoa cannot immediately fertilize an oocyte, but during residence in the female reproductive tract they undergo a series of biochemical and functional modifications collectively referred to as capacitation. These modifications are necessary before spermatozoa can bind to the zona pellucida, the extracellular glycocalyx that surrounds the mammalian oocyte, and penetrate it [1–3]. Although the precise details of these modifications are poorly understood, it is generally believed that the process involves a series of intracellular and extracellular changes, including the removal of seminal plasma proteins absorbed to the sperm surface, reorganization of the sperm surface molecules, and alterations of sperm surface glycoproteins [4].

The current knowledge on capacitation has been provided by the pioneering studies by Yanagimachi and Chang [2, 3]. These researchers were the first to report successful fertilization of hamster eggs in vitro through use of spermatozoa recovered from the female reproductive tract after mating or use of epididymal spermatozoa preincubated with oviductal secretions [3]. These studies suggest the presence of factor(s) in the female genital tract secretions responsible for the induction of capacitation. Lectins, a class of plant proteins capable of binding to terminal sugar residues with high affinity and specificity, have been used to examine carbohydrate modifications on the hamster spermatozoa during capacitation. Results have provided evidence that sperm surface glycoproteins are modified during capacitation in a time-dependent manner [5, 6]. Two sets of enzymes are capable of modifying the existing sperm plasma membrane glycoproteins either by adding sugar residues (glycosyltransferases) or by cleaving terminal sugar residues (glycohydrolases).

The purpose of the studies presented in this report was to investigate the existence of glycoprotein-modifying enzymes (glycosyltransferases and glycosidases) in the reproductive tract of the hamster during the estrous cycle. This animal has proven to be an excellent model for examining cellular processes involved in ovulation and fertilization because the estrous cycle in this species is relatively stable and can be timed accurately by simple observation of postestrous vaginal discharge. Data presented in this report indicate that the activity of glycosyltransferases (but not glycohydrolases) is modulated in a temporal manner during the

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estrous cycle. The temporal surge in the glycosyltransferase activities may be important in the regulation of the oviductal microenvironment during and after fertilization.

MATERIALS AND METHODS

Materials

Golden hamsters (130–150 g) were obtained from Sasco (Omaha, NE). The animals were divided into four groups on the basis of day of the estrous cycle as previously described [7]. Briefly, animals showing postestrous discharge in the vagina represented animals immediately after ovulation (Day 1). In these animals, ovulation had occurred at or near midnight between Day 4 and Day 1 (onset of ovulation) and was observed on the morning of Day 1. The other animal groups were Day 2 (diestrous Day 1), Day 3 (diestrous Day 2), and Day 4 (proestrus). All animals were given free access to food and water and were kept in our animal facility under a constant 14L:10D cycle. The uterine and oviductal fluids used were collected from all groups of animals between 1000 and 1130 h. The exogenous acceptor glycoproteins (asialo-ovomucoid, asialo-agalacto-ovomucoid, and asialo-agalacto-aglucosamino-ovomucoid) were prepared according to our published procedures [8, 9]. All *p*-nitrophenyl (PNP) glycosides and unlabeled nucleotide sugars were from Sigma Chemical Company (St. Louis, MO). Radiolabeled nucleotide sugars, namely UDP-[6-^{3}H-(*N*)]GlcNAc (6.6 Ci/mmol), UDP-[4,5-^{3}H(*N*)-galactose (43.3 Ci/mmol), GDP [^{14}C(U)]fucose (225 mCi/mmol), and CMP[9-^{3}H]sialic acid (25.5 Ci/mmol), were from Dupont (Boston, MA). Budget-solve scintillation cocktail was from Research Products International (Mount Prospect, IL). The Centricon 10 microconcentrator (10 000 molecular weight cutoff) was from Amicon (Beverly, MA). Heparin sodium (ultrapure) was from Fisher Scientific (Pittsburgh, PA). Dulbecco's PBS (10-fold concentrated; Gibco-BRL, Grand Island, NY) was diluted 10-fold in distilled water while the pH was adjusted to 7.4 to make a working PBS solution. All other chemicals were obtained commercially and were of the highest purity available.

Collection of Oviductal and Uterine Fluids

Animals were given an i.p. injection of heparin (133 IU/100 g BW) followed by an i.p. injection of sodium pentobarbital (4 mg/100 g BW). Fifteen minutes after the sodium pentobarbital injection, the animals were anesthetized with ether and perfused through the left ventricle with about 40 ml of PBS to remove most of the blood. The oviducts and uteri were removed and used for further studies. Briefly, the oviducts from 4 to 5 animals were trimmed free of connective tissue, blotted, mixed with a small volume (0.5 ml) of ice-cold PBS, and minced with scissors. The minced tissue was shaken (10 min, 4°C) to release oviductal fluid and then centrifuged at 1000 × *g* for 10 min. The supernatant (oviductal fluid) was removed by aspiration, saved on ice, and used for the quantification of enzyme activities and protein content. The possibility that glycosidase and glycosyltransferase activities present in the fluid originated from the cytoplasmic constituents of the oviductal epithelium seems unlikely since the enzymes are restricted to lysosomes and Golgi apparatus, respectively, and are not expected to be released by mincing of the oviducts [10, 11].

The uterus (see above) was flushed with ~0.5 ml of PBS, and the flushed fluid was used again to flush another uterus. The procedure was continued until 8–10 uteri were flushed. The fluid was centrifuged at 1000 × *g* for 10 min. The clear supernatant was removed by aspiration and stored at 4°C. The protocol achieves collection of concentrated uterine fluid for quantification of enzymatic activities and protein content.

Preparation of Serum for Hormonal Assays

Blood collected by cardiac puncture was allowed to clot at room temperature for 30 min and then centrifuged at 1000 × *g* for 30 min. The serum, collected by aspiration, was stored at −70°C until used. Concentration of estradiol-17β was measured with an in-house hamster RIA using charcoal-treated dextran. FSH was measured by RIA using reagents donated by Dr. A.F. Parlow, Pituitary Hormone and Antisera Center (Torrence, CA). Progesterone was measured by a direct RIA using commercially available reagents (Diagnostic Products Corp., Los Angeles, CA).

Enzyme Assays

All enzyme assays were carried out within 8 h after the collection of the sample. Glycosidase activities were quantified by measuring the release of *p*-nitrophenol in a standard incubation mixture (0.5 ml) containing 5 mM of the respective *p*-nitrophenyl glycoside substrate and the desired buffer as previously described [12]. The reaction was started by the addition of uterine or oviductal fluid and incubation at 37°C. After 30–60 min, the reaction was stopped by the addition of 1 ml of stopping buffer, and the released *p*-nitrophenol was quantitated by measuring absorbance at 400 nm. One unit is the amount of enzyme that catalyzes the release of 1 μmol of *p*-nitrophenol per hour.

Glycosyltransferase activities were assayed by measuring the amount of radiolabeled sugar transferred to an exogenous substrate essentially according to our published procedures [8, 9]. One unit of galactosyltransferase (GalTase) and *N*-acetylglucosaminyltransferase (GlcNAc-Tase) activity is the amount of enzyme that catalyzes the transfer of 1 pmol of the labeled galactose and *N*-acetylglucosamine per minute, respectively. One unit of fucosyltransferase (FucTase) and sialyltransferase (SialylTase) activity is the amount of

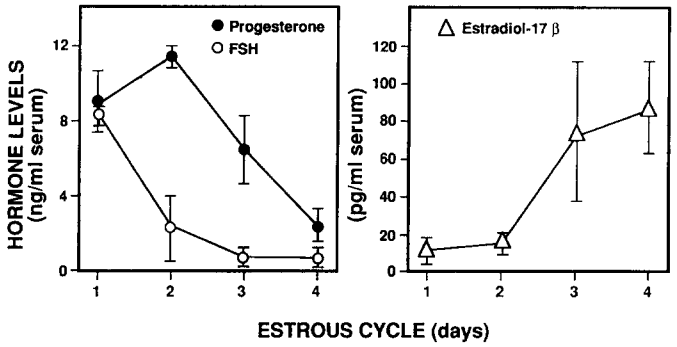

FIG. 1 Changes in serum concentrations of progesterone, FSH, and estradiol in the golden hamster during the estrous cycle. Data reported are the averages for four separate animals (n = 4); the vertical bar indicates SE.

enzyme that catalyzes the transfer of 1 pmol of the labeled fucose and sialic acid per hour, respectively.

Protein was measured by the colorimetric method of Bradford [13] according to Bio-Rad's instructions with BSA used as standard.

Statistical Analysis

Comparisons of the mean values in Figures 2–5 were done by one-way ANOVA. This was followed by the Tukey-Kramer multiple comparisons test to pinpoint extremely significant differences ($p > 0.001$).

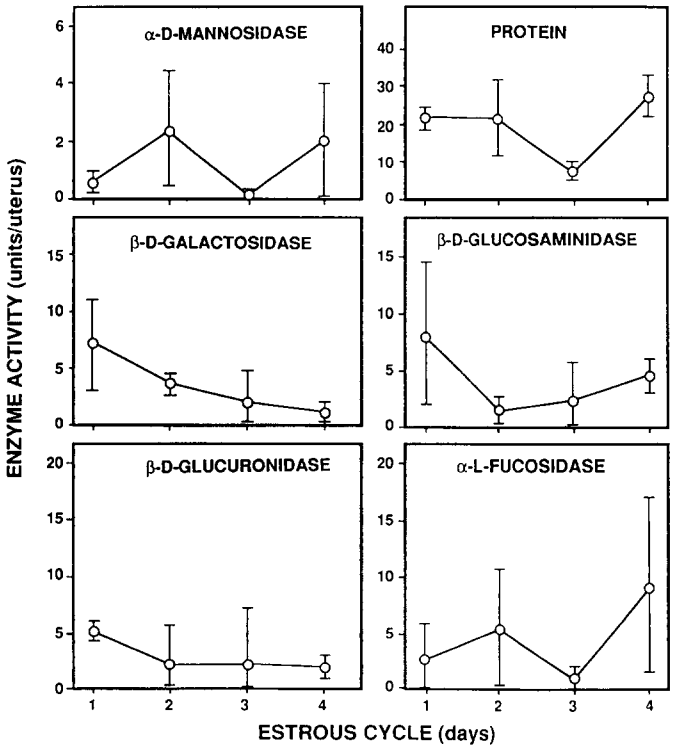

FIG. 2. Glycosidase activities and protein content in hamster uterine fluid during the estrous cycle. Data are the average of four pools (each pool containing fluid from 8–10 uteri); the vertical bar indicates SE. Protein units are milligrams per uterus. Values in various groups are not statistically different.

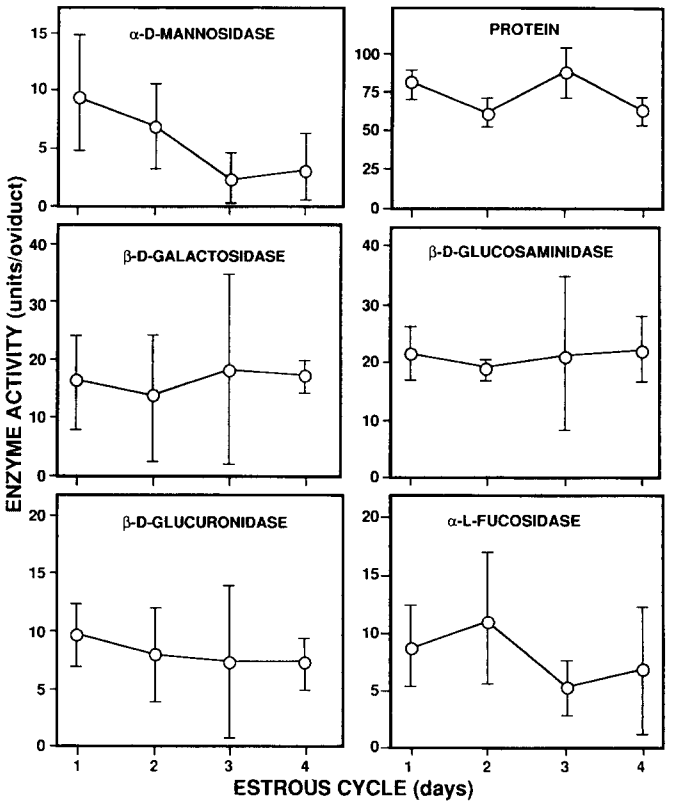

FIG. 3. Glycosidase activities and protein content in hamster oviductal fluid during the estrous cycle. Data reported are the average of four pools (each pool containing fluid from 8–10 oviducts); the vertical bar indicates SE. Protein units are milligrams per oviduct. Values in various groups are not statistically different.

RESULTS

Changes in Serum Estradiol-17β, FSH, and Progesterone Levels

Although use of the postestrous vaginal discharge criteria is a reasonably accurate method for timing the estrous cycle, it was necessary to confirm this classification by changes in the serum hormone levels during the cycle. The time course of changes in serum estradiol-17β, FSH, and progesterone levels is shown in Figure 1. As expected, serum FSH and progesterone levels were higher at the onset of ovulation (FSH, Day 1; progesterone, Day 1 and Day 2) and then showed a gradual decrease. In contrast, serum estradiol levels were very low on Day 1 and Day 2 of the estrous cycle but showed large increases during the last part of the cycle (Fig. 1).

Glycosidase Activities in Hamster Uterine and Oviductal Fluids

Activities of five glycosidases and protein levels were determined in the fluid prepared from hamster uteri and ovi-

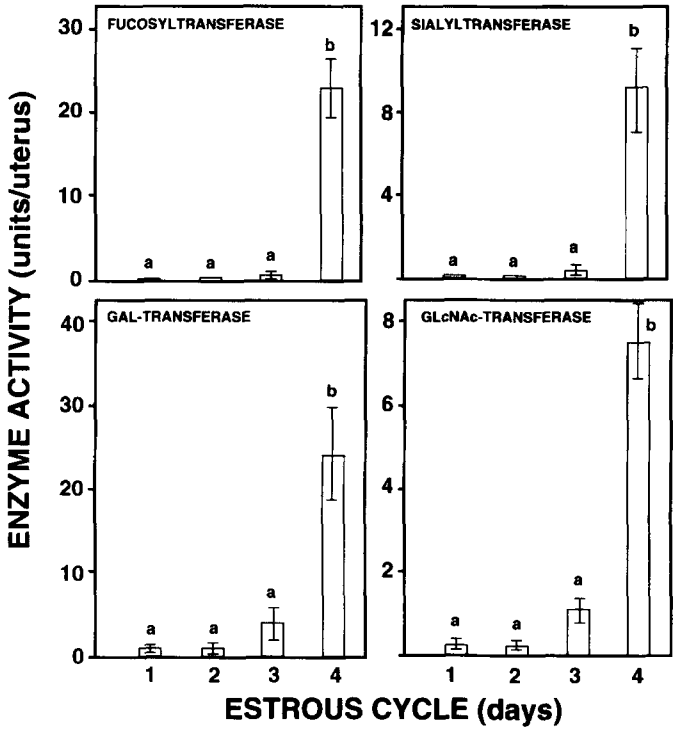

FIG. 4. Glycosyltransferase activities in uterine fluid during the estrous cycle. Data reported are the average of duplicate analyses carried out in four pools (each pool containing fluid from 8–10 uteri); the vertical bar indicates SE. Groups that are statistically different do not share the same letter.

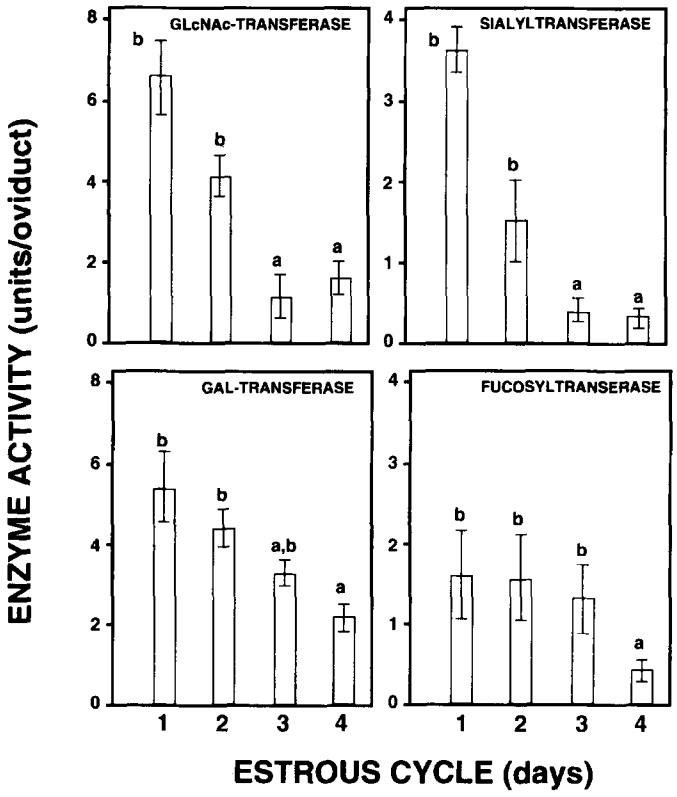

FIG. 5. Glycosyltransferase activities in oviductal fluid during the estrous cycle. Data reported are the average of duplicate analyses carried out in four pools (each pool containing fluid from 8–10 oviducts); the vertical bar indicates SE. Groups that are statistically different do not share the same letter.

ducts during the 4 days of the estrous cycle. As shown in Figure 2, levels of the five glycosidases (α-D-mannosidase, β-D-galactosidase, β-D-glucuronidase, *N*-acetyl β-D-glucosaminidase, and α-L-fucosidase) as well as protein levels were similar (not statistically different) in the uterine fluid during the estrous cycle. Similarly, in the oviductal fluid (Fig. 3), the levels of all glycosidases assayed (α-D-mannosidase, β-D-galactosidase, β-D-glucuronidase, *N*-acetyl β-D-glucosaminidase, and α-L-fucosidase) as well as the protein content showed no significant difference during the 4 days of the estrous cycle.

Glycosyltransferase Activities in Hamster Uterine and Oviductal Fluids

Activities of four glycosyltransferases and protein levels were quantified in fluid collected from the hamster uteri and oviducts during the estrous cycle; the results are presented in Figures 4 and 5, respectively. All glycosyltransferase activities were at negligible or low levels in the uterine fluid at onset of ovulation (Day 1) and remained relatively low during Days 2 and 3 of the estrous cycle (Fig. 4). However, on the day preceding ovulation (Day 4) there was a significant surge in activity of all four glycosyltransferases assayed. In contrast, glycosyltransferase activities in oviductal fluid were higher at the onset of ovulation and gradually decreased during the remaining 3 days of the cycle (Fig. 5). Thus the enzyme activities on the last day of the cycle (Day 4) were only 20–35% of those (significantly lower) measured at the onset of ovulation.

Since mammalian blood (serum) is known to contain glycosyltransferase activities [14], it was possible that some of the enzyme activities present in the uterine/oviductal fluids were contributed by blood present as a contaminant. Although this was unlikely because the animals were perfused, we quantified glycosyltransferase activities in the hamster serum and compared these values with the enzyme activities found in the uterine and oviductal fluid. Comparative data from these studies, reported as specific activities, are shown in Table 1. Values in the uterine and oviductal fluids were much higher than in the blood, a result indicating that most of the enzyme activities (> 95%) reported in Figures 4 and 5 were of tissue, not blood, origin.

DISCUSSION

The data presented here demonstrate a temporal surge in glycosyltransferase activities in uterine fluid preceding ovulation and in oviductal fluid at the onset of ovulation. The observed changes in glycosyltransferase activities may be attributable to an increased rate of synthesis and secre-

tion or to activation of these enzymes. Since the glycohydrolase activities and protein content remained similar during the 4 days of cycle, the surge in glycosyltransferase activity appears to be specific. The fact that regulation of the four glycosyltransferase activities within the uterus and oviduct is concurrent with changes in hormonal state of the animal suggests that the enzymes may be directly or indirectly regulated by reproductive hormones. In fact, current evidence suggests that steroids play an important role in the regulation of glycoprotein expression in the reproductive tract [15, 16]. These changes may be important in the regulation of the microenvironment during in vivo fertilization and early development of the embryo.

The glycosyltransferases, although typically present in the Golgi apparatus [11], are also present in soluble form in the epididymal luminal fluid, where they are believed to modify sperm plasma membrane glycoprotein(s) during epididymal maturation [9, 17]. The soluble transferases present in the uterine and oviductal fluids, like the enzymes in the epididymal luminal fluid, would be expected to mediate incorporation of sugar residues to the endogenous glycoproteins in the reproductive tract.

There is a vast literature on the functional significance of glycoproteins of the reproductive system [18]. The carbohydrate portion of several molecules is believed to mediate cell-cell adhesion, including sperm-egg interaction [19], sperm-oviductal adhesion [20], and implantation of the embryo [21, 22]. Thus depending on the reproductive event under study, modification of the terminal sugar residues of the functional glycoproteins in the reproductive tract is expected to alter their physiological roles.

One potential substrate of the soluble glycosyltransferases in the female genital tract is the sperm plasma membrane. Epididymal or ejaculated spermatozoa undergo a series of biochemical and functional changes in a defined culture medium or within the female genital tract that are collectively termed capacitation. The known changes include alterations in motility [23, 24], calcium influx [25], loss of sperm surface molecules [26], changes in lectin-binding properties [5, 27, 28], and other surface modifications [29].

TABLE 1. Glycosyltransferase activities in hamster reproductive tract fluids and serum.[a]

Enzyme	Uterine[b] fluid	Oviductal[c] fluid Specific Activity	Serum[c]
GalTase	0.83	0.08	0.02
GlcNAcTase	0.25	0.09	0.01
FucTase	1.57	0.02	Traces[d]
SialylTase	0.36	0.05	Traces[d]

[a]The fluid/serum prepared as described in *Materials and Methods* was assayed for glycosyltransferase activities and protein content. Data reported are average of three pools, each pool containing fluid/serum from 4–5 animals.
[b]Prepared from animals in Day 4 of estrus.
[c]Prepared from animals in Day 1 of estrus.
[d]Less than 0.001 units.

If the glycosyltransferase activities are functional in the uterine and oviductal fluids, they would be expected to alter the sperm surface glycoproteins by adding sugar residue(s) to the existing glycoproteins on sperm plasma membrane, thereby changing their lectin-binding properties.

Another obvious target of the glycosyltransferases could be the oocyte. Mammalian eggs exposed to the oviduct show structural changes in the zona pellucida glycoproteins as detected by lectin binding studies [30–32], a result consistent with the suggestion that oviductal fluid contains one or more factors that could bind to the oviductal oocytes. Although an oviduct-specific glycoprotein secreted from the epithelium cells in several species [33–39] is believed to bind to the egg surface and alter the lectin-binding properties of the extracellular coat, the zona pellucida, it is possible that the glycosyltransferase activities in the oviductal fluid are involved in the modification of the terminally exposed glycan units on the zona pellucida through transferring additional sugar residues. Since carbohydrate residues are implicated in the fertilization process in several species, including the hamster [40], any alteration of the exposed sugar residues of the zona pellucida will have an effect on its ability to bind spermatozoa. Moreover, after fertilization, glycosyltransferases may also mediate changes in the carbohydrate structure of the zona pellucida, preventing polyspermy.

It is noteworthy that glycosyltransferases, in addition to being glycoprotein-modifying enzymes, are also glycoproteins and possibly are able to bind to the surface of spermatozoa and/or zona pellucida in the reproductive tract. It is not currently known whether nucleotide sugar donors are present in the lumen of the genital tract. Without the nucleotide sugars, the transferases may act as ectoenzymes and tightly bind to the endogenous acceptor substrate. This binding would be expected to alter the lectin-binding properties of these cells as has been reported [5, 6, 30–32]. Thus, it will be important to determine whether and how the circulating hormones regulate the synthesis and/or secretion of these enzymes.

In addition to acting on the gametes, the soluble glycosyltransferases in the female genital tract may also target other soluble or cell surface glycoproteins in the uterus and oviduct. For instance, one such potential substrate could be the oviductal-specific glycoprotein that has been reported in the fluid of several species [33–39], including the hamster [41–43]. Interestingly, lectin binding studies have indicated the association of galβ1,3GalNAc- and *N*-acetylglucosaminyl residues with the estrus-associated glycoproteins in bovine oviductal fluid [37]. If a similar association occurs in the hamster oviductal fluid, this result would be consistent with the proposed role for the glycosyltransferases.

In summary, the present study provides the first set of data indicating a surge in transferase activities in the uterine fluid preceding ovulation and in the oviductal fluid at the

onset of ovulation. Since the activity of the synthetic enzymes is modulated during the estrous cycle, it is reasonable to suggest that the enzymes have a role in the regulation of microenvironments during fertilization and postfertilization events. It will be of interest to determine whether the observed surge occurs in other species, including the mouse (the most common species used in in vitro fertilization) and the human.

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