Uterine fluid proteins bind sperm cholesterol during capacitation in the rabbit

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Summary. Cholesterol from rabbit sperm cells was bound by uterine fluid proteins following intrauterine insemination into ovulating does. Serum albumin has been identified as a uterine sterol acceptor. Progesterone administration suppressed binding and elevated the concentration of cholesterol, phospholipid, and protein. Uterine fluid cholesterol affinity, therefore, correlated with sperm capacitation activity in utero.

Mammalian spermatozoa displayed a close correlation, in a recent study, between their cholesterol level and capacitation interval, which represents the interval in utero when they acquire fertilizing capacity[2]. This finding suggests there is a membrane/sterol barrier to fertilization in mammals. Furthermore, the rate of cholesterol depletion during sperm capacitation, inferred by analogy with the kinetics of cholesterol efflux from leukocytes suspended in a lipoprotein-free serum fraction, provided a quantitatively reasonable model of the period for this transformation in utero. Earlier experiments[3-5] with cauda epididymal rat spermatozoa demonstrated exchange diffusion of phospholipids and cholesterol between these cells and serum albumin, which was in a defined medium used for capacitation in vitro. Resultant alterations occurred in the sperm lipid make-up. In particular, a decrease was observed in the cholesterol/phospholipid ratio among postincubated spermatozoa and their plasma membrane. Depletion of cholesterol should reversibly destabilize the sperm plasma membrane. It is known that a Ca^{2+}-facilitated fusion between the plasma membrane of capacitated sperm and outer membrane of the acrosome vesicle in the sperm head initiates the acrosome reaction[6-8] and that the resulting exposure of hydrolytic acrosomal enzymes helps sperm penetration through the corona radiata, zona pellucida, and vitelline membrane[9-11]. Significantly, uterine-capacitated sperm cells lose their fertilizing ability, in a reversible manner, during exposure to membrane vesicles from seminal plasma and synthetic phospholipid vesicles, when they contain cholesterol[3,12,13]. The present investigation aimed to establish a) the presence of sterol-binding proteins in rabbit uterine fluid that could act as acceptors for sperm cholesterol during capacitation, b) an elevation in their affinity around the time of ovulation, and c) a decrease in uterine fluid cholesterol levels at ovulation, to provide sperm cells with a negative external cholesterol gradient.

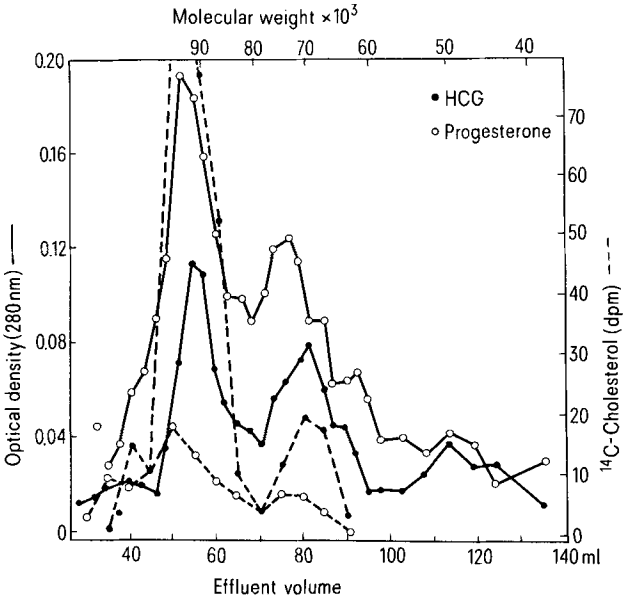
Spermatozoa (18×10^6 sperm/0.1 ml of Hanks solution) prelabeled (Davis et al.[5]) with 3400 dpm ^{14}C-cholesterol (0.05 Ci/mmole) were inseminated by injection into the uterus, after ligation near the cervix and uterotubal junction. During this procedure, the recipient was anesthetized by i.v. injection of 30 mg sodium pentobarbital/kg b.wt. Each uterine horn was then flushed twice with 4 ml of isotonic saline at 1.5 h postinsemination. After sedimentation, to remove cells, and concentration to 0.5 ml, the uterine fluid was fractionated on a Sephadex G-100 column (2.5 cm diameter, 36 cm length) with 0.05 M KCl and 0.005 M Tris, pH 7.4, as eluant. Protein peaks among these fractions were located by their absorbance at 280 nm. Radioactivity measurements were made in a liquid scintillation counter, after addition of 0.25 vols of 50 (v/v)% Biosolv (New England Nuclear). The authenticity of ^{14}C-cholesterol in these peaks was demonstrated through comigration with purified standard on a silica gel plate. Solute molecular weights were calibrated from the effluent volumes of albumin (68,000), ovalbumin (44,000), and lysozyme (13,500). Recipient does received either an i.v. injection of 70 IU human chorionic gonadotrophin (Squibb) preinsemination (−12 h) or 25 mg progesterone/day by s.c. injection for 6 days (days −6 to −1).

Uterine fluid from rabbits (New Zealand strain), induced to ovulate by an injection of human chorionic gonadotrophin, contains a significant amount of bound ^{14}C-cholesterol after chromatographic fractionation, 1.5 h postinsemination with sperm bearing the labeled sterol (fig.). Radioactivity is associated with a 6.8×10^4 dalton peak, which is tentatively identified as serum albumin. It comprises almost half the protein in uterine fluid from oestrus rabbits[14,15]. This peak accounted for 42% of absorbance at 280 nm by fluid from these rabbits. These uterine fluid fractions contained nearly 10% (324/3400 dpm) of sperm ^{14}C-cholesterol. Recovery decreased nearly 3-fold following administration of progesterone (Sigma). The steroid impedes transuterine permeation by serum proteins including albumin[14,15]. Binding also decreased in the leading peak

Composition of uterine fluid from rabbits administered human chorionic gonadotrophin (HCG) or progesterone

Hormone	No. of animals	Protein (mg/uterine horn)	Phospholipid	Cholesterol
HCG (70 IU)	4	0.85 ± 0.17	n.d.[a]	n.d.
Progesterone (25 mg/day × 6)	5	13.10 ± 0.88	1.84 ± 0.56	0.047 ± 0.005

[a] n.d., not detected.



Optical density and radioactivity profiles obtained by gel filtration chromatography of uterine fluid from rabbits given either human chorionic gonadotrophin (HCG), to promote uterine capacitation activity, or progesterone, to suppress capacitation, following intrauterine insemination of sperm cells bearing ^{14}C-cholesterol.

(fig.), and this suggests there is another serum-derived sterol acceptor in the vicinity of 10^5 daltons. The uterus excludes proteins much above this molecular weight. From the optical density profiles (fig.), there is more protein in the uterine fluid of progesterone-treated rabbits.

The table presents the results of protein, cholesterol, and phospholipid assays[5] with these uterine fluids. They reveal a 15-fold increase with progesterone treatment in protein concentration, from 0.85 to 13.1 mg/uterine horn. Phospholipids and cholesterol were not detected in uterine fluid from ovulating does. By contrast, steroid-treated rabbits had 1.84 ± 0.56 and 0.047 ± 0.005 mg/uterine horn of these lipids, respectively. This yields a cholesterol/phospholipid mole ratio of 0.05. In addition, there were 4 mg cells/uterine horn in this fluid. The cell fraction was nondetectable in fluid from ovulating does. Subcellular membrane components of uterine fluid show a similar pattern of occurrence among rabbits treated with these hormones[16].

These results corroborate a report[17] that progesterone elevates uterine fluid lipid levels among rats. A progesterone-induced increase in uterine fluid cholesterol concentration (table) presumably helped suppress ^{14}C-cholesterol efflux from prelabeled sperm in utero (fig.), despite this fluid having a lower cholesterol/phospholipid mole ratio than ejaculated rabbit sperm cells (0.05 vs 0.88). A rabbit sperm cell contains an estimated 8.47×10^8 cholesterol molecules[2] and from present binding data (fig.) about 10% could be removed after 1.5 h during capacitation in utero. Hence, around 10^{15} cholesterol molecules are removed from 10^7 sperm, which is approximately the number that enter the uterus after mating. Results in the table indicate, however, there are an additional 10^{17} cholesterol molecules in uterine fluid from rabbits given progesterone. In agreement with present findings, the distribution of capacitation activity, assayed using hamster sperm, after elution of serum from a Sephadex G-150 column[18] suggests that both albumin and α-globulins act as capacitation factors. Cholesterol affinity in the latter has been experimentally established[19]. In this connection, analbuminemic rats with elevated α-globulins in their serum display normal fertility[20]. It would be interesting to further characterize the uterine fluid sterol-acceptor proteins and to establish the kinetics of sperm cholesterol efflux in capacitation.

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Binding of actinomycin D and divalent cations to lipopolysaccharides of *Agrobacterium tumefaciens* as studied by fluorescence spectroscopy

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Summary. The binding of actinomycin D and divalent cations to lipopolysaccharides of *A. tumefaciens* was studied. Fluorimetric titrations revealed 2 binding sites (low and high affinity sites) for divalent cations, and 1 high-affinity site for actinomycin D.

The lipopolysaccharides (LPS) of many gram negative bacteria have been studied earlier by various investigators[2]. Moreover, it has also been established that LPS, located in the external leaflet of the outer membrane of gram negative bacteria, are important for the barrier function of the membrane[3]. It has been postulated that stabilization of the outer membrane takes place as a result of the formation of ionic bridges between adjacent LPS phosphate groups, mediated by divalent cations[3]. However, it may also be that 2-keto 3-deoxy octulosonate (KDO) residues form a high affinity site for divalent cations, as has been established for sialic acid[4,5]. Schindler and Osborn[3] have shown that 3 KDO residues of the LPS molecule do indeed form the divalent cation binding sites which bind 1 mole of divalent cation per moles of LPS. Such divalent cation binding studies have not been carried out with the LPS isolated from *Agrobacterium tumefaciens*. Further, Walker and Durham[6] have shown that *Pseudomonas fluorescens* cells grown in glucose are much less sensitive to actinomycin D than succinate grown cells. They have established that the glucose grown cells have a higher concentration of LPS than the ones grown in succinate; this can influence the binding of actinomycin D to the cell surface, and thus exhibit its function in the cells in respect of different permeability conditions which are reflected in the sensitivity to the antibiotic. However, no reports are available regarding the LPS-actinomycin D binding in relation to the site and affinity of binding. In this communication the interactions