Surfactant-like Materials (SF-phospholipids) in Fallopian Tubal Secretions and Their Physiologic Role in Reproduction

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Abstract: *The physiologic roles of the tubal endothelium and tubal secretions are as yet poorly understood. In this study, we examined the tubal surfactant-like materials (SF-phospholipids) found in the tubal endothelium and lumen in order to elucidate their roles in the maintenance and development of the zygote. Evaluation of tubal infertility based on the presence or absence of SF-phospholipids in human tubal endothelium and tubal secretions was carried out in order to determine the degree to which tubal function was intact. By using fluorescing phosphin E, we found fluorescence in both the endothelium and the tubal secretions throughout the entire length of the tubes. In electron microscopic observations of the tubal endothelium, we were able to identify high electron density, lamellar inclusion bodies, either scattered or clustered in the areas close to the tubal lumen, in both ciliated and secretory endothelial cells. These lamellar granules in the tubal secretions appear to be secreted by a merocrine process and are observed in the secretions as a dispersion. Tubes from the proliferatory phase and the secretory phase were evaluated with the following results. The constituents of the tubal secretions were found to be phospholipids and proteins. The phospholipids were most abundant at or around the time of ovulation. This material caused capacitation of sperm by inducing an acrosomal reaction in the hamster sperm penetration test.*

Key words: *Surfactant-like materials, SF-phospholipids, Fallopian tubal secretions, Hamster sperm penetration assay test.*

The fallopian tubes are intimately involved in the process of fertilization and zygote transport. The tubal ampulla is active in capturing ova expelled from the ovaries and transporting them medially to the area where fertilization is said to optimally occur. The tubal environment is reported to aid in the acrosomal reaction of sperm as it is being transported distally to fertilize ova in the medial ampulla. The isthmic portion of the tube is further involved in aiding the zygote to mature as it is being transported medially toward the uterine cavity. Although the tubal environment is known to be intimately involved in fertilization of the ova and transport of the zygote, many questions remain to be answered [1–4]. Recent *in vitro* studies on co-culturing ova and early zygotes have shown the importance of adding tubal epithelial cells, which enhance fertilization and zygote development [5–11]. This investigation involved electron microscopic evaluation of the cells of the tubal lining and of the changes observed in phase with the menstrual cycle. Electron micrographs revealed various orgenelles within the epithelial cells lining the tubes. We paid special attention to the surfactant-like materials which are extruded into the tubal lumen by a merocrine process [12]. The fact that these materials are most abundant around the time of ovulation, led us to assume that they are involved in the events of early pregnancy, especially as the surfactant-like materials are most abundant in the cervical mucus and in the tubal lumen, although they are also found throughout the internal reproductive tract. Nevertheless, as the actual roles of the tubal endothelium and tubal secretions are as yet poorly understood, we began to study the tubal surfactant-like material (SF-phospholipids) found in the tubal endothelium and lumen in order to elucidate their role in the maintenance and development of the zygote. Evaluation of tubal infertility based on the presence or absence of SF-phospholipids in human tubal endothelium and tubal secretions in order to

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determine the degree to which tubal function was intact, led us to make the following interesting observations.

Materials and Methods

A histochemical and electron microscopic evaluation of SF-phospholipids in human fallopian tubal secretions

Fallopian tubes obtained during hysterectomies carried out for various disorders in women aged 20–35, during the proliferatory, ovulation and secretory phases of the menstrual cycle, were used in this evaluation. All specimens were used with the written consent of the subjects who had undergone surgery. All tubes were fixed in a mixture of prefixation solution, consisting of 2.5% glutaraldehyde, 4% paraformaldehyde and 1% tannic acid, for 1 day, and then finely diced and fixed in 1% osmium acid. These pieces were embedded in an epoxy resin for electron microscopy, in an acetone resin for microscopic observation, or in paraffin for fluorescent staining with phosphin E.

Analysis of the tubal secretions

The fallopian tubes to be evaluated were obtained during surgery from patients undergoing hysterectomies, and were used with their written consent which was obtained prior to surgery. Tubal fluids were taken from women who had regular menstrual cyclicity in the age range of 25–35 yrs. They were divided into the proliferatory and secretory phases. The tubes were repeatedly lavaged with physiologic saline, freeze-dried, and then extracted with a mixture of chloroform methanol. The constituents of the tubal secretions and the composition of the phospholipids from the extracted material were assayed for surfactant by iatroscanning, as in the procedures for assaying pulmonary surfactant.

The effect of lung surfactant (Surfacten®) on human sperm capacitation in the hamster sperm penetration assay test

The male subjects selected for this study were the husbands of women under management for infertility. We selected 35 males with normospermia (sperm count>6.0 $\times$ 10^6, motility>60%, abnormal sperm <40%), and 30 males diagnosed as oligospermia. Our investigation involved the use of Surfacten® (Tokyo Tanabe Pharm.), a surfactant isolated from swine lung tissue, as a substitute for Ca^{++}-ionophore to determine its ability to capacitate sperm. We prepared two specimens from one semen sample, one exposed to Surfacten® 10 μg/ml, the other to Ca^{++}-ionophore by the conventional method, and compared their sperm activity by means of the hamster test. We further examined sperm by electron microscopy to examine morphological changes, and by a triple-staining method with Trypan blue, Bismark brown and Rose bengal.

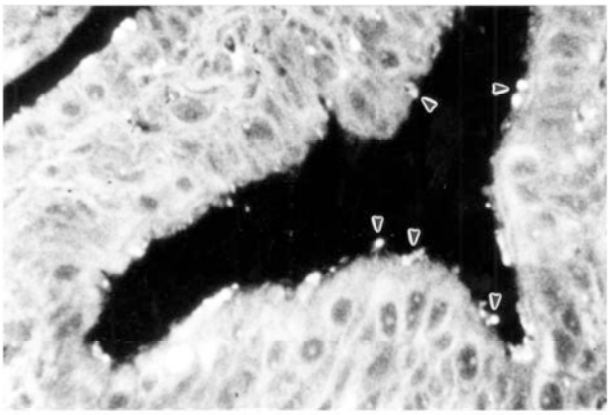

Fig. 1. The SF-phospholipids found in the tubal fluids fluoresced phosphin E. We were able to identify fluorescence in both the endothelium and the tubal secretions throughout the entire length of the tubes.

Results

The histochemical and electron microscopic evaluation of SF-phospholipids in the human Fallopian tubal secretions

Using fluorescing phosphin E, we observed fluorescence in both the endothelium and the tubal secretions throughout the entire length of the tubes. These fluorescent substances seem to be more abundant distally and scantier medially in the tubal endothelium and tubal secretions (Fig. 1). In electron microscopic observations of the tubal endothelium, we were able to identify high electron density, lamellar inclusion bodies, either scattered or clustered in the areas close to the tubal lumen, in both ciliated and secretory endothelial cells. These lamellar granules in the tubal secretions appear to be secreted by a merocrine process and are observed in the secretions as a dispersion (Fig. 2). These lamellar inclusion bodies containing large amounts of well-developed rER are best seen in the tubal endothelium in the ovulatory phase. Approaching the superficial areas of the cells, the lamellar inclusion bodies are found finely dispersed in the rER, but in the sER they tend to form larger clumps. These lamellar inclusion bodies are most abundant in the vesicles in the Golgi zone, suggesting that they are synthesized here. Under high magnification the inclusion bodies appear as laminated

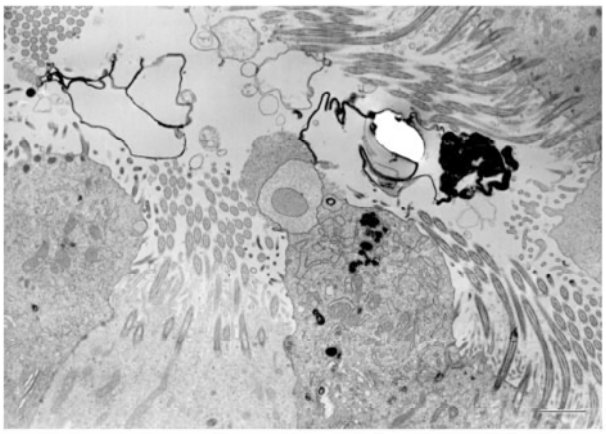

Fig. 2. These lamellar granules in the tubal secretions appear to be secreted by a merocrine process and have been observed in the secretions as a dispersion. An electron micrograph showing the SF-phospholipids in close approximation with the Golgi apparatus.

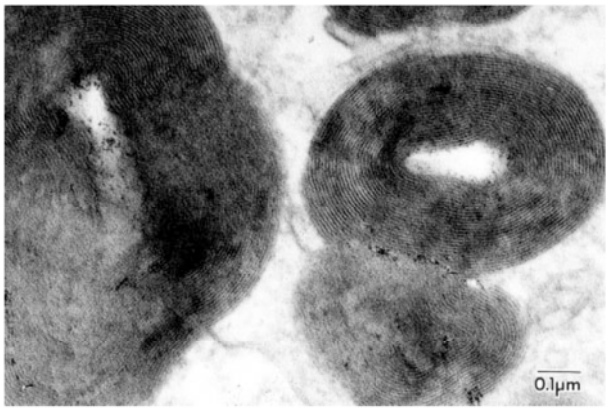

Fig. 3. These inclusion bodies under high magnification appear as laminated band or ribbonlike structures, with alternating dark and clear layers about 50 Å in width. These ribbon-like laminated structures are observed in the tubal endothelial cells and the secretions, and are most abundant at or around ovulation.

Table 1. Composition of tubal secretions

	Follicular phase (5 persons/10 tubes)	Postovulatory phase (6 persons/12 tubes)
Constituents of tubal secretion		
Total phospholipid	1,802 μg	1,528 μg
Protein	16,400 μg	18,825 μg
Extraction of phospholipids		
Total phospholipid	214.0 μg	142.5 μg
Phospholipid/Person	42.8 μg	23.6 μg
PC/PL	41.6%	54.9%
DSPC/PL	16.9%	12.9%
DSPC/PC	40.6%	24.3%
Composition of phospholipids		
Phosphatidylcholine	41.6%	54.9%
Phosphatidylethanolamine	14.5%	10.5%
Phosphatidylglycerol	2.2%	3.5%
Sphingomyelin	21.0%	18.1%
Lisophospholisin	2.3%	3.9%
Others	18.4%	9.1%

band or ribbon like structures, with alternating dark and clear layers about 5OÅ in width (Fig. 3.). In the tubal secretions, these ribbon-like inclusion bodies separate into membranous filaments and whorls, and finally degrade into clumps of ultrafine granules. An evaluation of these tubal secretions reveals that phosphatidylcholine during the proliferatory phase increases with the approach of ovulation.

Analysis of the tubal secretions

Five patients in the proliferatory phase (10 tubes) and 6 patients (12 tubes) in the secretory phase were evaluated with the following results (Table 1). The constituents of the tubal secretions were total phospholipids of 1,802 μg and 1,528 μg, respectively, and total protein of 16,400 μg and 18,825 μg, respectively. Composition of phospholipids and phosphatidylcholine was 46.1% and 54.9%, followed by sphingomyeline at 21.0% and 18.1%, and phosphatidylethanolamine at 14.5% and 10.5%. In terms of the phases of the menstrual cycle, the total phospholipids per tube were 21.4 μg at ovulation, and 11.8 μg during the luteal phase. In terms of phosphati-

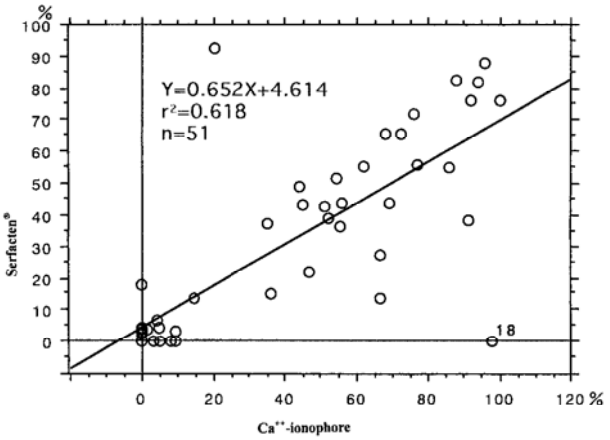

Fig. 4. The effects of Ca^{++}-ionophore and Surfacten® in enhancing spermal capacitation as evaluated by the golden hamster ovum penetration test.

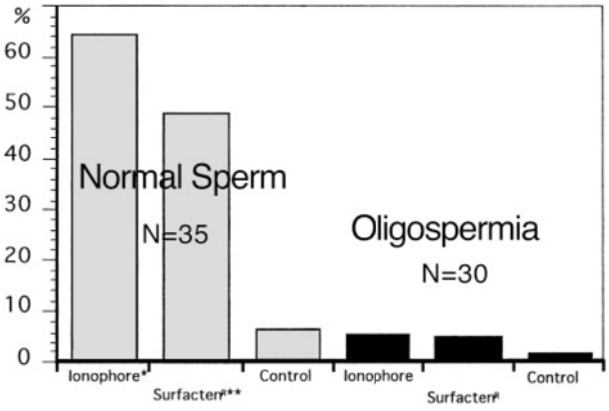

Fig. 5. The effect of lung surfactant on human sperm capacitation in the hamster sperm penetration assay test. *Ca-Ionophore: 5 μMol, **Surfacter®: 10 μg/ml.

dylcholine at ovulation, they were 9.0 μg and 6.5 μg, respectively (per tube). Thus the results indicate that the volume of phospholipids is most abundant, especially phosphatidylcholine, with a statistically significant increase at ovulation, as compared to the other phases of the menstrual cycle.

The effect of Surfacten® on human sperm capacitation in the hamster sperm penetration assay test

We found that Surfacten® capacitated sperm in terms of positive hamster ova and the penetration rate was roughly 90% that of Ca^{++}-ionophore capacitated sperm. Thus we established that Surfacten® is capable of capacitating sperm, although not as efficiently as Ca^{++}-ionophore (Figs. 4, 5). Our electron microscopy findings showed that the acrosome reaction had occurred in sperm capacitated by Surfacten®. The results of the triple staining method also demonstrated that the acrosomal reaction had occurred.

Discussion

In vitro fertilization and embryo transfer (IVF&ET) was first successful in humans in '78, and since then has maintained an important position in the management of infertile couples. The success rates with this procedure are gradually improving, but, even with high fertilization rates, rates of nidation and later development remain low, resulting in "take home baby" rates of only 15–20%. One procedure to improve success rates is the co-culture method, which involves the culturing of zygotes with other cells in order to obtain good quality zygotes. The tissue said to offer the most similar physiologic conditions when co-cultured is the epithelial lining of the fallopian tubes. Since 1987, reports on co-culturing zygotes with bovine and ovine tubal endothelium have appeared [5–10]. *In vivo*, the ovum is fertilized in the ampullaris portion of the tube, and then undergoes cleavage while passing through the tube, to arrive in the uterine cavity as a blastocyst. It is reported that in humans the ovum is retained in the ampullar portion of the oviduct for 70–80 hrs, and, under optimal conditions, undergoes fertilization. After this period of retention in the ampulla, the fertilized ovum is passed medially through the oviduct reaching the uterine cavity in about 10 hrs. [2, 3]. Therefore, one approach to improving IVF&ET success rates is to culture the zygote to the blastocyst stage before performing ET. At present, a common clinical procedure is to perform ET at the 4 to 8-cell stage, or at roughly 48 hrs. post-insemination. The reason for this is that prolonged culturing to obtain zygotes beyond the 8-cell stage, results in a high rate of degenerative changes. Since the advent of GIFT, PROST, co-cultures and other techniques used in assisted reproduction, the role of tubal physiology has become of interest because of its apparent function in the maintenance of the zygote en route to the uterus. We began to study the tubal surfactant-like materials (SF-phospholipids) found in the tubal endothelium and lumen in order to elucidate their role in the maintenance and development of the zygote. These ribbon-like laminated structures observed in the tubal endothelial cells, and the secretions in the tubal lumen structurally, closely resemble surfactant found in pulmonary alveolar studies. The chemical structure of this tubal material also

resembles that of alveolar surfactant, leading to the assumption of the presence of a phospholipid similar to surfactant and which is involved in the normal function of the fallopian tubes. These lamellar inclusion bodies are a conjugate of tannin and the lipid phosphatidylcholine and are consistent with the major constituents of pulmonary surfactant, and show signs of similar surface activity. We were able to structurally and biochemically identify an SF-phospholipid in the endothelium and tubal secretions of normal human fallopian tubes. Our findings led us to postulate that this SF-phospholipid substance is necessary for the maintenance of tubal function and environment, and may be involved in other as yet unexplained functions. These phospholipids in the tubal secretions reveal a cyclicity in phase with ovarian cyclicity, suggesting that they play a role in reproductive physiology. Many authors have discussed the influence of the steroid hormones in inducing increased oviductal fluid near estrus (ovulation) [13–16]. Borland reports that the phosphorus concentrations in tubal fluids are similar to those in blood serum [17]. Lippes reports lower levels of phosphorus in tubal fluids than in blood serum [18]. Zhu has reported on the effects of human cervical mucus, oviductal fluids and follicular fluids on sperm function [19] but, to date, we have found only Killian reporting on cattle and referring to the phospholipids in response to menstrual cyclicity, nor have any investigators identified the phosphorus containing compounds in the tubal fluids as being similar to pulmonary surfactants [20]. The functions ascribed to pulmonary surfactants include: the reduction of alveolar surface tension, the maintenance of alveolar form (configuration), reduction of the viscosity of bronchial fluids, and antimicrobial activity, by the activation of macrophages, stimulation of IgA synthesis, and enhancement of bacterial autolysis [21]. The phospholipids in the tubal secretions, which closely resemble pulmonary surfactants, are believed to reduce the surface tension of tubal secretions, which may aid or facilitate the transport of the sperm distally and the zygote medially. This material is also capable of inducing spermal capacitation and activation of the acrosome reaction. It is also believed to be involved in the maintenance of the tubal environment to facilitate fertilization of ova in the medial ampulla, provide a media for the early development of the zygote, while providing assistance in transporting these zygotes medially toward the uterine cavity. It also seems to have antimicrobial functions similar to pulmonary surfactant, while simultaneously suppressing the zygotes antigenicity while in the tube. In male infertility due to oligospermia or reduced motility, IVF&ET have required the additional development of a microfertilization technology. Among the techniques which have been developed are zona drilling, partial zona dissection, and intracytoplasmic sperm injection (ICSI). But this latest development, ICSI, has tended to make the older techniques of opening the zona pellucida obsolete. The conventional methods of assessing male fertility are by semen volume, motility, etc. The present method of evaluating sperm function is the sperm penetration assay or hamster test, where the ability of the capacitated sperm to penetrate hamster ova can be observed. The ethical considerations involved make the use of human ova to assess sperm activity impractical. Thus, as an alternative, golden hamster ova stripped of the zona pellucida has become an acceptable substitute when evaluating human sperm activity. The hamster ova are exposed to trypsin to strip the zona pellucida, to which Human sperm capacitated by Ca^{++}-ionophore is introduced, to evaluate whether the sperm can penetrate the ovum's outer membrane. A clinical application in which we used Surfacten® capacitated sperm has resulted in pregnancies which were not achieved by Ca^{++}-ionophore capacitated sperm. We therefore believe that the surfactant-like material endogenous to the fallopian tubal lining is of great importance in causing the acrosome reaction in sperm as they traverse the tube, leading to our conclusion that the use of Surfacten®, and eventually human tubal surfactant-like materials, will greatly improve pregnancy rates in infertile couples requiring IVF & ET.

Conclusions

In conclusion, we found that the surfactant-like materials in the reproductive tract facilitate the transport of sperm distally through the tube on its way toward the medial ampulla where fertilization is optimally achieved. During this distal migration of the sperm, it is capacitated by these materials to make it more capable of fertilization. When considering an ovum and an early zygote, they are provided with an adequate environment, while also being provided with assistance in their medial transport by the reduction of the surface tension of the tubal contents. The surfactant-like materials also appear to suppress the antigenicity of the early zygote, allowing it to arrive in the uterine cavity where it is able to undergo further growth and development. We therefore believe that these materials are of major importance when considering treatment to enhance fertility.

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