# Inhibitory Effects of Progesterone on Plasma Membrane Fluidity and Tumorigenic Potential of Ovarian Epithelial Cancer Cells

ANNA C. MCDONNEL, EDWARD A. VAN KIRK, DALE D. ISAAK, AND WILLIAM J. MURDOCH[1]

*Reproductive Biology Program, University of Wyoming, Laramie, Wyoming 82071*

**The lethality of common (surface) epithelial ovarian cancer is contingent on its metastatic capacity. Dissemination of the neoplasia throughout the abdominal cavity has been associated with secretion of proteolytic enzymes from vesicles shed by ovarian cancer cells. We report that the lipophilic steroid hormone progesterone decreases the fluid dynamics of plasma membranes of human SKOV-3 adenocarcinoma cells. The decrease in membrane fluidity was related to an inhibition *in vitro* of exocytotic vesicle release, cellular invasiveness into Matrigel, and colony formation in three-dimensional collagen matrix. Tumorigenesis was suppressed by progesterone in immunocompromised nude mice inoculated intraperitoneally with SKOV-3 cells. Progestins could therefore be of benefit in the prevention and(or) treatment of early-stage ovarian carcinomatosis.** Exp Biol Med 228:308–314, 2003

Key words: progesterone; plasma membrane fluidity; epithelial ovarian cancer

Steroid hormones alter cellular functions by diverse mechanisms. The classic mode of action involves hormonal binding to intracellular receptors, to initiate gene transcription (1). Receptors localized within plasma membranes elicit rapid nongenomic responses (2, 3). Because of their lipophilicity, steroid hormones (particularly progesterone) also intercalate into the backbones of membrane phospholipid bilayers and can thereby perturb fluid dynamics; shifts in the molecular order of the lipid environment of plasma membranes affect signal transduction, enzymatic activation, ion flux, and secretory processes (4, 5).

Urokinase-type plasminogen activator (uPA) and matrix metalloproteinases (MMPs) are involved in the metastatic progression of ovarian cancers of surface epithelial origin (6). Ovarian cancer cells liberate uPA with MMPs from exocytotic vesicles derived from the plasma membrane (7). Plasmin, the byproduct of uPA cleavage of plasminogen, activates latent MMPs, which consequently digest basement membranes and interstitial connective tissue matrices—providing an avenue for tumor cell invasion (8). High physiological concentrations of progesterone (but not testosterone or estradiol-17β) suppress secretion of uPA by SKOV-3 ovarian adenocarcinoma cells; this reaction was not influenced by the progesterone receptor antagonist RU486 or the transcriptional inhibitor actinomycin D (9). Indeed, it appears that progesterone protects against the development of common epithelial ovarian cancer (10).

We hypothesized that progesterone invokes an antitumorigenic effect by decreasing plasma membrane fluidity. The initial objective of this investigation was to determine the dose–response effects of progesterone on fluidity of ovarian epithelial cancer cells. Membrane rigidificaton was related to reduced shedding of secretory vesicles, *in vitro* invasiveness, colony formation in collagen matrix culture, and tumorigenesis in athymic nude mice.

## Materials and Methods

Reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless indicated otherwise.

**Ovarian Cancer Cells/Progesterone.** The highly invasive, tumor-forming, progesterone receptor negative SKOV-3 (11) epithelial cell line (American Type Culture Collection, Rockville, MD) was used as an experimental paradigm. Cells were propagated to confluence in T-75 flasks (Corning Costar, Cambridge, MA) at 37°C under an atmosphere of 5% $CO_2$ in 15 ml of RPMI-1640 (R 6504) medium supplemented with 10% charcoal-stripped/heat-inactivated fetal calf serum (Atlanta Biological, Norcross, GA), 10 μg/ml insulin (I 6634), and 1% antibiotic/antimycotic solution (A 9909). A trypsin (0.25%)/EDTA (0.03%) solution was used to harvest cells (>95% viabilities as indicated by trypan blue exclusion).

Progesterone was initially dissolved in ethanol (stock solution). The final concentration of ethanol in treatments

This work was supported by the National Institutes of Health Grant CA-97796-01.

[1] To whom requests for reprints should be addressed at University of Wyoming, P.O. Box 3684, Laramie, WY 82071. E-mail: wmurdoch@uwyo.edu

Received September 9, 2002.
Accepted November 29, 2002.

1535-3702/03/2283-0308$15.00

Copyright © 2003 by the Society for Experimental Biology and Medicine

was 0.05%. No effects of progesterone on proliferation or death of cultured SKOV-3 cells were observed in preliminary studies.

**Effect of Progesterone on Plasma Membrane Fluidity.** SKOV-3 cells were diluted in phosphate-buffered saline to $2 \times 10^5$/ml and incubated for 30 min or 6 hr in the absence or presence of progesterone (1, 10, 100, 1000 ng; $n = 5$–6). Doses of progesterone represented follicular/early luteal phase peripheral venous (1 ng/ml), mid-luteal peripheral venous (10 ng/ml), and local ovarian/preovulatory follicular fluid (1 μg/ml) concentrations ascribed to the human menstrual cycle (12, 13).

Membrane fluidity of living cells was assessed by anisotropy (rotational freedom) of an incorporated hydrophobic fluoroprobe (14). Cells were labeled with 1-(4-trimethylammonium-phenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH; T 0775; $5.0 \times 10^{-7}$ M, 1 min). Fluorescence was measured with an ISA/JY-Spex spectrofluorometer (Fluorolog-3) system equipped with automated polarizers (Model FL-1008; Edison, NJ). Excitation and emission wavelengths were set to the optimal intensities for TMA-DPH excitation (355 and 430 nm, respectively) (15). Membrane fluidity was inferred by determining the ratio between emission intensities parallel and perpendicular to the excitation plane (16). Anisotropies (r) for each sample were measured three times over 10 sec and corrected for background light-scattering and autofluorescence. Greater r values equate to lesser degrees of lipid mobility. Positive controls were conducted by subjecting cells to temperature (22, 32°C) changes (17). Assay coefficients of variation were <1%. Progesterone does not interfere with the spectroscopic properties of TMA-DPH (preliminary study).

**Membrane Release of Exocytotic Vesicles.** Subconfluent flasks of SKOV-3 cells were cultured for 24 hr without or with 100 ng/ml progesterone ($n = 9$). Supernatants were collected after centrifugation (500 g, 10 min; 800 g, 15 min) and vesicles therein pelleted (100,000 g, 1 hr; 4°C; 18). Vesicles were suspended in PBS and labeled ($5.0 \times 10^{-7}$ M TMA-DPH, 1 min) for spectrofluorometric analysis. Fluorescence intensity is proportional to the concentration of lipid bilayer present in the sample (16).

***In Vitro* Invasion Assay.** An analysis of cellular invasive activity was performed using BioCoat Matrigel chambers (Becton Dickinson, Bedford, MA). Cells ($1 \times 10^5$/ml) were plated onto coated wells (0.3 $cm^2$ membrane surface area, 8-μm pore size) for 1 hr and then incubated for 12 hr (37°C) without or with progesterone (1, 10, 100 ng/ml serum and phenol red-free RPMI [R 8755]; $n = 3$). Medium within the chambers was supplemented with 5% fetal calf serum as a chemoattractant. Cells that penetrated the membrane were fixed, stained with Wright-Giemsa, and counted (×200 magnification; six fields per specimen) with the aid of image scanning software (Optimas, Bothell, WA).

**Colony Formation in Three-Dimensional Collagen Culture.** SKOV-3 cells were suspended in a solution containing type-I collagen ($2.5 \times 10^5$/ml; ECM 675; Chemicon International, Temecula, CA) and transferred (0.1 ml) into a 96-well culture plate. After polymerization occurred (37°C, 60 min), culture medium without or with progesterone (10, 100 ng/ml; $n = 10$–14) was added (0.1 ml) to cover the collagen gels. Matrices were cultured for 14 days at 37°C in an atmosphere of 5% $CO_2$. Media were replaced daily. Matrices were removed from the plate, fixed for 48 hr in Histochoice (Amersco, Solon, OH), dehydrated in a graded series of ethanol, cleared in xylene, infiltrated with paraffin, sectioned at 12-μm thickness, rehydrated, stained in hematoxylin and eosin, and examined by light microscopy. Aggregates of greater than 10 cells were counted within 9–10 $mm^2$ fields per specimen.

**Tumor development in Immunocompromised Nude Mice Inoculated with SKOV-3 Cells.** The following experiment was conducted with the approval of the University of Wyoming Animal Care and Use Committee. Athymic (nu/nu) female and male mice (BALB/c strain) at 6–8 weeks of age were anesthetized by intraperitoneal injection of sodium pentobarbital (Abbott Laboratories, North Chicago, IL) and implanted subcutaneously with two (bilateral sites between the ears and shoulders) placebo or progesterone (25 mg)-containing ($n = 6$) pellets (Innovative Research of America, Sarasota, FL). SKOV-3 cells ($1 \times 10^7$ suspended in 0.1 ml of culture medium) were injected into the abdominal cavity the day after implant insertions. Animals were maintained in a pathogen-free environment under controlled temperature (24°C) and lighting (12L:12D) conditions. Sterilized rodent chow and water were supplied *ad libitum*.

Tail bleeds were performed at weekly intervals until animals were killed (cervical dislocation) on day 42 post-inoculation. Serum samples were analyzed for progesterone using a radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA). The assay was sensitive to 0.0156 ng of progesterone. Dilutions of mouse serum were parallel to the standard curve. Progesterone recoveries from spiked samples were >98%. Assay coefficients of variation were <6.1%.

Numbers of tumor nodules on the external surfaces of the duodenum and associated mesentery were counted (1-cm segments, three sites per animal) on day 42. Tissues were excised and processed (paraffin-embedding/hematoxylin and eosin; 7-μm sections) for light microscopic histopathological inspection; tumor-intestinal interfaces of four specimens per animal were examined.

**Statistical Analyses.** Assignments to treatments and selections of microscopic fields were made at random. Subsample data were averaged. Discrete group means were compared by one-way analysis of variance and protected least significant difference or Student's *t* test. Serum progesterone patterns were contrasted using a spilt-plot analysis of variance procedure for repeated measures. Differences were considered significant at $P < 0.05$.

## Results

Plasma membrane fluidities (1/r) deduced from fluorescence polarization in TMA-DPH-labeled SKOV-3 ovarian cancer cells were markedly attenuated by exposure to progesterone. There was a linear increase in anisotropy readings with increasing progesterone concentrations to 100 ng/ml; differences between 100 and 1000 ng/ml were not significant. Reactions after 30-min and 6-hr incubations were not different; combined r data are shown in Figure 1. The decrease in plasma membrane fluidity observed upon short-term incubations with 100 ng/ml progesterone equated to a decrease (after a 24-hr hormonal exposure) in cellular shedding of secretory vesicles (Fig. 2).

Invasion of SKOV-3 cells into Matrigel membranes also was restricted by treatments with progesterone (1, 10, and 100 ng/ml). Diminished chemotactic responses were most pronounced for cells subjected to the highest concentration of progesterone (Fig. 3). Formation of colonies by SKOV-3 cells cultured for two weeks in collagen matrices was inhibited significantly by 100 (but not 10) ng/ml progesterone (Fig. 4).

Mice with progesterone implants exhibited elevated systemic serum concentrations over time; amplitudes, however, did fluctuate. Progesterone increased to approximately 25 ng/ml during the first week after implantation of the hormone-releasing pellets. Concentrations then declined to day 21, rebounded (to about 15 ng/ml) by day 28, and fell again to day 42 (levels on days 21 and 42 were still greater than the corresponding control values; $P < 0.05$, *t* test; Fig. 5).

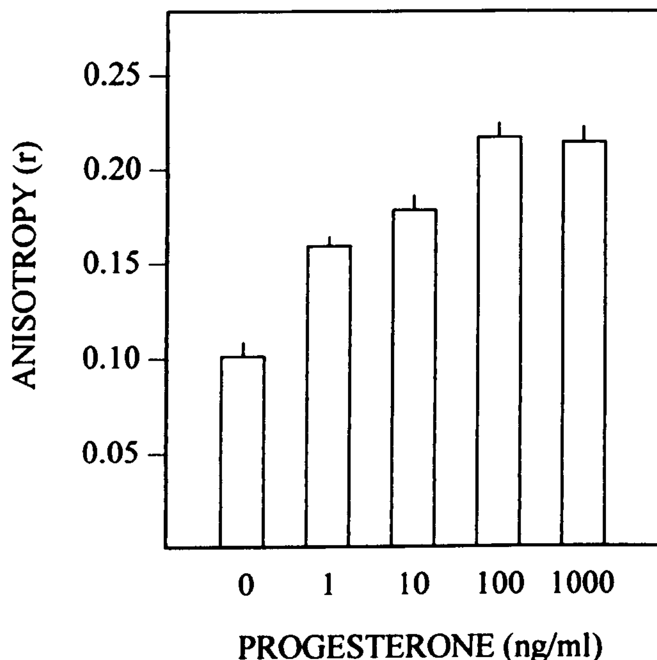


**Figure 1.** Anisotropy measurements of SKOV-3 cells incubated with progesterone. Numerical r values are inversely related to plasma membrane fluidity. Each progesterone treatment is different ($P <$ 0.0001) from control (0). Means + SE ($n = 11$) are plotted.

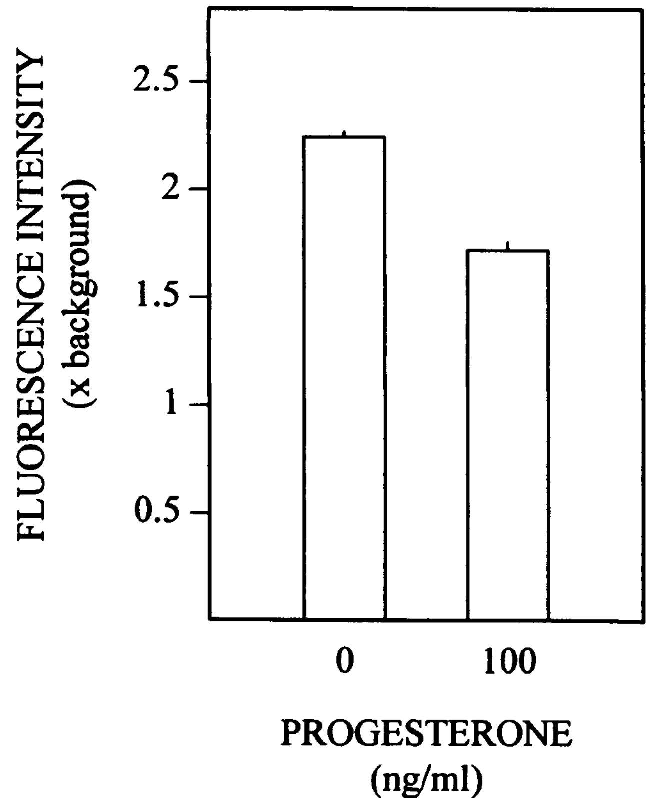


**Figure 2.** Inhibition by progesterone ($P < 0.0001$) of vesicular shedding from SKOV-3 cells. Values are expressed relative to a mean blank score ($n = 8$).

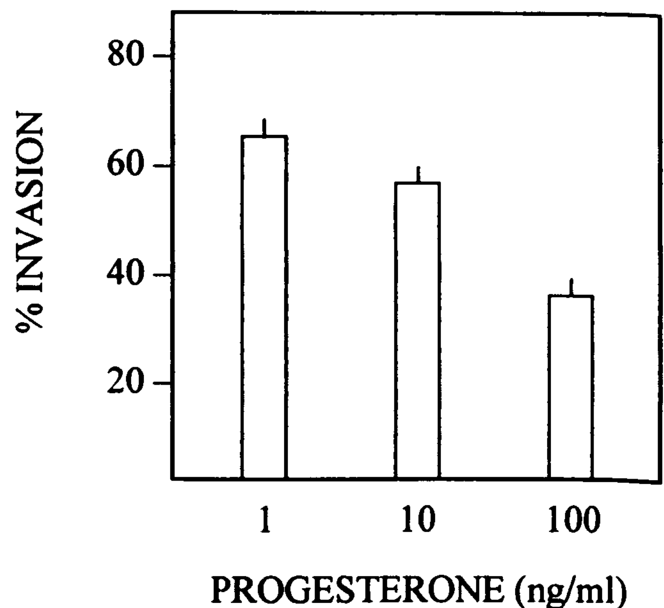


**Figure 3.** Inhibition by progesterone of the *in vitro* invasive capacity of SKOV-3 cells. Data (Matrigel infiltration rates) are expressed as percentages of the 0 ng/ml control (0 vs 1 or 10 ng/ml: $P < 0.05$; 0 vs 100 ng/ml: $P < 0.01$).

Animals treated with progesterone had fewer ovarian tumors on the surfaces of their intestines and mesenteries than control animals (Fig. 6). There was no evidence by day 42 of intraperitoneal accumulation of ascites fluid in either

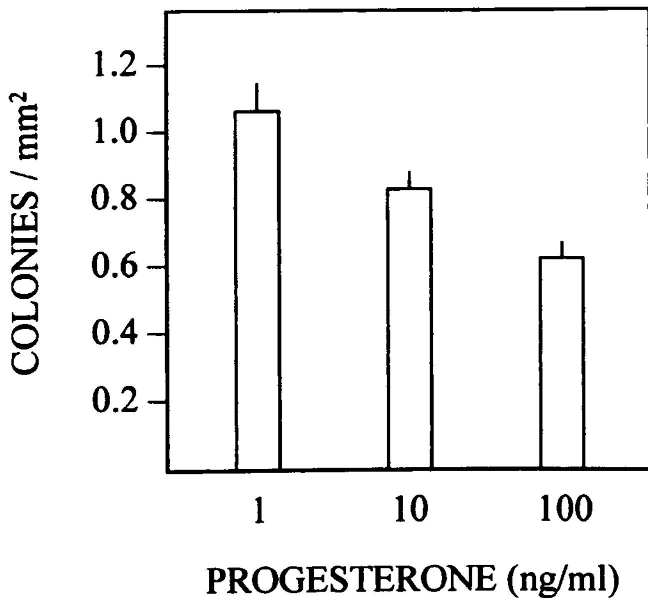


**Figure 4.** Effects of progesterone on formation of cellular colonies in collagen matrices (14-day culture). A statistically-different depression ($P < 0.05$ vs 0) was distinguished at 100 ng/ml progesterone.

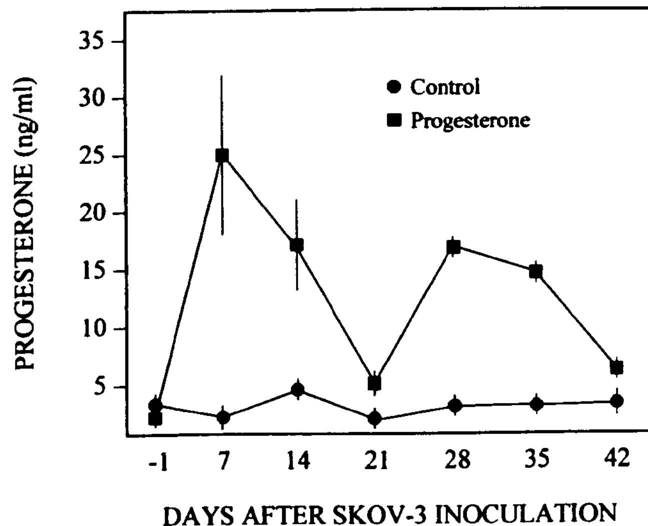


**Figure 5.** Circulatory progesterone profiles in control and progesterone-treated mice (time x treatment: $P < 0.001$).

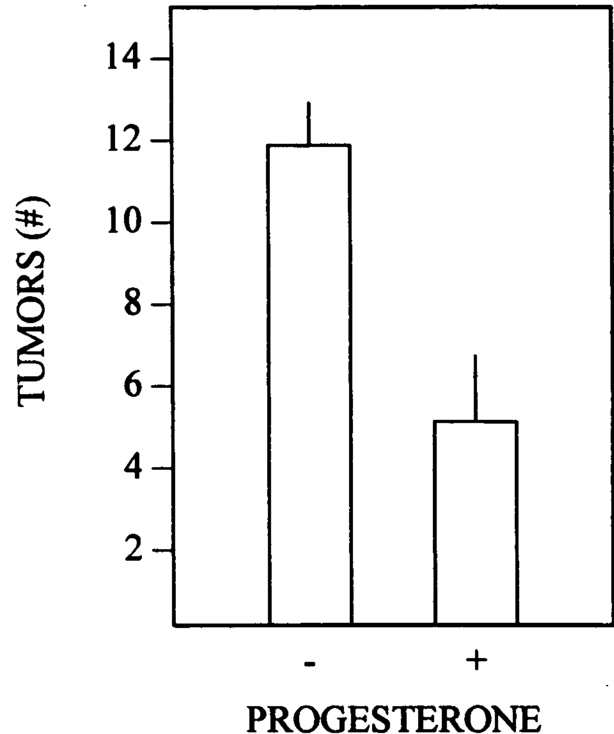


**Figure 6.** Day-42 intraperitoneal (per 1-cm length intestine/mesentery) tumor burdens in control and progesterone-treated mice. Mean contrasts are different ($P < 0.05$).

group. Microscopic examination of cross-sections along regions of tumor contact revealed that the serosal covering and underlying muscularis externa of the intestines were disrupted/degraded in all samples from control animals; these tissues typically (67% of cases) were intact in progesterone-treated mice (Fig. 7).

## Discussion

Physical state of the plasma membrane (equilibrium between molten/liquid crystal and solid gel) is a crucial determinant of cellular structure-function. Fluidity of biological membranes is affected primarily by integral fatty acid and cholesterol contents. Unsaturated fatty acids, which contain kinks in their hydrocarbon tails that minimize packing with contiguous lipids, increase fluidity. Cholesterol generally restricts lateral movements of membrane molecules. A propensity for metastatic dissemination of tumor cells has been related to increased plasma membrane fluidity (19).

Results of this investigation indicate that progesterone decreases the fluidity of plasma membranes of transformed ovarian surface epithelial cells, and that this translates into an inhibition of carcinogenic potential. It is predicted that the hydrophobic planar ring system of progesterone interacts with and immobilizes the core acyl side-chains of membrane phospholipids (20), thereby hindering the rotational mobility and transmembrane migration of exocytotic vesicles. Apparently, cell–cell adhesive interactions (colony formation) are likewise impeded by progesterone. Progestogens decreased plasma membrane fluidity in breast cancer cells (21) and the *in vitro* mobility and invasive activities of OMC-3 ovarian adenocarcinoma cells (22).

The multifactorial courses of episodes that can lead to common epithelial ovarian cancer have not been defined. Several aberrant stages are undoubtedly required to generate a malignant ovarian clone with a distinct growth advantage. It appears that a first step in tumorigenesis involves disturbances to the ovarian surface stemming from ovulation (23–26). Ovarian surface epithelial cells are exposed to DNA-damaging stimuli (inflammatory mediators and reactive oxidants) produced during the biomechanics of ovulatory follicular rupture (27–31). Cells within the immediate vicinity of the formative ovulation site (stigma) become apo-

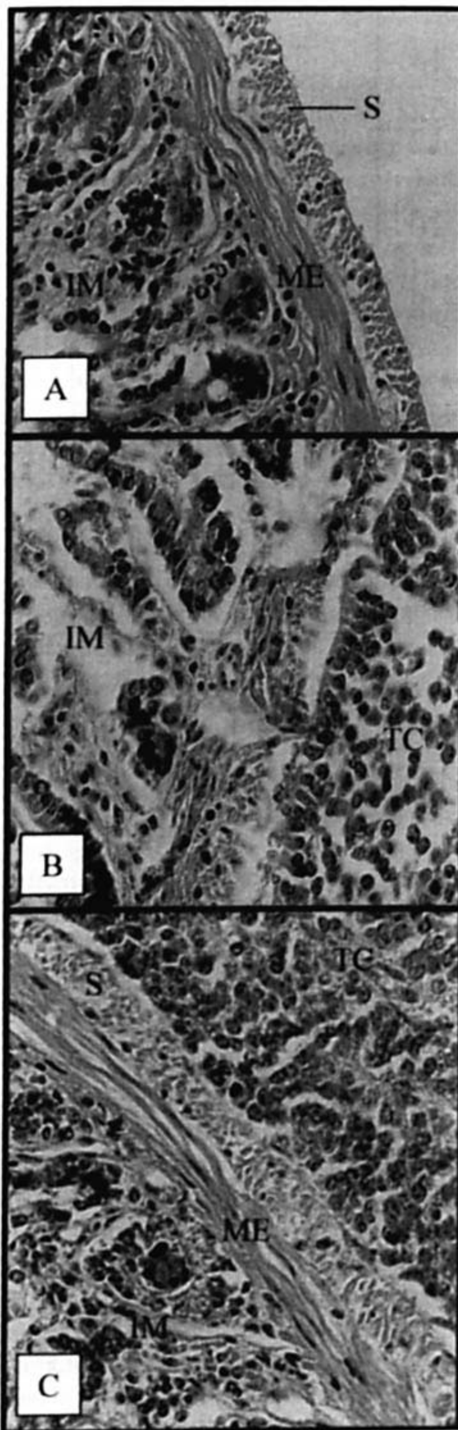


**Figure 7.** Representative photomicrographs of histological sections of intestinal tracts without (A) and with tumor implants (B, control; C, progesterone). Note the loss of tissue integrity at the tumor interface in B. S, serosa; ME, muscularis externa; IM, intestinal mucosa; TC, tumor cells. Magnification ×200.

ptotic and are sloughed (32). Proliferating bystander cells mend the ovarian surface defect after corpus luteum involution (33). It is conceivable that distresses to DNA, which are inflicted upon ovarian surface epithelial cells within a limited diffusion radius of the ovulatory rupture site, if uncorrected, could yield a tumorigenic progenitor. Precursor lesions of malignancy evidently arise from an ovarian inclusion cyst that contains surface epithelial cells that have undergone Mullerian metaplasia (34). Inclusion cysts are formed when surface cells become entrapped within the ovarian wound created at ovulation or during luteal absorption (33). With cystic rupture, cells are extruded into the peritoneal cavity. Peritoneal spread, metastatic colony formation, and development of ascites fluid (tumor deposits occlude lymphatics and impair venous drainage causing transudation) are hallmarks of disease advancement (35). Circumstances that avoid ovulation (oral contraceptive use, pregnancy, lactation) protect against ovarian neoplasia (36–38).

Phenotypes of ovarian tumors with high malignant and recurrent competencies accumulate uPA and progelatinases (39, 40). Secretion of uPA into ascites by membrane vesicles is a marker of tumor aggressiveness (41, 42). Modulation of uPA bioactivity involves a complex interplay of its receptor (uPAR) and inhibitors (PAI-1 and -2). Urokinase is synthesized and secreted as a proenzyme, which upon binding to uPAR, is proteolytically activated (43). The uPA receptor associated with ovarian cancer exists in cell-surface and ligand-free soluble forms (44–46). Localization to the cell surface directs the catalytic (i.e., plasminogen activator) function of urokinase (43). Elevated levels of uPAR and PAI-1 in ascites fluid were correlated with prolonged survival in ovarian cancer patients (40). Immunoneutralization of uPA inhibited ovarian cancer cell invasion *in vitro* (47) and the spread of human ovarian cancer in immunodeficient mice was reduced by antisense inhibition of uPA (48).

Ovarian cancer of surface epithelial origin accounts for more than 90% of all ovarian cancers and is the fifth leading cause of cancer-related deaths in women. It is the most prevalent cause of death from a gynecologic malignancy. Lethality of ovarian cancer is related to the clinical silence of pathogenesis (late diagnosis)—as a sequel of intraperitoneal carcinomatosis (49). Unfortunately, progestogens have been of limited value in the therapy of ovarian cancer; notwithstanding, treatments have been applied to patients with advanced chemorefractory illness (50, 51). Observations from these fundamental studies provide a conceptual basis for the prophylactic and therapeutic applications of progesterone in individuals deemed at high risk for the development of ovarian carcinoma or after diagnosis of early-stage disease, respectively. High doses of progesterone would theoretically be required to achieve beneficial outcomes. Albeit, side effects with (natural) progesterone (e.g., as compared with synthetic progestins, such as medroxyprogesterone acetate) are expected to be low (52).

In our experience, untreated immunodeficient mice bearing intraperitoneal SKOV-3 tumors generally develop ascites and die of gastrointestinal complications within 50–60 days of inoculation (unpublished observations). The hormonal pellets used in the *in vivo* study were designed (according to the manufacturer) to release progesterone at a consistent rate for 60 days; it is unclear why there were

acute decreases in circulatory levels on days 21 and 42. Interactions of inocular burdens and of dosages, modes of delivery, and durations of progesterone exposure on life spans of xenografted mice are under investigation.


1. Beato M, Sánchez-Pacheco A. Interaction of steroid hormone receptors with the transcription initiation complex. Endocr Rev **17**:587–609, 1996.
2. Revelli A, Massobrio M, Tesarik J. Nongenomic actions of steroid hormones in reproductive tissues. Endocr Rev **19**:3–17, 1998.
3. Pietras RJ, Nemere I, Szego CM. Steroid hormone receptors in target cell membranes. Endocrine **14**:417–427, 2001.
4. Brann DW, Hendry LB, Mahesh VB. Emerging diversities in the mechanism of action of steroid hormones. J Steroid Biochem Mol Biol **52**:113–133, 1995.
5. Golden GA, Mason PE, Rubin RT, Mason RP. Biophysical membrane interactions of steroid hormones: A potential complementary mechanism of steroid action. Clin Neuropharmacol **21**:181–189, 1998.
6. Stack MS, Ellerbroek SM, Fishman DA. The role of proteolytic enzymes in the pathology of epithelial ovarian carcinoma. Int J Oncol **12**:569–576, 1998.
7. Dolo V, D'Ascenzo S, Violini S, Pompucci L, Festuccia C, Ginestra A, Vittorelli ML, Canevari S, Pavan A. Matrix-degrading proteinases are shed in membrane vesicles by ovarian cancer cells in vivo and in vitro. Clin Exp Metast **17**:131–140, 1999.
8. Danø K, Andreasen PA, Grøndahl-Hansen J, Kristensen P, Nielsen LS, Skriver L. Plasminogen activators, tissue degradation, and cancer [review]. Adv Cancer Res **44**:139–266, 1985.
9. McDonnel AC, Murdoch WJ. High-dose progesterone inhibition of urokinase secretion and invasive activity by SKOV-3 ovarian carcinoma cells: evidence for a receptor-independent nongenomic effect on the plasma membrane. J Steroid Biochem Mol Biol **78**:185–191, 2001.
10. Risch HA. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. J Natl Cancer Inst **90**:1774–1786, 1998.
11. Hua W, Christianson T, Rougeot C, Rochefort H, Clinton GM. SKOV3 ovarian carcinoma cells have functional estrogen receptor but are growth-resistant to estrogen and antiestrogens. J Steroid Biochem Mol Biol **55**:279–289, 1995.
12. Abraham GE, Odell WS, Swerdloff RS, Hopper R. Simultaneous radioimmunoassays of plasma FSH, LH, progesterone, 17-OH progesterone and estradiol during the menstrual cycle. J Clin Endocrinol Metab **45**:312–318, 1972.
13. McNatty KP, Hunter WM, McNeilly AS, Sawers RS. Changes in the concentration of pituitary and steroid hormones in the follicular fluid of human graafian follicles throughout the menstrual cycle. J Endocrinol **64**:555–571, 1975.
14. Kuhry J, Duportail G, Bronner C, Laustriat G. Plasma membrane fluidity measurements on whole living cells by fluorescence anisotropy of trimethylammoniumdiphenylhexatriene. Biochim Biophys Acta **845**:60–67, 1985.
15. Haugland RP. Handbook of Fluorescent Probes and Research Chemicals. Eugene: Molecular Probes Inc., 1996.
16. Shinitzky M, Barenholz Y. Fluidity parameters of lipid regions determined by fluorescence polarization. Biochim Biophys Acta **515**:367–394, 1978.
17. Horváth I, Glatz A, Varvasoszki V, Török Z, Páli T, Balogh G, Kovács E, Nádasdi L, Benkö S, Joó F, Vígh L. Membrane physical state controls the signaling mechanism of the heat shock response in Synechocystis PCC 6803: Identification of hsp17 as a fluidity gene. Proc Natl Acad Sci USA **95**:3513–3518, 1998.
18. Dolo V, Ginestra A, Ghersi G, Nagase H, Vittorelli ML. Human breast carcinoma cells cultured in the presence of serum shed membrane vesicles rich in gelatinolytic activities. J Submicr Cytol Pathol **26**:173–180, 1994.
19. Sherbet GV. Membrane fluidity and cancer metastasis. Exp Cell Biol **57**:198–205, 1989.
20. Whiting KP, Restall CJ, Brain PF. Steroid hormone-induced effects on membrane fluidity and their potential roles in non-genomic mechanisms. Life Sci **67**:743–757, 2000.
21. Van Bommel T, Marsen T, Bojar H. Effects of high-dose medroxyprogesterone acetate and various other steroid hormones on plasma membrane lipid mobility in CAMA-1 mammary cancer cells. Anticancer Res **7**:1217–1223, 1987.
22. Ueda M, Fujii H, Yoshizawa K, Kumagai K, Ueki K, Terai Y, Yangaihara T, Ueki M. Effects of sex steroids and growth factors on invasive activity and 5'-deoxy-5-fluoruridine sensitivity in ovarian adenocarcinoma OMC-3 cells. Jpn J Cancer Res **89**:1334–1342, 1998.
23. Berchuck A, Carney M. Human ovarian cancer of the surface epithelium. Biochem Pharmacol **54**:541–544, 1997.
24. Auersperg N, Edelson MI, Mok SC, Johnson SW, Hamilton TC. The biology of ovarian cancer. Semin Oncol **25**:281–304, 1998.
25. Baker VV. The pathogenesis of epithelial ovarian cancer. J Clin Ligand Assay **21**:438–441, 1998.
26. Murdoch WJ, McDonnel AC. Roles of the ovarian surface epithelium in ovulation and carcinogenesis. Reproduction **123**:743–750, 2002.
27. Shukovski L, Tsafriri A. The involvement of nitric oxide in the ovulatory process in the rat. Endocrinology **135**:2287–2290, 1994.
28. Murdoch WJ. Plasmin-tumour necrosis factor interaction in the ovulatory process. J Reprod Fertil Suppl **54**:353–358, 1999.
29. Ness RB, Cottreau C. Possible role of ovarian epithelial inflammation in ovarian cancer. J Natl Cancer Inst **91**:1459–1467, 1999.
30. Kodaman PH, Behrman HR. Endocrine-related and protein kinase C-dependent generation of superoxide by rat preovulatory follicles. Endocrinology **142**:687–693, 2001.
31. Murdoch WJ, Townsend RS, McDonnel AC. Ovulation-induced DNA damage in ovarian surface epithelial cells of ewes: Prospective regulatory mechanisms of repair/survival and apoptosis. Biol Reprod **65**:1417–1424, 2001.
32. Murdoch WJ. Programmed cell death in preovulatory ovine follicles. Biol Reprod **53**:8–12, 1995.
33. Murdoch WJ. Ovarian surface epithelium during ovulatory and anovulatory ovine estrous cycles. Anat Rec **240**:322–326, 1994.
34. Feeley KM, Wells M. Precursor lesions of ovarian epithelial malignancy. Histopathology **38**:87–95, 2001.
35. Hamilton TC. Ovarian cancer, biology. Curr Prob Cancer **16**:5–57, 1992.
36. Cramer DW, Welch WR. Determinants of ovarian cancer risk. II. Inference regarding pathogenesis. J Natl Cancer Inst **71**:717–721, 1983.
37. Mant JWF, Vessey MP. Ovarian and endometrial cancers. Cancer Surv **19**:287–307, 1994.
38. Siskind V, Green A, Bain C, Purdie D. Beyond ovulation: Oral contraceptives and epithelial ovarian cancer. Epidemiology **11**:106–110, 2000.
39. Gleeson NC, Hill BJ, Moscinski LC, Mark JE, Roberts WS, Hoffman MS, Fiorica JV, Cavanagh D. Urokinase plasminogen activator in ovarian cancer. Eur J Gynaecol Oncol **17**:110–113, 1996.
40. Schmalfeldt B, Prechtel D, Harting K, Spathe K, Rutke S, Konik E, Fridman R, Berger U, Schmitt M, Kuhn W, Lengyel E. Increased expression of matrix metalloproteinase (MMP)-2, MMP-9, and the urokinase-type plasminogen activator is associated with progression from benign to advanced ovarian cancer. Clin Cancer Res **7**:2396–2404, 2001.
41. Chambers SK, Gertz RE, Ivins CM, Kacinski BM. The significance of urokinase-type plasminogen activator, its inhibitors, and its receptor in ascites of patients with epithelial ovarian cancer. Cancer **75**:1627–1633, 1995.
42. Ginestra A, Miceli D, Dolo V, Romano FM, Vittorelli ML. Membrane vesicles in ovarian cancer fluids: A new potential marker. Anticancer Res **19**:3439–3445, 1999.
43. Behrendt N, Rønne E, Danø K. The structure and function of the

urokinase receptor, a membrane protein governing plasminogen activation on the cell surface. Biol Chem **376**:269–279, 1995.

44. Kobayashi H, Moniwa N, Sugimura M, Shinohara H, Ohi H, Terao T. Increased cell-surface urokinase in advanced ovarian cancer. Jpn J Cancer Res **84**:633–640, 1993.
45. Pedersen N, Schmitt M, Rønne E, Nicoletti MI, Høyer-Hansen G, Conese M, Giavazzi R, Danø K, Kuhn W, Jänicke F, Blasi F. A ligand-free, soluble urokinase receptor is present in ascitic fluid from patients with ovarian cancer. J Clin Invest **92**:2160–2167, 1993.
46. Wahlberg G, Høyer-Hansen G, Casslen B. Soluble receptor for urokinase plasminogen activator in both full-length and a cleaved form is present in high concentration in cystic fluid from ovarian cancer. Cancer Res **58**:3294–3298, 1998.
47. Kobayashi H, Ohi H, Sugimura M, Shionhara H, Fujii T, Terao T. Inhibition of in vitro ovarian cancer cell invasion by modulation of urokinase-type plasminogen activator and cathepsin B. Cancer Res **52**:3610–3614, 1992.
48. Wilhelm O, Schmitt M, Hohl S, Senekowitsch R, Graeff H. Antisense inhibition of urokinase reduces spread of human ovarian cancer in mice. Clin Exp Metast **13**:296–302, 1995.
49. Perez RP, Godwin AK, Hamilton TC, Ozols RF. Ovarian cancer biology. Semin Oncol **18**:186–204, 1991.
50. Rao BR, Slotman BJ. Endocrine factors in common epithelial ovarian cancer. Endo Rev **12**:14–26, 1991.
51. van der Vange N, Greggi S, Burger CW, Kenemans P, Vermorken JB. Experience with hormonal therapy in advanced ovarian cancer. Acta Oncol **34**:813–820, 1995.
52. de Ziegler D, Fanchin R. Progesterone and progestins: Applications in gynecology. Steroids **65**:671–679, 2000.