0021-972X/80/5106-1239$02.00/0
Journal of Clinical Endocrinology and Metabolism
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Vol. 51, No. 6
Printed in U.S.A.

Biochemical Characterization of Peritoneal Fluid in Women during the Menstrual Cycle*

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ABSTRACT. Peritoneal fluid was collected at laparoscopy in women during the menstrual cycle and was assayed for protein and steroid hormone content.

The total protein concentration in peritoneal fluid and the concentrations of the steroid hormone-binding proteins, transcortin and sex hormone-binding globulin, the polypeptide hormones, LH, FSH, and PRL, correlated with the plasma concentration but were lower; they were, respectively, 68%, 71%, 68%, 42%, and 34% of the plasma concentration.

The concentrations of steroid hormones secreted by the ovary, *i.e.* 17β-estradiol, progesterone, androstenedione, and testosterone, were always equal or higher in peritoneal fluid than in plasma. In contrast, the concentration of cortisol, a nonovarian steroid hormone, was 40% lower in peritoneal fluid than in plasma.

No cyclic variations were observed in the peritoneal fluid concentrations of androstenedione and testosterone, two steroid hormones secreted by the stromal component of the ovary. On the contrary, the concentrations of 17β-estradiol and progesterone secreted by the follicular apparatus of the ovary increased sharply in peritoneal fluid after ovulation, reaching values of 44000 pg/ml and 3000 ng/ml, respectively. They declined progressively, whereas in plasma, peak concentrations were achieved only in the midluteal phase.

In conclusion, the concentrations of 17β-estradiol and progesterone are much higher in peritoneal fluid than in plasma for at least 1 week after ovulation. We suggest that the secretion of the early, not yet vascularized, corpus luteum is directed preferentially toward the peritoneal cavity, creating a specific hormonal environment for the released oocyte and the oviduct. (*J Clin Endocrinol Metab* **51:** 1239, 1980)

THE EXISTENCE of peritoneal fluid in normal women was described as early as 1922 by Novak (1), but little is known about its possibly very important significance in reproductive physiology. Indeed, this fluid is the physiological environment for the Fallopian tube and the oocyte immediately after ovulation. It might thus influence oocyte maturation, fertilization, and tubal transport processes, since the concentrations of progesterone and of 17β-estradiol during the luteal phase were reported to be much higher in peritoneal fluid than in plasma (2). Moreover, we recently reported that the assay of these steroid hormones could differentiate (3) between women with a normal ovulatory cycle and women with infertility due to the luteinized unruptured follicle syndrome, a newly discovered but frequent cause of infertility (4–6).

Therefore, we studied the composition of this fluid during the menstrual cycle.

Received December 11, 1979.

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* This work was supported in part by a grant from the Fonds Derde Cyclus (to K.U.L.).

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Materials and Methods

Patients

All women studied had regular biphasic cycles (26–35 days), and laparoscopies were performed during the investigation for primary or secondary infertility. The day of the cycle was determined by endometrial biopsy dating, as previously described (7).

Laparoscopy and peritoneal fluid collection

During laparoscopy under general anesthesia (CO_2 pneumoperitoneum) and with the patient in the supine position, the peritoneal fluid, collected in the pouch of Douglas, was aspirated and its volume was measured. The mean contamination with blood was estimated to be 4.2% by hemoglobin assay: 124 ± 16 g/liter (n = 11) in blood and 5.2 ± 5.6 g/liter (range, 0–17 g/liter; n = 15) in peritoneal fluid. Peritoneal fluids which were heavily blood stained were discarded. A blood sample taken at the same time was allowed to clot at room temperature for 1 h and was centrifuged together with the peritoneal fluid. The supernatants were frozen until assayed. The different numbers of blood and peritoneal fluid samples assayed for progesterone by competitive protein-binding assay (CPB) or RIA and for 17β-estradiol (by RIA), as indicated in Fig. 1, resulted, on the one hand, from the fact that the collection of paired blood samples was not continued up to the end of the study and, on

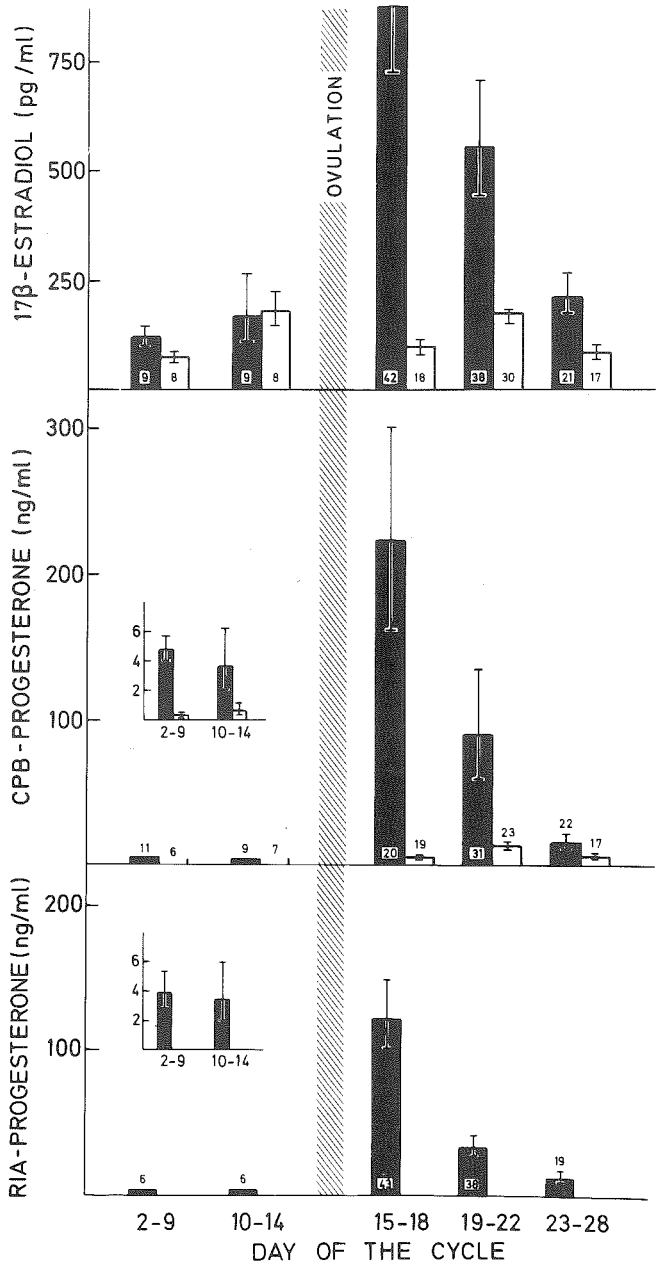

FIG. 1. Concentrations of 17β-estradiol and progesterone, assayed by CPB and RIA, in peritoneal fluid (■) and paired plasma samples (□). The $mean_{log}$ ± SE and the number of determinations are indicated.

the other hand, from the fact that the CPB progesterone assay was replaced at about the same time by a RIA.

The ovaries were carefully inspected for the presence or absence of a corpus luteum and an ovulation stigma. Pelvic endometriosis was classified as mild (superficial pelvic implants), moderate (ovarian implants), and severe (ovarian endometriomata of more than 1 cm in diameter) according to Acosta *et al.* (8), but no differences were found between women with and without endometriosis (9).

Assays

Sodium, potassium, chloride, carbon dioxide, calcium, phosphorus, urea, and total protein were assayed on an SMAC analyzer (Technicon Instrument Co., San Francisco, CA). Transcortin was assayed by radial immunodiffusion (10), and the 17β-hydroxy-5α-androstan-3-one (DHT)-binding capacity of sex hormone-binding globulin (SBHG) was assayed by ammoniumsulfate precipitation (11). PRL, LH, and FSH were assayed by RIA (12), and parallelism was demonstrated between serial dilutions of peritoneal fluid and standard curves using the MRC standards 68/40, 68/39, and 71/222. Cortisol was assayed by CPB (13). Peritoneal fluids which contained more than 100 ng progesterone/ml were not assayed for cortisol in order to prevent cross-reaction.

Progesterone was initially assayed by CPB and later by RIA. For the CPB assay (13), a semispecific extraction was performed with 5 vol *n*-hexane-ethanol (98:2); the recoveries of progesterone, 20α-hydroxy-pregn-4-en-3-one, and cortisol were, respectively, 104 ± 3% (n = 6), 106 ± 5% (n = 6), and 0.6 ± 0.04% (n = 6). The within- and between-assay variances at 50% of the initial binding level were 1.5% and 2.2% (n = 15). In some experiments, progesterone, 20α-hydroxy-pregn-4-en-3-one, and 17α-hydroxy-pregn-4-ene-3,20-dione were separated on LH-20 columns (30 × 1 cm), as described by Murphy (14), and then assayed separately by CPB.

The usefulness of CPB for the assay of progesterone was limited, however, by the presence in peritoneal fluid of unidentified products which compete actively for transcortin. At least some of these unidentified products gave blue UV-adsorbing spots (fluorescence, 240 nm) with R_F values of 0.58, 0.55, and 0.38 (R_F of progesterone, 0.51) on precoated F_{254} silica gel glass plates (0.25 mm thick) developed by methylenechloride-ether (70:30).

For this reason, a RIA was developed. The antiserum used was a rabbit 6β-hydroxy-pregn-4-en-3,20-dione hemisuccinate-bovine serum albumin antibody specific for progesterone, with 25% cross-reaction with 5α-pregnane-3,20-dione, 20% with 3α-hydroxy-5α-pregnan-20-one, and 1% with 17α-hydroxy-pregn-4-en-3,20-dione. Cross-reactivities with 20α-hydroxy-pregn-4-en-3-one, 5α-pregnane-3α,20α-diol, 3α-hydroxy-androst-5-en-17-one, DHT, androsterone, and 17β-estradiol were less than 0.1%. RIA of progesterone was performed without prior chromatography of the extract, since after thin layer chromatography, elution, and correction for procedural losses, the results for progesterone were 93 ± 5% (n = 20) of those obtained with the direct assay. The sensitivity of the assay was 100 pg, and the within- and between-assay variances were 1.9% and 3.0% (n = 15).

For the RIA of testosterone and androstenedione, the samples were extracted with 4 vol cyclohexane-ethylacetate (1:1) and chromatographed on LH-20 columns (20 × 1 cm) using chloroform-*n*-heptane-ethanol-H_2O (50:50:1:1) as eluent. Results were corrected for procedural losses by internal standards. The antiserum for androstenedione had a cross-reaction of 85% with 5α-androstane-3,17-dione and 2% with testosterone (15). The antiserum for testosterone had a cross-reaction of 13% with DHT and 0.5% with 3α,17β-dihydroxy-5α-androstane. Cross-reactions with androstenedione, DHEA, progesterone, 17β-estradiol, and 5α-pregnane-3α,20α-diol were less than 0.1%. The sensitivity of the assay was 50 pg for androstenedione and 12.5 pg for testosterone. The within- and between-assay variances

were, respectively, 0.8% and 1.6% (n = 3) and 0.8% and 2.0% (n = 15).

Estrogens were assayed by RIA, as described (16). Cyclohexane-ethylacetate (1:1) extracts were chromatographed on Sephadex LH-20 columns (0.5 × 8 cm) using benzene-methanol (9:1) as eluent. Results were corrected for procedural losses by internal standards.

Specificity of the progesterone, 17β-estradiol, androstenedione, and testosterone RIAs were performed, as described by Anderson *et al.* (17), using the Sephadex LH-20 columns mentioned.

Standards

The MRC standards 68/40, 68/39, and 71/222 were used for LH, FSH, and PRL, respectively. By RIA, 1 mg of the NIH standard LER 907 corresponded to 42–50 IU LH and 25 IU FSH (12).

Statistics

Means and SDs of hormone concentrations were calculated after logarithmic transformation (18). The mean ± SD are indicated unless stated otherwise. Statistical significance was evaluated according to Student's paired and unpaired *t* tests and by regression analysis.

Results

Electrolytes

Differences between the concentrations of electrolytes in plasma and peritoneal fluid, although significant, were small (Table 1). No differences were found between women in the luteal and those in the follicular phase of the cycle.

Total protein, steroid hormone-binding proteins, and polypeptide hormones

The concentrations of various proteins in peritoneal fluid correlated with the serum concentrations, but were about 35% lower, except for LH and PRL (Table 2).

TABLE 1. Electrolyte concentrations in peritoneal fluid and plasma

	Plasma		Peritoneal fluid		P value
Sodium (mmol/liter)	139.0 ± 3.2	(28)	136 ± 0.7	(36)	0.017
Potassium (mmol/liter)	5.2 ± 0.7	(28)	3.9 ± 0.1	(36)	0.051
Chloride (mmol/liter)	102.8 ± 1.4	(67)	110.6 ± 4.2	(86)	<0.001
Carbon dioxide (mmol/liter)	16.3 ± 0.3	(67)	22.4 ± 2.1	(86)	0.012
Calcium (mg/dl)	8.3 ± 0.2	(28)	7.4 ± 0.1	(36)	<0.001
Phosphorus (mg/dl)	2.9 ± 0.1	(67)	2.6 ± 0.1	(86)	0.003
Urea (mg/dl)	20.4 ± 0.7	(67)	21.1 ± 0.6	(86)	NS
Creatinine (mg/dl)	0.80 ± 0.02	(67)	0.70 ± 0.01	(86)	0.001

The mean ± SE and the number of determinations (in parentheses) are indicated.

During the luteal phase, the total protein, transcortin, and SHBG (Table 3) concentrations were significantly higher than during the follicular phase. In plasma, no significant differences were found during the menstrual cycle.

Progestins

Progesterone concentrations, whether measured by RIA or CPB, were higher in peritoneal fluid than in plasma throughout the menstrual cycle. In peritoneal fluid, the concentration increased sharply after ovulation and declined progressively thereafter (days 15–18 *vs.* days 19–22: P = NS for progesterone CPB assay and $P < 0.0001$ for progesterone RIA; days 19–22 *vs.* days 23–28: $P = 0.03$ for progesterone CPB assay and $P = 0.01$ for progesterone RIA), whereas in plasma the highest concentrations were found during the midluteal phase (Fig. 1).

In plasma, progesterone concentrations measured by CPB assay (CPB-progesterone) (nanograms per ml) were about 30% higher than progesterone concentrations measured by RIA; $y_{CPB} = 1.3x_{RIA} + 0.24$; r = 0.94; n = 12; $P < 0.001$). Also, in peritoneal fluid, CPB-progesterone concentrations measured in unchromatographed extracts were sometimes higher than RIA-progesterone concentrations (Fig. 2) and were higher than CPB-progesterone concentrations measured after chromatography (y = 1.4x + 20.4; r = 0.81; n = 27; $P < 0.001$). This could be explained, at least partially, by the presence of 20α-hydroxy-pregn-4-en-3-one (19); the relative concentrations of progesterone, 20α-hydroxy-pregn-4-en-3-one, and 17α-hydroxy-pregn-4-ene-3,20-dione were, respectively, 65.7 ± 12.6%, 29.7 ± 12%, and 3.2 ± 2.5% (n = 23). Moreover, the concentration of 20α-hydroxy-pregn-4-en-3-one correlated with the progesterone concentration (y = 0.60x − 5.2; r = 0.78; n = 27; $P < 0.002$). Occasionally, however, much higher concentrations (up to 6000 ng/ml) were found by CPB assay, due to the presence of unidentified products which competed for transcortin.

Estrogens

The estrogens found in peritoneal fluid (n = 15) were almost exclusively 17β-estradiol (85.9 ± 7.9%), with only minor amounts of estrone (7.6 ± 5.0%) and estriol (6.0 ± 4.5%), as evaluated after LH-20 chromatography.

During the follicular phase, the concentrations of 17β-estradiol were comparable in peritoneal fluid and plasma. After ovulation, however, the concentration in peritoneal fluid increased sharply (Fig. 1) and declined thereafter (days 15–18 *vs.* days 19–22, P = NS; days 19–22 *vs.* days 23–28, $P = 0.010$). The correlation between 17β-estradiol and RIA-progesterone concentrations in peritoneal fluid

TABLE 2. Concentrations of total protein, steroid hormone-binding proteins, and polypeptide hormones in peritoneal fluid and plasma

	Plasma	Peritoneal fluid	% in peritoneal fluid	Correlation	
				r	*P*
Total protein (g/liter)	63.8 ± 0.9 (67)	43.2 ± 0.8 (85)	68	0.04	NS
Transcortin (mg/liter)	34.3 ± 0.9 (53)	24.1 ± 0.7 (68)	71	0.49	<0.001
SHBG (μg/liter)	16.6 ± 0.7 (45)	11.2 ± 0.5 (61)	68	0.81	<0.001
LH (mU/ml)	4.8 ± 1.6 (28)	2.0 ± 0.4 (40)	42	0.86	<0.001
FSH (mU/ml)	3.0 ± 0.4 (41)	1.9 ± 0.1 (93)	63	0.71	<0.001
PRL (mU/ml)	3.4 ± 0.1 (43)	1.2 ± 0.1 (86)	34	0.15	NS

The mean ± SE and the number of determinations (in parentheses) are indicated.

TABLE 3. Concentrations of total protein, transcortin, and SHBG DHT-binding capacity in peritoneal fluid during the menstrual cycle

	Total protein (g/liter)	Transcortin (mg/liter)	SHBG (μg/liter)
Follicular phase	37.0 ± 1.2 (17)	20.3 ± 1.6 (19)	9.8 ± 1.4 (17)
Luteal phase			
Days 14–20	44.9 ± 0.8 (39)[a]	25.1 ± 0.7 (29)[b]	12.1 ± 0.5 (29)[a]
Days 21–28	44.0 ± 0.8 (29)[a]	26.9 ± 1.5 (20)[b]	11.1 ± 1.1 (15)

The mean ± SE and the number of determinations (in parentheses) are indicated.

[a] $P < 0.001$ *vs.* follicular phase.

[b] $P < 0.005$ *vs.* follicular phase.

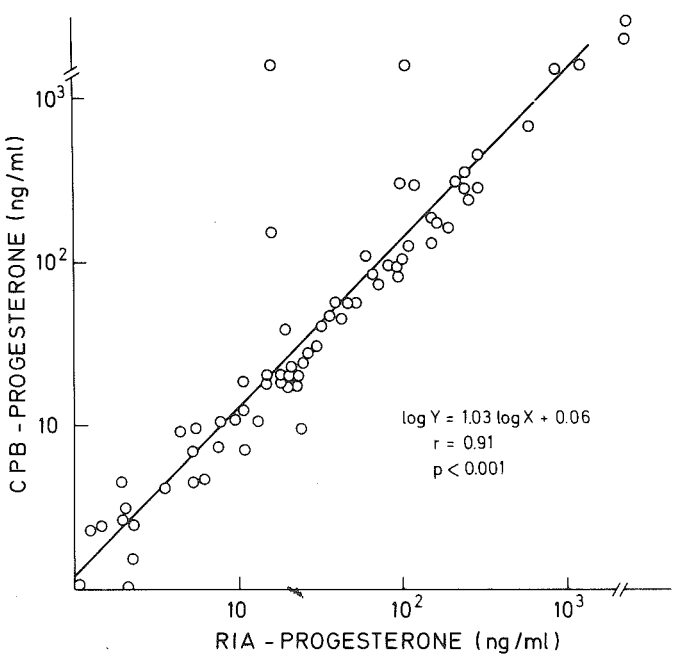

FIG. 2. Correlation of progesterone concentrations in peritoneal fluid measured by RIA and CPB on unchromatographed extracts.

was highly significant (Fig. 3), demonstrating that the high concentrations of both hormones occurred in the same women. No correlation could be found between the plasma and the peritoneal fluid concentrations or during the follicular (r = 0.033; n = 15) or luteal phase (days 17–18, r = 0.128 and n = 12; days 19–20, r = 0.186 and n = 17).

Androgens

The concentration of androstenedione was higher in peritoneal fluid than in plasma throughout the menstrual cycle. The concentration in peritoneal fluid correlated significantly with the plasma concentration and with the testosterone concentration in peritoneal fluid (Table 4). The concentrations of testosterone were comparable in peritoneal fluid and plasma during both phases of the cycle.

Cortisol

The concentration of cortisol was 137 ± 81 ng/ml in plasma (n = 52) and 54.7 ± 33 ng/ml in peritoneal fluid (n = 36). No differences were found between the two phases of the cycle.

Discussion

Steroid hormones

It was suggested (20) that although some peritoneal exudation cannot be excluded, peritoneal fluid is mainly formed by ovarian exudation, since 1) the volume of peritoneal fluid is not affected by the absence or presence of the Fallopian tube and 2) in cycling women the volume increases *pari passu* with ovarian activity, while the volume is uniformly low in women with suppressed ovarian activity, *i.e.* postmenopausal women and women taking combined oral contraceptives or Lynoestrenol (5 mg daily).

Direct and continuous secretion of sex steroid hormones from the ovary into the peritoneal cavity must be postulated in order to explain the steroid hormone concentrations. Indeed, throughout the menstrual cycle a concentration gradient is present for the steroid hormones of ovarian origin, *i.e.* progesterone, 17β-estradiol, androstenedione, and testosterone, since in peritoneal fluid the concentrations of these hormones are always equal to or higher than the plasma concentrations despite the lower concentrations of their binding proteins. On the contrary, the concentration of cortisol, a nonovarian steroid hormone, is 60% lower in peritoneal fluid than in plasma.

TABLE 4. Concentrations of androstenedione and testosterone in peritoneal fluid and plasma during the follicular and luteal phases of the menstrual cycle

	Plasma	Peritoneal fluid	Correlation		
			r	n	*P*
Follicular phase					
Androstenedione (ng/ml)	1.9 (1.1 – 3.5) [5]	2.3 (0.9 – 5.9) [10]	0.16	5	NS
Testosterone (ng/ml)	0.30 (0.25 – 0.38) [5]	0.23 (0.11 – 0.44) [10][a]	0.46	5	NS
Luteal phase					
Androstenedione (ng/ml)	1.5 (1.1 – 2.1) [17]	3.7 (1.6 – 8.2) [21]	0.87	10	<0.005
Testosterone (ng/ml)	0.30 (0.20 – 0.44) [16]	0.29 (0.18 – 0.47) [21][b]	0.76	7	<0.05

The $mean_{log}$ (mean − 1 SD, mean + 1 SD; in parentheses) and the number of determinations (in brackets) are indicated.
[a] r = 0.92, n = 10, $P < 0.001$ (correlation with peritoneal fluid androstenedione concentration).
[b] r = 0.67, n = 21, $P < 0.005$ (correlation with peritoneal fluid androstenedione concentration).

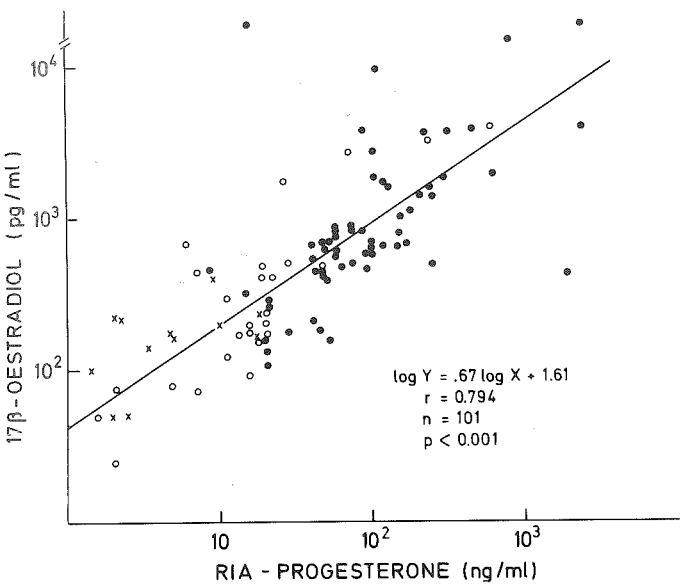

FIG. 3. Correlation between progesterone and 17β-estradiol concentrations in peritoneal fluid during the follicular (X) and luteal (●, days 15–20; ○, days 21–28) phases of the cycle.

The 17β-estradiol and progesterone contents of follicular fluid (21, 22) could explain the very high concentrations found in peritoneal fluid immediately after ovulation. Without continuous secretion, however, the concentrations would equilibrate rapidly with the plasma concentrations, since small molecules diffuse freely through the peritoneum (23). To explain the important concentration gradient during at least 1 week and the lag time between peak concentrations in plasma and peritoneal fluid after ovulation, we suggest the following mechanism. The secretion products of the early corpus luteum, which is not yet vascularized, are preferentially directed toward the peritoneal cavity by exudation of fluid from the thecal capillaries through the corpus luteum and the ovulation ostium. After vascularization of the corpus luteum, by capillary in-growth, secretion is mainly directed toward the blood stream. The progressively rising plasma progesterone concentrations during the early luteal phase would thus reflect vascularization of the corpus luteum.

Similarly, we suggest that during the follicular phase, steroid hormone-laden fluid is drained through the avascular area of the ovary, *i.e.* the follicle. Indeed, it is likely that a similar permeability of the follicle wall is present where the follicle is in contact with the theca layer and where the follicle reaches and protudes above the ovarian surface; exchange between follicular fluid and thecal capillaries has been shown to occur rapidly (24). In this way, we can understand that 1) concentrations of steroid hormones derived from the follicular apparatus are relatively higher than those derived from the stromal component of the ovary and 2) the time course of peritoneal and plasma concentrations of progesterone and 17β-estradiol are not contemporaneous.

Total protein, steroid hormone-binding proteins, and polypeptide hormones

The concentrations of total protein, steroid hormone-binding protein, and FSH were 30% lower in peritoneal fluid than in plasma. The PRL concentration, however, was much lower in peritoneal fluid (66% lower than in plasma). This can be explained by the fact that the plasma concentrations which were very high were probably acutely elevated by stress and/or anesthesia, and equilibrium with peritoneal fluid was not yet reached at the moment of sampling. Indeed, diffusion of proteins through the peritoneum is relatively slow (23). The lower PRL concentrations are probably not caused by degradation, since after incubation of peritoneal fluid at 37 C for 4 h, the concentrations remained unchanged.

Clinical and biological significance

Steroid hormone concentrations in peritoneal fluid can be used for the diagnosis of the luteinized unruptured follicle syndrome, a frequent cause of infertility. Indeed, after ovulation, the concentrations of 17β-estradiol and progesterone are only slightly elevated in the peritoneal

fluid of women with this syndrome (25).

The biological significance of these extremely high steroid hormone concentrations in reproductive physiology is not yet known but warrants further investigation. Indeed, it will have to be evaluated 1) whether blood stream concentrations or peritoneal fluid concentrations are most important for tubal function, 2) if oocyte maturation and fertilization are influenced by these hormone concentration, and 3) how endometriosis is related to peritoneal fluid hormone concentrations.

Acknowledgments

We thank Prof. M. Renaer, Prof. A. Van Assche, Dr. P. Ide, and Dr. F. De Wolf for their help in collecting peritoneal fluid; Prof. Dixon (Bristol, England) and Prof. Robertson (London, England) for fruitful discussions; and Miss V. Celis, Mrs. T. Valkenborgh, Mrs. M. R. Van Gossum, Mrs. B. Minten, Mr. B. Vanhaleweyk, Mr. J. Cailleau, and Mr. I. Jans for excellent technical assistance. The standards were a gift of the NIH and the Medical Research Council. The androstenedione antiserum was generously donated by Prof. Vermeulen, Gent, Belgium.

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