Protein synthesis and secretion by the human endometrium during the menstrual cycle and the effect of progesterone *in vitro*

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Summary. To identify markers of endometrial differentiation specimens of endometrium from the menstrual cycle were incubated *in vitro* with [³⁵S]methionine, in the absence or presence of progesterone, and protein synthesis and secretion were studied by fluorographic analysis of one dimensional SDS/gradient polyacrylamide gels. Changes were demonstrated in the rate of synthesis and secretion of a number of endometrial proteins (EP) during the cycle and in response to progesterone. Endometrial proteins were classified into three groups: Group I—synthesized and secreted throughout the menstrual cycle and unaffected by progesterone exposure; Group II—synthesis and secretion associated with histological type of endometrium and unaffected by progesterone exposure, e.g. EP 13 (M_r 33 000) with proliferative, EP 15 (M_r 28 000) with secretory and EP 14 (M_r 32 000) with late secretory endometrium; Group III—synthesis and secretion regulated by progesterone exposure irrespective of source of endometrium, e.g. EP 9 (M_r 54 000) and 11 (M_r 45 000). The Group II proteins EP 14 and 15 were also the major secretory protein products of endometrium from first and second trimester pregnancy respectively, the native forms referred to as pregnancy-associated endometrial α_1 - and α_2 -globulins (α_1 - and α_2 -PEG). We conclude that EP 15 (α_2 -PEG) represents a human analogue of uteroglobin.

Introduction

The human endometrium under the influence of oestrogen and progesterone exhibits dramatic histological and biochemical alterations during the menstrual cycle (Robertson, 1981). Progesterone is the principal steroid hormone involved in the differentiation of the endometrium during the post-ovulatory secretory phase of the cycle characterized by initiation of glandular epithelial secretion, decidual transformation of stromal fibroblasts and appearance of endometrial granulocytes. It is generally assumed that secretion associated with epithelial differentiation may contribute to the interuterine environment and affect the blastocyst before and during its implantation (O'Grady & Bell, 1977). Although in many species several specific secretory proteins in luminal fluid have been identified, including uteroglobin (Beier, 1968), studies on human uterine luminal fluid have been less successful (Schumacher, Holt & Reale, 1979). A number of specific functions have been ascribed to secretory protein products of the endometrium and decidua of laboratory rodents (Bell, 1983a, b).

Many studies have been directed toward the identification of alterations in cytosolic protein synthesis during the human menstrual cycle and its *in-vitro* regulation by steroid hormones. These studies have consistently detected the increased synthesis of a protein of M_r 50–55 000 by the secretory endometrium and induced *in vitro* by progesterone (Hirsch, Fergusson & King, 1977; O'Grady, Bell, Govan & Black, 1978; Shapiro & Forbes, 1978; Iacobelli *et al.*, 1981). However,

whether this protein is secreted is unknown. More recently, two dimensional polyacrylamide gel electrophoretic techniques have identified a number of proteins synthesized and secreted by the proliferative endometrium during culture (Strinden & Shapiro, 1983). The rate of synthesis and secretion of a number of these proteins is modulated by exposure to progesterone *in vitro*. The secretory endometrium has been demonstrated to secrete prolactin (Riddick & Daly, 1982) and indirect evidence suggests that it secretes the cytosolic protein, "progestagen-dependent endometrial protein", PEP (Joshi, 1983). However, whether these represent major secretory products of this tissue is unknown.

As part of a programme to identify proteins synthesized and secreted by the endometrium, to be used as markers of endometrial differentiation (Bell, 1985), the two major secretory protein products of the endometrium during pregnancy have been characterized, i.e. EP 14 and EP 15 (Bell, Hales, Patel, Kirwan & Drife, 1985a; Bell, Patel, Hales, Kirwan & Drife, 1985b). These two proteins, in their native forms termed pregnancy-associated endometrial α_1 - and α_2 -globulins (α_1 - and α_2 -PEG), exhibited different profiles of synthesis and secretion during pregnancy (Bell & Drife, 1985). We have now extended this study and examined the synthesis and secretion of proteins by the endometrium during the menstrual cycle and the effect of progesterone *in vitro*.

Materials and Methods

Tissues. Tissues were obtained from outpatients attending the Leicester Royal Infirmary. Portions of all tissues were forwarded to the University Department of Pathology for histological examination. Endometrial specimens were obtained from women undergoing laparoscopic sterilization or hysterectomy for benign pelvic conditions. All patients were of proven fertility and had normal menstrual cycles. Patients previously receiving oral contraceptive agents discontinued treatment at least 6 weeks before to operative procedures.

Specimens were classified as follows: early proliferative, Days 5–7; mid-proliferative, Days 8–10; late proliferative, Days 11–14; early secretory, Days 15–18; mid-secretory, Days 19–23 and late secretory–premenstrual, Days 24–28 according to the description of Noyes, Hertig & Rock (1950) and Wynn (1977).

Incubation and labelling of proteins. Tissues were rinsed in cold saline (9 g NaCl/l) and, after excess saline was removed onto filter paper on ice, were cut into pieces of about 2 mm² and weighed. Then 75–100 mg tissue were placed in vials containing incubation medium (2 ml) consisting of Trowell's T-8 (Gibco Europe) supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml) and fungizone (0.25 µg/ml). Vials were preincubated for 2 h at 37°C and medium was replaced. When sufficient tissue was available, pairs of vials were set up, the medium in one vial being replaced with progesterone-containing medium (dissolved in ethanol and present at 0.1 µg/ml). To label proteins, 40 µCi [³⁵S]methionine (Amersham Int., Bucks, U.K.; sp. act. 800 Ci/mmol) were added to the medium and the incubation was carried out at 37°C in an atmosphere of 95% O₂–5% CO₂.

After incubation, the secreted proteins were analysed in the incubation medium as previously described (Bell *et al.*, 1985a). Briefly, the medium was removed and centrifuged at 100 000 g for 1 h and the supernatant fraction stored at 20°C. Aliquants to be used for gel electrophoresis and fluorography were lyophilized and resuspended in the appropriate volume of sample buffer to achieve adequate loading of radioactivity. To determine incorporation of labelled amino acids into protein, aliquants of medium (100 µl) were mixed with bovine serum albumin (BSA) to a final concentration of 0.12% (w/v) BSA. Proteins were precipitated by adding 300 µl ice-cold 10% (w/v) trichloroacetic acid (TCA) and vortexing. The precipitates were kept at 4°C for 30 min and centrifuged at 2000 g for 5 min. The supernatants were discarded and precipitates washed three times with 300 µl ice-cold 5% TCA. The pellets were then dissolved in 500 µl 0.2 M-NaOH; 5 ml scintillation fluid (Fisofluor 1, Fisons Ltd) were added and the tubes were counted in a LKB Beta-Rac Scintillation Counter.

The colorimetric method of Bradford (1976) was used to determine total proteins using BSA as a reference standard.

Protein separation. For one-dimensional SDS-gradient gel electrophoresis up to 75 μ l volumes of medium (containing up to 100 000 c.p.m.) were mixed with an equal volume of buffer containing β -mercaptoethanol (10%), SDS (5%), glycerol (20%), Trizma (0.125 M, pH 6.8) and bromophenol blue (0.002%). Samples were heated at 100°C for 3 min and analysed on 1.5 mm thick 5–15% acrylamide slab gel (containing 0.26–0.82% bisacrylamide) with a 4.5% stacking gel (containing 0.12% bisacrylamide) both containing 0.1% SDS.

Gels were stained for 1–2 h in Coomassie blue G (0.04% in 3.5% perchloric acid, 20% methanol) then destained for 24 h using several changes of 5% acetic acid, 10% methanol. All gels to be processed for fluorography were soaked in 1 M-sodium salicylate, dried, and exposed to Kodak X-Omat X-ray film using Harmer intensifying screens (Chamberlain, 1979). The rates of synthesis and secretion of individual proteins were estimated by: (i) scanning the fluorographs with a densitometer and determining the percentage of total incorporation represented by a particular band on the fluorograph, (ii) and multiplying this percentage by the total rate of incorporation of radioactivity into TCA-precipitable material in the medium.

The molecular weights of proteins were estimated by their mobilities relative to standard proteins of known molecular weight (Sigma Chemical Co., Poole, Dorset, U.K.).

Results

Radioactive incorporation into medium and identification of secreted proteins

Incorporation of radioactive methionine into protein was measured during incubations of menstrual cycle endometrium in medium with or without progesterone (0.1 μ g/ml) in paired vials when sufficient tissue was available. However, during the early proliferative phase, sufficient material did not always permit such analysis and all the specimen was incubated with or without progesterone. Progesterone was included to determine whether the synthesis and secretion of proteins was regulated by progesterone during the culture period. Analysis of radioactivity in TCA-precipitable proteins in the medium revealed that, under these conditions, radioactive proteins were secreted after a lag phase of 2–4 h and then linearly for 24 h. No effect on total incorporation into secreted proteins in the medium was observed with and without progesterone.

To determine the nature of proteins synthesized and secreted by menstrual cycle endometrium during the incubation, aliquants of culture medium were analysed by one-dimensional SDS-gradient polyacrylamide gel electrophoresis and the gels processed for fluorography. Figure 1 is a typical fluorograph of a gel in which culture medium from late proliferative (Day 11) and mid-secretory (Day 21) endometrium specimens after 24 h in culture were analysed. Track 4 is a culture medium from a first trimester pregnancy endometrium (Week 9–10) for an internal comparison. As in the previous study on protein secretion by early pregnancy endometrium, 17 endometrial proteins were synthesized and secreted by the endometrium during the normal menstrual cycle and, although no protein species was uniquely and specifically associated with any stage of the cycle or pregnancy, dramatic alterations in the quantitative rate of synthesis and secretion of certain protein species were observed during the menstrual cycle and in their response to progesterone *in vitro*. An analysis of the rates of synthesis and secretion of these proteins was therefore performed in greater detail.

Synthesis and secretion of endometrial proteins EP 13, 14 and 15

Examination of fluorographs revealed a group of endometrial proteins that appeared to exhibit an increased rate of synthesis and secretion associated with a particular stage of the menstrual

Fig. 1. Fluorograph of one dimensional SDS/gelatin PAGE analysis of medium after incubation of endometrium with [^{35}S] methionine. Track 1, late proliferative endometrium (Day 11) without progesterone; Track 2, mid-secretory endometrium (Day 21) with progesterone; Track 3, secretory endometrium (Day 21) without progesterone; Track 4, first trimester pregnancy endometrium (Week 9–10) without progesterone. Left hand axis refers to M_r of protein standards as visualized by Coomassie Blue staining of gel before processing for fluorography.

cycle, e.g. EP 13 (M_r 33 000) was associated with the follicular phase, EP 15 (M_r 28 000) with the luteal phase and EP 14 (M_r 32 000) with the late luteal phase. The rates of in-vitro synthesis and secretion of these proteins were determined during the menstrual cycle. Aliquants of culture medium were removed at timed intervals, analysed by one dimensional SDS gradient PAGE and fluorography (see 'Materials and Methods'), and the rate was determined from slope of incorporation versus incubation time (Fig. 2). Synthesis and secretion of EP 13 was principally associated with proliferative endometrium (Fig. 3a), with synthesis dramatically declining in the early secretory endometrium ($P < 0.01$). During the follicular phase, this protein represented a major secretory protein of the endometrium (Fig. 1, track 1). In contrast, a major secretory protein product during the luteal phase was EP 15, representing up to 35% of the total [^{35}S]methionine incorporated into secretory proteins by the mid to late secretory endometrium (Fig. 3c). Little synthesis and secretion

was noted by the proliferative or early secretory endometrium. This protein continued to represent a major secretory protein of the endometrium during early pregnancy. However, after Week 6–7, synthesis declined (Bell *et al.*, 1985a).

Although in direct contrast to the situation in pregnancy EP 14 was never a major secretory protein of the endometrium during the menstrual cycle, an increase in synthesis and secretion by the late secretory endometrium containing decidualized endometrium compared to mid-secretory endometrium was observed ($P < 0.05$) (Fig. 3b).

None of the proteins was affected by in-vitro exposure of menstrual cycle endometrium to progesterone under the culture conditions used in this study. Similar results were previously obtained for EP 14 and 15 during early pregnancy (Bell *et al.*, 1985a).

Synthesis and secretion of progesterone-regulated endometrial proteins EP 9 and 11

The synthesis and secretion of certain endometrial proteins appeared to be affected by short-term exposure to progesterone *in vitro*. The in-vitro rate of synthesis and secretion of EP 9 (M_r 54 000) was decreased by progesterone (decrease of $40.3 \pm 16.6\%$ for 13 specimens, mean $\pm$ s.d.). It was a major secretory protein of proliferative phase endometrium incubated in the absence of progesterone (Fig. 1, track 1; Fig. 4a) but not in specimens incubated with progesterone (Fig. 4a). In mid-secretory endometrial specimens, although in the presence of progesterone little incorporation into EP 9 was observed (Fig. 1, track 2; Fig. 4a), in paired specimens from the same patient cultured in the absence of progesterone EP 9 synthesis and secretion were pronounced (Fig. 1, track 3; Fig. 4a).

Fig. 2. Estimation of rates of synthesis and secretion of endometrial proteins during incubation of secretory endometrium. Aliquants of culture medium at timed intervals were analysed by SDS/gradient polyacrylamide gel electrophoresis and gels were processed for fluorography. Fluorographs were scanned in a densitometer to obtain an estimate of percentage of total incorporation represented by each protein band.

Fig. 3. Rate of synthesis and secretion of endometrial proteins EP 13(a), 14(b), and 15(c) during the menstrual cycle. Open circles and bars represent mean and s.e.m. of observations. Specimens during the menstrual cycle were proliferative (P) (early, Day 5–7; mid, Day 8–10; or late, Day 11–14) or secretory (S) (early, Day 15–18; mid, Day 19–23; or late, Day 24–28). Specimens of pregnancy endometrium (Pg) obtained during the first trimester (Weeks 6–12) were also analysed for comparison. Data were compared using Student's *t* test and levels of significance between groups as indicated by bars are as follows: $P < 0.01$ (◐), $P < 0.02$ (☆), $P < 0.05$ (★) and not significant (○).

Fig. 4. Rates of synthesis and secretion of endometrial proteins EP 9 (a) and 11 (b) during the menstrual cycle and the effect of progesterone *in vitro*. Lines between observations represent an experiment performed on paired specimens cultured with (●) or without (○) progesterone. Specimens were early (EP, Days 5–7), mid (MP, Days 8–10) or late (LP, Days 11–14) proliferative endometrium, and early (ES, Days 15–18), mid (MS, Days 19–23) or late (LS, Days 24–28) secretory endometrium.

In direct contrast, EP 11 (M_r 45 000) synthesis and secretion was increased by short-term exposure to progesterone *in vitro* (increase by $160 \pm 159\%$ in 13 specimens, mean $\pm$ s.d.). It was low when proliferative-phase endometrium was cultured in its absence and appeared in its presence (Fig. 1, track 1; Fig. 4b). EP 11 was a major secretory protein product of mid-secretory endometrium incubated with progesterone (Fig. 1, track 2; Fig. 4b) but its synthesis and secretion fell dramatically in its absence (Fig. 1, track 3; Fig. 4b). Under the incubation conditions employed no alteration in the histology of endometrium occurred whether in the presence or absence of progesterone.

Discussion

This study has demonstrated that, during the normal menstrual cycle, dramatic alterations in the synthesis and secretion of specific proteins occur and also that *in-vitro* exposure of the endometrium to progesterone can markedly affect the rate of synthesis and secretion of other protein species. We have therefore classified the proteins synthesized and secreted *in vitro* by the endometrium into three groups (Table 1) which, if detectable in sera, may be of clinical value in assessing endometrial function and differentiation and the *in-vivo* effect of progesterone upon the endometrium. These proteins are suggested to represent species synthesized and actively secreted and

Table 1. Classification of endometrial proteins synthesized and secreted by human endometrium during menstrual cycle and pregnancy

Group	Protein number	Associated with histologically-defined endometrial type	Affected by short-term exposure to progesterone <i>in vitro</i>
I	EP 6	No	—
	EP 12	No	—
II	EP 13	Yes, proliferative	—
	EP 14 (α_1 -PEG)	Yes, decidualized	—
	EP 15 (α_2 -PEG)	Yes, secretory	—
III	EP 9	No	+ (↓)
	EP 11	No	+ (↑)

not the result of cell lysis during the incubation since few low molecular weight proteins were observed.

Group II endometrial secreted proteins are those that appear to be associated with a histologically-defined endometrial type, unaffected by short-term *in-vitro* progesterone exposure, and they include the two major secretory proteins of the endometrium of pregnancy, i.e. EP 14 (α_1 -PEG) and EP 15 (α_2 -PEG) (Bell *et al.*, 1985a, b). EP 15 (α_2 -PEG) represents the major secretory protein of the mid- to late secretory endometrium and, because it appeared to be associated with the decidua spongiosa region of the decidua parietalis during pregnancy (Bell *et al.*, 1985a), it presumably originates in the secretory glandular epithelium. α_2 -PEG has been purified from a cytosolic fraction of this tissue and exhibits identical properties to the secretory form of α_2 -PEG. In first trimester pregnancy its major route of secretion appears to be into the amniotic fluid (Bell & Drife, 1985), whereas during the menstrual cycle its profile makes it a prime candidate for a uterine lumen component potentially able to affect the blastocyst before and during implantation. This pattern of synthesis and tissue origin also suggests that α_2 -PEG may be the human analogue of rabbit uteroglobin or blastokinin (Beier, 1968). EP 14 (α_1 -PEG) was not a major secretory protein during the menstrual cycle and its small increase in synthesis and secretion by the late secretory and premenstrual endometrium is interpreted as due to its association with pre-decidualization occurring during this period. In pregnancy it is the major secretory protein of the decidualized decidua compacta layer of the decidua parietalis (Bell *et al.*, 1985a). The third protein of this group, EP 13, a minor secretory protein product during pregnancy is, however, a major product during the follicular phase of the cycle and may be associated with cell division in the tissue, synthesis and secretion declining during the luteal period of differentiation. Although the synthesis and secretion of none of these proteins were affected by short-term exposure to progesterone *in vitro*, presumably EP 14 and 15 are ultimately dependent upon this steroid hormone since endometrial differentiation itself is principally progesterone dependent.

Group III endometrial secreted proteins represent proteins for which rate of synthesis and secretion is modulated by progesterone exposure *in vitro*. Both types of effects are observed in this group with EP 9 being repressed and EP 11 induced by progesterone *in vitro*. Since these effects are caused within 24 h *in vitro*, it is suggested that the rate of synthesis and secretion of those proteins *in vivo* may closely reflect the endogenous concentration of progesterone to which the uterus is exposed. These effects of progesterone are observed throughout the menstrual cycle independent of the histological type of endometrium and are therefore not linked to progesterone-dependent differentiation. If these proteins are detectable in peripheral sera, they may form the basis for examining the *in-vivo* response of the endometrium to exogenous progesterone.

Table 2. Proteins demonstrated to be synthesized and/or secreted by the human endometrium during the menstrual cycle and early pregnancy

Name of polypeptide or protein and possible equivalents described	Group	M_r ($\times 10^{-3}$)*	Endometrium†			Synthesis and secretion	Radio label‡	Ref.§
			Proliferative	Secretory	Pregnancy			
(1) S1	III	130	+/- (f)	+(f)		+	L	1
(2) EP9	III	54	++ (l)	+(l)	+/-	+	M	2,3
S2		58	++ (l)	+(l)		+	L	1
(3) EP10	II	50	+/-	+/-	+	+	M	2
(4) EP11	III	45	+/- (f)	+(f)	+	+	M	2,3
S3		50	+/- (f)	+/- (f)		+	L	1
51.5K		51.5	-(f)	++			L	4
50K		50		(f)		+	L	5
51K		51		(f)		+	M	6
50K		50	—	++		+	L	7
(5) S4	III	35	+(f)	+(f)		+	L	1
(6) EP13	II	33	++ (-)	+ (-)	+/-	+	M	2,3
(7) EP14 (α_1 -PEG)	II	32 (36)	+/- (-)	+/- (-)	++	+	M	2,3,8
(8) EP15 (α_2 -PEG)	II	28 (56)	+/- (-)	++ (-)	++	+	M	2,3,8
PEP/AUP		27 (48-50)	—	+	++	+	L	9-11
(9) S5	III	28	+(l)			+	L	1

* As most techniques of protein analysis employed SDS-polyacrylamide gel electrophoresis under reducing conditions, M_r value refers to denatured molecular weight except for those in parentheses which refer to native values.

† Arrows in parentheses refer to effect of progesterone in short-term culture upon polypeptide synthesis and/or secretion.

‡ M = [35 S]methionine; L = [3 H]leucine.

§References: 1, Strinden & Shapiro (1983); 2, Bell *et al.* (1985a); 3, Bell *et al.*, this paper; 4, Shapiro & Forbes (1978); 5, Hirsch *et al.* (1977); 6, Iacobelli *et al.* (1981); 7, O'Grady *et al.* (1978); 8, Bell *et al.* (1985b); 9, Joshi *et al.* (1980); 10, Sutcliffe *et al.* (1980); 11, Sutcliffe *et al.* (1982).

A few studies have been directed towards the identification of secretory proteins of the endometrium by such techniques and more studies have examined the synthesis of cytosolic proteins. In Table 2 the possible relationship between these proteins we have described and those reported has been examined. Two of the progesterone-regulated secretory proteins described by Strinden & Shapiro (1983) appear similar to those described in this paper, i.e. S2 $\equiv$ EP 9 and S3 $\equiv$ EP 11, and the latter species may indeed represent the secretory form of the progesterone-induced cytosolic protein described by a number of authors (see 'Introduction'). However the lower molecular weight secretory proteins EP 13–15, of which EP 13 and 15 appear from our study to represent major secretory proteins of the menstrual cycle endometrium, were not described in the similar study by Strinden & Shapiro (1983). This would imply that these proteins possess a low leucine or high methionine content since Strinden & Shapiro (1983) used [^3H]leucine as the radiolabelled amino acid. Another possibility is that Strinden & Shapiro (1983) examined secretory endometrium before the period of substantial synthesis and secretion for EP 14 and 15, i.e. Day 18. Three of the secretory proteins described in the present study also exhibited reduced molecular weights similar to those of 3 cytosolic proteins described by Umapathysivam & Jones (1981), i.e. EP 11 $\equiv$ 48 K protein, EP 14 $\equiv$ 36 K protein and EP 15 $\equiv$ 26 K protein. Their rates of in-vitro synthesis and secretion also reflected the pattern of total endometrial tissue content during the menstrual cycle and pregnancy described by these authors (see Table 2).

The relationship between two of the proteins described in our study and the two soluble proteins extracted from the term placenta, i.e. PP 12 and 14 (Bohn, Kraus & Winckler, 1982) is of interest. α_1 -PEG (EP 14) and α_2 -PEG (EP 15) have been demonstrated to be biochemically and immunochemically related to PP 12 and PP 14 respectively (Bell & Drife, 1985). Immunoreactive PP 12 has been detected in soluble extracts of the secretory endometrium during the cycle, although this has not been characterized, and synthesis or secretion have been demonstrated by term decidua (Rutanen *et al.*, 1984, 1985). Similarly, immunoreactive chorionic α_2 -microglobulin (CAG-2) isolated from first trimester placental tissue which is immunochemically identical to PP 14 has been demonstrated in extracts of secretory endometrium (Petrinin, Kozljaeva, Mesrjankina & Shevchenko, 1980). It is likely that EP 15 (α_2 -PEG) is also similar to PEP since this biochemically similar protein has been detected in extracts of secretory endometrium (Joshi, Bank & Szarowski, 1981) as well as pregnancy endometrium.

We have now described the major in-vitro secretory protein products of the endometrium during the menstrual cycle and early pregnancy. At present, we have only determined the native forms of the two major secretory proteins during early pregnancy, i.e. EP 15 as α_2 -PEG and EP 14 as α_1 -PEG, although α_2 -PEG has been shown to be the major secretory product during the secretory phase of the menstrual cycle. Both of these proteins are found during pregnancy at highest concentrations in the amniotic fluid (Bell & Drife, 1985), similar to endometrially-derived prolactin, diamine oxidase (Tujvesson, 1978) and PEP (Joshi, Smith & Stokes, 1980). In pregnancy, therefore, these proteins are principally secreted into the lumen and traverse the chorion and amnion to gain access to the amniotic fluid, and not into the vascular system. It would be anticipated that in the cycle α_2 -PEG (EP 15) would therefore be secreted into the uterine lumen. The native forms of the remaining proteins remain to be determined and whether they also contribute to the intrauterine milieu. The identification of two groups of secretory proteins whose rate of synthesis and secretion is either linked with progesterone-dependent differentiation or are progesterone-regulated short-term offers an opportunity to assess the role of endometrial dysfunction in implantation failure and to examine progesterone-dependent gene expression in the human endometrium.

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