Vascular endothelial growth factor (VEGF) and angiopoietin regulation by gonadotrophin and steroids in macaque granulosa cells during the peri-ovulatory interval

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The role of endothelial cell-specific growth factors in the vascularization of the primate peri-ovulatory follicle was examined. Experiments were designed firstly to detect expression of vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) in granulosa cells and secondly, to determine whether gonadotrophins and/or steroids regulate their expression during the peri-ovulatory interval. Granulosa cells and follicular fluid were collected from rhesus macaques undergoing ovarian stimulation before (0 h), 12, or 36 h after a bolus of ovulatory human chorionic gonadotrophin (HCG), with or without steroid ablation and progestin replacement. VEGF, Ang-1 and Ang-2 mRNA were all detected prior to the ovulatory stimulus. Whereas follicular fluid VEGF concentrations increased 6-fold (P < 0.05) between 0 and 12 h, VEGF mRNA values were unchanged and were unaffected by steroid ablation. Ang-1 mRNA decreased from 0 to 12 h (P < 0.05), followed by a 30-fold increase (P < 0.05) at 36 h, while Ang-2 mRNA values were unchanged between 0, 12 and 36 h. Steroid ablation decreased (P < 0.05) Ang-1 mRNA at 36 h, and Ang-2 mRNA at 12 h, while only Ang-1 was restored by progestin replacement. These data suggest a dynamic expression of vascular-specific growth factors in a gonadotrophin-dependent, steroid-independent (VEGF) or steroid-dependent (Ang-1) manner in granulosa cells of peri-ovulatory follicles of primates.

Key words: angiopoietin-1/angiopoietin-2/granulosa cells/VEGF

Introduction

Recently, considerable attention has focused on endothelial cell specific factors (Koos, 1993; Ferrara and Davis-Smyth, 1997) that appear to be essential for angiogenesis (Suri et al., 1996). The mitogenic/permeability agent, vascular endothelial growth factor (VEGF), is associated with rapid vascular growth in both pathological and physiological conditions (Leung et al., 1989; Ferrara and Davis-Smyth, 1997). Also, two new angiogenic factors unrelated to the heparin-binding VEGF family have been recognized, angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) (Davis et al., 1996; Maisonpierre et al., 1997). Unlike VEGF, Ang-1 unable to stimulate endothelial cell proliferation (Davis et al., 1996), but instead required for the recruitment of peri-vascular cells that aid in the maturation and stabilization of newly developed capillaries (Suri et al., 1996; Hanahan, 1997; Maisonpierre et al., 1997). Ang-2 is a natural antagonist for Ang-1, opposing the effect of Ang-1-mediated stabilization by promoting a more plastic state for capillary endothelium, which can lead to endothelial migration and neovascularization (Maisonpierre et al., 1997).

Although VEGF and the angiopoietins are prevalent in many tissues during embryonic development, angiogenesis in the healthy adult is generally restricted to the physiological processes of follicular development, corpus luteum formation, and uterine endometrial proliferation during the ovarian cycle (Findlay, 1986; Reynolds et al., 1992; Augustin et al., 1995). Previous studies demonstrating the expression of VEGF, Ang-1 and Ang-2 mRNA in the ovary of the rat (Phillips et al., 1990; Koos, 1995; Maisonpierre et al., 1997), as well as VEGF mRNA in the non-human primate ovary (Ravinadranath et al., 1992), suggest a role for these factors in ovarian angiogenesis. Notably, Maisonpierre et al. (1997), compared the temporal and spatial expression patterns of Ang-1, Ang-2, and VEGF in the rat ovary, and proposed that these substances act in concert to promote or inhibit vascular restructuring. However, information about tissue- and time-specific expression of these angiogenic factors during the ovarian cycle, particularly in mono-ovular species such as primates, is lacking.

Recent studies also suggest that VEGF expression is regulated by gonadotrophins and steroids (Ravinadranath et al., 1992; Koos, 1995; Christenson and Stouffer, 1997; Lee et al., 1997; Anasti et al., 1998; Hyder et al., 1998). VEGF mRNA values in the rat ovary (Koos, 1995), as well as VEGF production by primate granulosa cells in vitro (Christenson and Stouffer, 1997), increased after in-vivo administration of a bolus of human chorionic gonadotrophin (HCG), suggesting that VEGF expression by peri-ovulatory follicles is stimulated by the midcycle gonadotrophin surge. Further, VEGF production by T47-D breast cancer cells (Hyder et al., 1998), and retinal pigment epithelial cells (Sone et al., 1996) increased after incubation in the presence of progesterone. However, detailed studies investigating the regulation of VEGF or the angiopoietins by the gonadotrophin surge or steroids during
the cascade of events leading to ovulation of the primate follicle have not been reported.

The specific objectives of this study were: (i) to determine the temporal expression of VEGF and the angiopoietins by granulosa cells in the primate follicle during the peri-ovulatory interval; and (ii) to test the hypothesis that the ovulatory gonadotrophin surge or locally-produced steroids regulate VEGF/Ang expression by primate granulosa cells. Granulosa cells were obtained from rhesus monkeys undergoing controlled ovarian stimulation before (0 h), 12, or 36 h after administration of an ovulatory HCG bolus. This model provides multiple pre-ovulatory follicles that are capable of ovulating (Hibbert et al., 1996), and are steroidogenically similar to those of natural cycles (Chaffin et al., 1999). Further, administration of a 3β-hydroxysteroid dehydrogenase (3β-HSD) inhibitor during the peri-ovulatory interval depletes intrafollicular steroid concentrations (Chaffin and Stouffer, 1999), permitting analysis of local steroid requirements for mRNA expression (Chaffin and Stouffer, 1999), including those for angiogenic factors.

Materials and methods

Animals

The general care and housing of rhesus monkeys at the Oregon Regional Primate Research Center (ORPRC) was described previously (Wolf et al., 1990). Animal protocols and experiments were approved by the ORPRC Animal Care and Use Committee, and studies were conducted in accordance with the NIH guide for the care and use of laboratory animals. Adult female rhesus monkeys exhibiting normal menstrual cycles of ~28 days were stimulated with recombinant human gonadotrophins, i.e. recombinant human follicle stimulating hormone (rFSH), 30 IU i.m. twice daily for 8 days; and recombinant human luteinizing hormone (rLH), 30 IU twice daily on days 7 and 8 (Laboratoires Serono SA, Aubonne, Switzerland) beginning 1–3 days after the onset of menses in order to promote the development of multiple pre-ovulatory follicles (VandeVoort et al., 1989). Monkeys also received a daily s.c. injection of the gonadotrophin-releasing hormone (GnRH) antagonist Antide [08:00, 0.5 mg/kg body weight, total RNA was isolated from 10^4 granulosa cells using the TRIzol method. A 1 μg sample was treated with RNase-free DNase (BRL) to remove contaminating genomic DNA, before reverse transcription using 200 IU of Molony murine leukemia virus reverse transcriptase (MMLV; BRL). PCR was performed in a 75 μl reaction containing 1% (w/v) gum tragacanth (Sigma). TRL was administered 6 h prior to HCG administration and every 12 h thereafter until the time of follicular aspiration to suppress endogenous steroid production (Chaffin and Stouffer, 1999), which ultimately inhibits ovulation (Hibbert et al., 1996). A third group of animals (n = 3/time point) received TRL plus the non-metabolizable progestin R5020 (Promegestrone; DuPont/NEN; 2.5 mg in sesame oil, s.c., once daily starting at the time of HCG) to restore progestin concentrations necessary to reinstate the ovulatory process (Hibbert et al., 1996). The TRL and TRL+R5020 groups were aspirated only at 12 and 36 h post-HCG.

Follicle aspiration and granulosa cell preparation

Granulosa cells were obtained by follicle aspiration as previously described (Wolf et al., 1990). After the oocytes were removed for other studies (Lanzendorf et al., 1990), somatic cells were harvested from follicular aspirates by centrifugation at 277 g for 15 min at 4°C. The resulting follicular fluid was divided into aliquots of 25–100 μl and stored at −80°C. The cell pellet was resuspended in Tyrode’s albumin lactate pyruvate medium (TALP)–HEPES (Bavister et al., 1983), and enriched for granulosa cells as described by Chaffin and Stouffer (1999). In brief, cells were centrifuged at 190 g for 10 min at 4°C, and resuspended in Ham’s F-10 medium (Life Technologies, Grand Island, NY, USA) plus 0.1% bovine serum albumin (BSA). The resuspension was layered onto a gradient of 40% Percoll (Sigma Chemical Corporation, St Louis, MO, USA) and 60% Hank’s balanced salt solution with 0.1% BSA and centrifuged at 470 g for 30 min at 4°C. The resulting layer of granulosa cells was resuspended in Ham’s F-10, cell numbers were determined using a haemacytometer, and cell viability was assessed by Trypan Blue exclusion.

VEGF assay

Follicular fluid VEGF concentrations were determined by enzyme-linked immunosorbent assay (ELISA) (Quantikine Human VEGF Immunoassay; R&D Systems Inc, Minneapolis, MN, USA). This highly specific sandwich assay displays no cross-reactivity with >90 cytokines tested (R&D Systems), and was validated for macaque studies using serum (Christensen and Stouffer, 1997). The lower limit of detectability was 30 pg/ml. Pooled samples of human follicular fluid were used in a single assay to determine the intra-assay coefficient of variation (typically <5%).

Total RNA isolation and reverse transcription–polymerase chain reaction (RT–PCR)

Total RNA was isolated from 10^4–10^5 granulosa cells using the Trizol reagent (BRL, Gaithersburg, MD, USA) as per manufacturers instructions. Quality and quantity of RNA was determined by electrophoresis of samples against known concentrations of total ovarian RNA in a 2% agarose gel stained with ethidium bromide. RNA samples were all reverse transcribed and subjected to PCR as previously described (Chaffin and Stouffer, 1999). Briefly, granulosa cell RNA (0.5–1.0 μg in 10 μl) was treated with RNase-free DNase I (BRL) to remove contaminating genomic DNA, before reverse transcription using 200 IU of Moloney murine leukaemia virus reverse transcriptase (MMLV; BRL). PCR was performed in a 75 μl volume containing experimental + internal standard primers of which the concentrations were determined as part of the validation process with appropriate buffers (7.5 μl of 10X Taq buffer, 2 mmol/l MgCl2, 2 μl of 10 mmol/l dNTPs and 3 IU of Taq DNA polymerase; Promega Biotech, Madison, WI, USA). Each reaction was overlayed with 40 μl of mineral oil, and placed in a thermal cycler (MJ Research, Watertown, MA, USA) for an empirically determined number of cycles of denaturing at 94°C for 30 s, annealing at 60°C for 1 min and primer extension at 72°C for 1 min. Aliquots of each PCR reaction (20 μl) were electrophoresed through a 2% agarose gel stained with 0.1 μg/ml ethidium bromide. Gels were visualized on a UV transilluminator and photographed using 667 Polaroid film, and the photographs were analysed by densitometry. All values were
normalized to the internal standard cyclophilin; no apparent changes were observed in granulosa cell expression for the standard between time-points following administration of HCG.

Validation of the RT–PCR assay was performed (see Ma et al., 1994) using granulosa cells aspirated 27 h following HCG during routine in-vitro fertilization (IVF) protocols as a source of RNA (data not shown). Briefly, the amount of co-amplified product for experimental and internal standard primer sets was linear and parallel with increasing amounts of cDNA and both sets of primers were in the exponentially increasing phase relative to the number of cycles. In order to control for between assay variability, total RNA from granulosa cells of three monkeys was combined and reverse transcribed as described to form a pool that was amplified as four or five replicates during each PCR with the appropriate set of primers. Intra-assay variability, calculated using the four or five replicate pool samples, typically ranged from <1 to 12%. Because data for each set of primers was collected in two rounds of PCR reactions, the pool replicates were also used to normalize data between reactions. Sequence analysis (performed by ORPRC Molecular Biology Core) confirmed the identity of the cDNA fragments. Homology of the cDNA fragments to the published sequences of corresponding cDNAs from other species was determined using DNASTAR software (Madison, WI, USA).

**Oligonucleotide PCR primers**

Oligonucleotides used for PCR were synthesized by Gibco-BRL Custom Primers. Table I lists the primer sequences designed from published DNA sequences (DNASTAR, Inc., Madison, WI, USA), with the determined optimal primer concentrations used for each specific reaction.

**Statistical analysis**

Data were subjected to a Bartlett’s test, and subsequently transformed (log +2) prior to one-way analysis of variance, followed by the Newman–Keuls test for means comparison. Because TRL and TRL+R5020 data were collected at only 12 and 36 h post-HCG, comparisons were made between treatments within a time-point by separate one-way analysis of variance (ANOVA). P < 0.05 was considered to be significant and values are presented as mean ± SEM.

**Results**

VEGF concentrations in follicular fluid from control or TRL-treated animals at 0, 12, and 36 h after HCG administration are depicted in Figure 1. Appreciable amounts of VEGF were detectable at time 0. Control (HCG alone) animals displayed a 6-fold increase (P < 0.05) in follicular fluid VEGF concentrations within 12 h after receiving a standard gonadotrophin bolus (1000 IU HCG) to initiate peri-ovulatory events. VEGF concentrations remained elevated at 36 h, but values were intermediate between 0 and 12 h values. Treatment with TRL did not alter concentrations of VEGF at either 12 or 36 h after HCG administration. Since there was no effect of the 3β-HSD inhibitor on VEGF concentrations, the limited follicular fluid samples from TRL+R5020-treated animals were not assayed.

Figure 2 summarizes VEGF mRNA expression by granulosa cells harvested from control, TRL, and TRL with progesterin replacement (R5020) animals at 0, 12 or 36 h after a bolus injection of HCG. VEGF mRNA values were appreciable at time 0. No changes in VEGF mRNA were observed in the control group at any time point. Likewise, treatments with either TRL or TRL+R5020 did not alter VEGF mRNA expression at either 12 or 36 h after HCG.

Ang-1 mRNA expression by granulosa cells from control, TRL, or TRL+R5020-treated animals was also analysed at 0, 12, and 36 h after HCG administration (Figure 3). Ang-1 mRNA values were detectable in controls at time 0. Gonadotrophin exposure initially decreased Ang-1 mRNA expression between 0 and 12 h (P < 0.05), followed by a 30-fold increase (P < 0.05) by 36 h after HCG. Treatment with TRL or TRL+R5020 had no effect on the decline in Ang-1 mRNA expression at 12 h after HCG. However, the elevated Ang-1 expression at 36 h was markedly decreased (P < 0.05) by TRL treatment, and partially restored to control levels by R5020.

### Table I. Primer sequences and optimal concentrations used for polymerase chain reaction (PCR)

<table>
<thead>
<tr>
<th>Target</th>
<th>Sequence (5'→3')</th>
<th>Primer concentration</th>
<th>Cycle no.</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>VEGFa</td>
<td>up: GGTGAAAACTGACTGGCATCAGGGT</td>
<td>VEGF: 20 pmol</td>
<td>37</td>
<td>Houck et al., 1991</td>
</tr>
<tr>
<td></td>
<td>dn: GGTGAAAACTGACTGGCATCAGGGT</td>
<td>Cyclo: 80 pmol</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>up: CAGCTGACTGGCAGGCTCC</td>
<td>Ang-1: 40 pmol</td>
<td>33</td>
<td>Davis et al., 1996</td>
</tr>
<tr>
<td></td>
<td>dn: CAGCTGACTGGCAGGCTCC</td>
<td>Cyclo: 90 pmol</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>up: CATGCTGAGGAGAACACAGC</td>
<td>Ang-2: 5 pmol</td>
<td>38</td>
<td>Maisonpierre et al., 1997</td>
</tr>
<tr>
<td></td>
<td>dn: CATGCTGAGGAGAACACAGC</td>
<td>Cyclo: 50 pmol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophilin up: GGGAAGTCCATCTACGGCA Duffy and Stouffer, 1995</td>
<td></td>
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*Detects all five forms of vascular endothelial growth factor (VEGF).
Figure 2. Changes in vascular endothelial growth factor (VEGF) mRNA values in granulosa cells aspirated either before (0) or after administration of human chorionic gonadotrophin (HCG) / steroid depletion and progestin replacement. The top panel is a composite polymerase chain reaction (PCR) experiment of time and steroid treatments, and the bottom panel represents the densitometrically analysed data. Letters above bars indicate significance across time. Brackets with asterisks denote significance between groups (P < 0.05). For other details and abbreviations, see Figure 2.

Although Ang-2 mRNA expression was also detected at time 0 (Figure 4), there was no difference compared to values at 12 or 36 h post-HCG. Treatment with TRL decreased (P < 0.05) Ang-2 mRNA at 12 h compared with time-matched controls, but R5020 replacement did not affect Ang-2 mRNA values. Neither TRL nor TRL + R5020 altered Ang-2 mRNA expression at 36 h after HCG compared with controls.

To determine the similarity of the Ang-1 and Ang-2 products of the monkey to the corresponding section of the human sequence, the granulosa cell RT–PCR product was cloned into pGEM-T and sequenced. There was 96.1% homology between the nucleotide sequence for the monkey Ang-1 cDNA and that of the corresponding region of the hAng-1 cDNA, while monkey Ang-2 was 96.5% homologous with the hAng-2 cDNA (sequence data not shown).

Discussion

This study is the first to demonstrate gonadotrophin regulation of VEGF secretion in vivo in the pre-ovulatory follicle of the primate ovary. Follicular fluid VEGF concentrations increased 6-fold by 12 h after the administration of an ovulatory stimulus of HCG, and remained elevated at 36 h. These data are consistent with evidence that in vivo exposure to a midcycle bolus of gonadotrophin (hLH, HCG or hFSH) increased in vitro VEGF production by macaque granulosa cells more than 8-fold (Christensen and Stouffer, 1997). Furthermore, incubation of non-luteinized granulosa cells with LH or HCG progressively increased VEGF, as well as progesterone concentrations, during 4 days of culture (Christensen and Stouffer, 1997). Experiments in the human also demonstrated minimal immunocytochemical staining for VEGF in the granulosa cells of developing follicles (except in cumulus cells) during the pre-ovulatory stage, but intense staining in the newly-formed corpus luteum (Kamat et al., 1995; Gordon et al., 1996). Collectively, the data support the concept that the midcycle gonadotrophin surge promotes the production of the angiogenic factor VEGF by luteinizing granulosa cells in the primate pre-ovulatory follicle.

Unlike VEGF secretion, VEGF mRNA expression was not altered by the gonadotrophin surge during the 36 h pre-ovulatory interval. Interestingly, HCG stimulated expression of VEGF mRNA in human luteinized granulosa cells during 10 days of culture (Neulen et al., 1998). In addition, VEGF mRNA values in granulosa cells increased 10 h after HCG administration in the rat (Koos, 1995). These discrepancies may be time- and species-dependent. Alternatively, differences in the primers utilized for PCR in these studies may be relevant. While the rat study utilized a primer set that resulted...
in multiple-sized products (Koos, 1995), our study utilized a primer set that encompassed all five VEGF isoforms. Although VEGF_{165} is generally the most abundant isoform (Houck et al., 1991; Ferrara and Davis-Smyth, 1997; Soker et al., 1997), the relative expression of VEGF_{121}, VEGF_{145}, VEGF_{165} and VEGF_{206} may depend on the cell line or library screened (Houck et al., 1991). The possibility that cellular expression of VEGF isoforms changes during the peri-ovulatory interval, perhaps to equilibrate total VEGF expression, cannot be ruled out. Nevertheless, the increase in follicular fluid VEGF secretion during the peri-ovulatory interval without an apparent increase in cellular VEGF mRNA suggests a post-transcriptional site of regulation of VEGF production by gonadotrophins.

Although gonadotrophins clearly promote VEGF production by granulosa cells in the ovulatory follicle, other factors may mediate this effect or operate concomitantly to regulate production. Serum progesterone concentrations also increase during the peri-ovulatory interval and are essential for LH-induced ovulation and luteinization in primates (Hibbert et al., 1996). A correlation between VEGF and progesterone concentrations in follicular fluid and in media from granulosa cell cultures has been established in primates (Christensen and Stouffer, 1997; Lee et al., 1997; Anasti et al., 1998). Although progesterone may act locally in the peri-ovulatory follicle to regulate various processes, such as the expression of interstitial collagenase (matrix metalloproteinase-1; MMP-1) and its tissue inhibitor (TIMP-1) (Chaffin and Stouffer, 1999), the current data from TRL-treated animals supports the concept that VEGF mRNA and protein production is steroid-independent. Similarly, Lee et al. (1997) reported that in-vitro incubation of human luteinized granulosa cells with Trilostane or Trilostane plus the progesterone receptor antagonist ZK137.316 did not alter VEGF production. Whether LH action to promote VEGF production is a direct effect, mediated by non-steroidal local factors, or modulated by other changes in the follicular milieu, such as hypoxia (Friedman et al., 1997), awaits further study.

This is the first report detecting Ang-1 and Ang-2 in the primate ovary, notably in granulosa cells during the peri-ovulatory interval. Patterns of Ang-1 and Ang-2 expression differed during the peri-ovulatory interval; Ang-2 expression remained at a constant level, whereas Ang-1 expression initially declined 12 h after the gonadotrophin surge with a subsequent dramatic increase close to the time of ovulation. The constant expression of Ang-2 in the face of decreasing Ang-1 expression 12 h after HCG, may represent the initiation of events that lead to increased plasticity or loosening of the capillary support matrix associated with angiogenesis (Hanahan, 1997). The subsequent robust increase in Ang-1 expression around ovulation, suggests that a concomitant increase in Ang-1 protein may occur to increase vascular stability by recruiting pericytes to developing capillaries (Hanahan, 1997) as the basement membrane breaks down. Although this study focused on granulosa cells, Ang-1 and Ang-2 expression also occur in the thecal layer of large pre-ovulatory follicles in the rat (Maisonpierre et al., 1997). Therefore, expression of angiogenic factors in theca, as well as the granulosa, cells may regulate and fine-tune the formation and establishment of capillaries into the avascular tissue. Further studies addressing VEGF, Ang-1 and Ang-2 mRNA and protein expression patterns in all compartments of the primate pre-ovulatory follicle are warranted.

Gonadotrophin and steroid regulation of Ang-1 and Ang-2 mRNA expression during the peri-ovulatory interval is distinctly different immediately following the surge (≤ 12 h) compared to just prior to follicle rupture (≥ 36 h). First, Ang-1 expression decreases 10-fold in primate granulosa cells 12 h after the administration of HCG. Interestingly, the decline in Ang-1 expression coincides with an increase in progestosterone and VEGF concentrations in the follicular fluid. If progesterone was responsible for down-regulating Ang-1, a progestin synthesis inhibitor should restore Ang-1 expression to control values. However, treatment with Trilostane did not alter Ang-1 expression 12 h after HCG. Thus, the initial reduction in Ang-1 expression in the peri-ovulatory follicle may be directly regulated by gonadotrophins secreted around the time of ovulation. Conversely, Ang-2 expression was not altered by HCG administration, and appears to be continually expressed in the maturing follicle during the peri-ovulatory interval. Unlike Ang-1, Ang-2 is steroid-regulated early in the peri-ovulatory interval as Trilostane decreased Ang-2 expression 12 h after HCG. However, progestin replacement did not restore Ang-2 expression to control values. Second, Ang-1 expression undergoes a 30-fold induction by 36 h after the

**Figure 4.** Changes in angiopoietin-2 (Ang-2) mRNA values in granulosa cells aspirated either before (0) or after administration of human chorionic gonadotrophin (HCG) ± steroid depletion and progestin replacement. The top panel is a composite PCR experiment of time and steroid treatments, and the bottom panel represents the densitometrically analysed data. For other details and abbreviations, see Figure 2.
ovulatory gonadotrophin stimulus, suggesting that initial repression of Ang-1 transcription yields to up-regulation. Steroids also play a role in regulating Ang-1 mRNA expression, as treatment with Trilostane decreased Ang-1 mRNA expression 6-fold in the late peri-ovulatory interval. In this case, progesterone is likely responsible, at least in part, for Ang-1 expression at 36 h after HCG, as the addition of a synthetic progesterin was able to partially restore Ang-1 mRNA expression to time-matched control values. However, the lack of progesterin effect on Ang-2 expression at 12 h, and only partial restoration of Ang-1 mRNA at 36 h suggests possible regulation by another steroid. Whether androgens, oestrogens or other factors (Chaffin and Stouffer, 1999), operate alone or synergistically with progesterone to modulate angiopoietin mRNA or protein expression during the peri-ovulatory interval awaits further study.

In conclusion, an ovarian stimulation protocol was used in rhesus monkeys to examine the expression of endothelial-specific factors in pre-granulosa cells. VEGF, Ang-1 and Ang-2 mRNA were present in granulosa cells of pre-ovulatory follicles, but displayed different patterns of gonadotrophin and steroid regulation after administration of the ovulatory HCG bolus. VEGF protein and Ang-1 mRNA, but not VEGF or Ang-2 mRNA, are gonadotrophin-regulated during the peri-ovulatory interval. Depletion of follicular fluid steroids using a 3β-HSD inhibitor and replacement with a progesterin replacement revealed that Ang-2 expression was steroid-dependent early (12 h) but not late (36 h), whereas Ang-1 mRNA was steroid-dependent late but not early in the peri-ovulatory interval. In contrast, VEGF mRNA and protein production were steroid-independent. Additional studies are needed to localize the mRNA and protein for these vasoactive factors in the primate ovary, as well as to elucidate their role(s) in ovulation and luteinization of the primate follicle.

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