CYTOKINES AND THE HUMAN OVARY

LING JIA WANG, MD.

A thesis submitted for the Degree of Doctor of Philosophy at the University of Adelaide

Reproductive Medicine Unit,
Department of Obstetrics and Gynaecology,
The Queen Elizabeth Hospital,
The University of Adelaide
Australia

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>viii</td>
</tr>
<tr>
<td>Declaration</td>
<td>xiii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>xiv</td>
</tr>
<tr>
<td>Publications</td>
<td>xvi</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>xvii</td>
</tr>
</tbody>
</table>

## Chapter One

### 1.1 Introduction

### 1.2 Overview of the Immune System

#### 1.2.1 The major components and functions of the immune system

#### 1.2.2 Cytokines

#### 1.2.2.1 Functions of macrophage-derived cytokines

#### 1.2.2.2 Functions of T lymphocyte-derived cytokines

### 1.3 Review of Ovarian Function

#### 1.3.1 Evolution of follicles and formation of corpus luteum

#### 1.3.2 Ovarian secretory products

#### 1.3.2.1 Steroid Hormones

#### 1.3.2.1a Estrogens

#### 1.3.2.1b Progestins

#### 1.3.2.1c Androgens

#### 1.3.2.2 Nonsteroidal products

#### 1.3.3 Receptors in ovary

#### 1.3.3.1 FSH receptor

<table>
<thead>
<tr>
<th>Literature Review</th>
<th>1</th>
</tr>
</thead>
</table>
1.3.3.2 LH receptor
1.3.3.3 PRL receptor
1.3.3.4 Steroid receptors
1.3.3.5 Growth factor receptors
1.3.4 Regulation of ovarian function
1.3.4.1 Mechanisms of hormone action
1.3.4.2 Regulation of the follicular development and ovulation
1.3.4.2a Hypothalamus–pituitary–ovarian (H–P–O) axis
1.3.4.2b Intraovarian paracrine regulation
1.3.4.3 Regulation of steroidogenesis
1.3.4.3a FSH
1.3.4.3b LH
1.3.4.3c PRL
1.3.4.3d Ovarian steroids
1.3.4.3e Regulatory peptides

1.4 Cytokines and the Reproductive System
1.4.1 Cytokines and endometrium–decidua and placenta
1.4.1.1 Leukocyte subpopulation in endometrium–decidua and placenta
1.4.1.2 Cytokine production from endometrium–decidua and placenta
1.4.1.3 Effects of cytokines on endometrium–decidua and placenta
1.4.1.4 Effects of endometrium–decidua and placenta on lymphocyte proliferation
1.4.2 Cytokines and the embryo
1.4.3 Cytokines and the uterine cervix

1.5 Cytokines and Ovary
1.5.1 Distribution and action of leukocyte subpopulations in the ovary
1.5.1.1 Macrophages
1.5.1.2 Lymphocytes
1.5.1.3 Other leukocytes

1.5.2 Expression and action of cytokines in ovary

1.5.2.1 IL-1

1.5.2.2 TNFα

1.5.2.3 Other cytokines

1.5.3 Interaction between gonadotrophins, ovarian steroids and immune cells

1.5.3.1 LHRR

1.5.3.2 LH

1.5.3.3 Sex Steroids

1.6 Aims of This Thesis

Chapter Two Materials and Methods

2.1 Equipment

2.2 Chemicals and Reagents

2.3 Antibodies and Sera

2.4 Immunocytochemistry

2.5 3H-thymidine Uptake

2.6 Human Granulosa–Lutein Cell Culture

2.6.1 Preparation of material and solutions

2.6.1.1 Preparation of silicon pipette

2.6.1.2 Preparation of cell culture media

2.6.1.3 Preparation of 50% percoll solution

2.6.2 Collection of granulosa–lutein cells

2.6.3 Cell culture procedures

2.6.4 Evaluation of culture system

2.6.4.1 Identification and morphology

2.6.4.2 Cell growth in–vitro
2.6.4.3 Daily and accumulative P4 secretion 57
2.6.4.4 Response to hCG on different days of culture 61
2.6.4.5 Thirty days of cell culture exposed to continual hCG stimulation 61
2.6.4.5 P4 secretion from cultured granulosa-lutein cells in serum-free media 63

2.7 Leukocyte Culture 63
2.7.1 Separation of peripheral blood mononuclear (PBM) cells 65
2.7.2 Preparation of conditioned medium (CM) 65

2.8 Radioimmunoassays 66
2.8.1 IL-1β RIA 67
2.8.2 IL-2 RIA 67
2.8.3 TNFα RIA 68
2.8.4 cAMP RIA 68
2.8.5 P4 RIA 69
2.8.6 E2 RIA 69

2.9 Ethical Considerations 70
2.10 Statistics 70

Chapter Three Distribution of Leukocyte Subpopulations in The Human Corpus Luteum 71

3.1 Introduction 71
3.2 Materials and Methods 72
3.2.1 Preparation of tissue section 72
3.2.2 Immunocytochemistry 73
3.3 Results 73
3.4 Discussion 78
Chapter Four  Cytokine Determination in Human Follicular Fluid

4.1 Introduction

4.2 Materials and Methods

4.2.1 Collection of follicular fluid and blood

4.2.2 Immunassays

4.2.3 TNFa Bioassays

4.2.4 Specificity of the assays for interleukins

4.2.5 Analysis of Data

4.3 Results

4.3.1 IL-1 and IL-2

4.3.2 TNFa

4.4 Discussion

Chapter Five  Interleukin-2 Effects on Gonadotropin-Stimulated Progesterone Production and Cell Proliferation by Human Granulosa-Lutein Cells

5.1 Introduction

5.2 Materials and Methods

5.2.1 Granulosa-luteal Cell Collection and Culture Method

5.2.2 [3H] Thymidine Incorporation into Granulosa-lutein cells

5.2.3 The Preparation of Conditioned Media from MLA-144 Cell Line and Human Peripheral Blood Leukocytes

5.2.4 RIA
5.2.5 Data Analysis
5.3 Results
5.3.1 The effects of CM with α without hCG on progesterone production from granulosa-lutein cells
5.3.2 The effect of IL-2 with or without hCG or FK on progesterone production
5.3.3 IL-2 effects with or without hCG on granulosa-lutein cell proliferation
5.3.4 The effect of IL-1 with or without hCG on P4 production
5.4 Discussion

Chapter Six Tumor Necrosis Factor α Effects on Gonadotrophin-stimulated Progesterone Production and Cell Proliferation By Human Granulosa-lutein Cells

6.1 Introduction
6.2 Materials and Methods
6.2.1 Collection and culture of human Granulosa-lutein Cells
6.2.2 [3H] Thymidine Incorporation into Granulosa-lutein Cells
6.2.3 RIA
6.2.4 Data Analysis
6.3 Results
6.3.1 TNFα Concentrations in Medium from Cultured Human Granulosa-lutein Cells
6.3.2 The Effect of TNFα on Cell Proliferation
6.3.3 The Effect of TNFα on PG
6.3.4 The Effect of TNFα on P4 Production in Granulosa-
Chapter Seven  Cellular Composition of Primary Cultures of Human Granulosa-lutein Cells and the Effect of Cytokines on Cell Proliferation 119

7.1 Introduction 119
7.2 Materials and Methods 120
7.2.1 Granulosa-lutein cell collection and culture method 120
7.3.2 Immunocytochemistry 121
7.2.3 Data Analysis 121
7.3 Results 121
7.3.1 Cellular composition of unstimulated human granulosa-lutein cells 124
7.3.2 Changes in cellular composition in response to IL-2 and TNFα during a 48h culture 125
7.3.3 Changes in cellular composition over a 6 day culture period 125
7.4 Discussion 131

Chapter Eight Conclusions 135
Appendices 141
References 154
ABSTRACT

Studies reported in this thesis examined aspects of the distribution of leukocyte subpopulation in human corpus luteum, cytokine determination in human preovulatory follicular fluid, as well as the effects of cytokines on human granulosa-lutein cells with the aim of investigating one of the ovarian regulatory systems, which may be controlled by immune cell-derived cytokines.

In the first series of experiments, distribution of leukocyte subpopulation in human corpus luteum was demonstrated by immunocytochemistry. Ten intact corpora lutea were collected from female patients who had no apparent ovarian disease. The mean age of these patients was 37 years (range 23–55 years). Frozen and paraffin sections were subjected to analysis using monoclonal antibodies which were specific to leukocyte marker antigens. The results showed that there are macrophages, cells positive for leukocyte common antigen (LCA), T lymphocytes including T helper/inducer (T(H)) cells, T cytotoxic/suppressor (T(s)) cells and activated T (T(a)) cells (interleukin-2 receptor-positive cells), monocytes and natural killer (NK) cells but B lymphocytes were not found to be present in the human corpus luteum. The distribution of leukocytes present in the different parts of the corpus luteum was found to be in the order of: theca-luteal area > loose connective tissue area > granulosa-luteal area. Macrophages and T lymphocyte subsets comprised the main components of the total leukocytes in the human corpus luteum. T(α) cells were only localized in the loose connective tissue of the corpus luteum. In most cases, macrophages, LCA cells and Tα cells tended to be situated in a single cell layer on the edge of the thecal-luteal area and surrounding the granulosa-luteal area. These results suggest that the leukocytes may act to a greater extent in the theca-luteal area than in the granulosa-luteal area. A large concentration of leukocytes in the corpus luteum reinforces the view that cytokines may play a significant role in ovarian physiology.
In the second series of experiments, the presence of immunoreactive interleukin (IL)–1, IL–2 and tumor necrosis factor α (TNFα) in human follicular fluid obtained at the time of oocyte collection for in–vitro fertilization was ascertained by radioimmunoassay. Results obtained from group I (20 fluids of 20 patients) showed that the concentrations of IL–1 were 9±0.6 pmol/l and 19±0.4 pmol/l in follicular fluid and plasma respectively. A positive correlation existed between IL–1 levels in follicular fluid and plasma (r = 0.56, p<0.01). Concentrations of IL–2 were 35±2 pmol/l and 61±3 pmol/l in follicular fluid and plasma respectively. A positive correlation of IL–2 levels was also found between follicular fluid and plasma (r = 0.65, p<0.01). There was no association between IL–1, IL–2 and steroid levels, regardless of whether they were compared in follicular fluid or plasma. Group II was composed of a series of fluids (2 to 7 samples for each of seven patients) in which the follicular concentrations of IL–1 and IL–2 did not show a positive correlation with the volume of follicular fluid or the concentrations of follicular fluid steroids. For measurement of immunoreactive TNFα levels, a total of 32 fluids were selected from 9 patients (2–7 samples from each patient). The concentration of TNFα in follicular fluid was 20±1 pmol/l and there were no significant correlations between levels of TNFα and of steroids or follicular volume. However, bioactivity detected by bioassay for TNFα in human follicular fluid was considerably less. These results suggested that human follicular fluid contains immunoreactive IL–1, IL–2 and TNFα at the preovulatory stage.

The following series of experiments examined the effects of cytokines on the human ovary, where the human granulosa–lutein cell culture was used as a model. These cells were collected from follicular fluid obtained from women undergoing in vitro fertilization procedures and cultured with or without conditioned media, cytokines or human chorionic gonadotrophin (HCG) etc., up to 7 days before supernatant measurement of progesterone, cAMP or prostaglandins (PGs).
Lymphokine-rich conditioned media was prepared from cultured human peripheral blood leukocytes (HPL-CM) or from the culture of a Gibben T cell line MLA-144 (MLA-CM). The influence of HPL-CM and MLA-CM1 stimulated by mitogens on granulosa-luteal cell progesterone production was inhibitory. In contrast, MLA-CM1, without mitogen stimulation, stimulated basal progesterone secretion. Human recombinant IL-2 (from 0.26 to 2500pmol/l) alone did not change progesterone levels, compared to control values, after 24h of cell culture. However, 26, 250 and 2500pmol IL-2 significantly inhibited progesterone secretion from cells stimulated by 5iu hCG (p<0.01). The enhanced progesterone levels stimulated by forskolin were also significantly inhibited by 250pmol IL-2 (p<0.01). This effect was not mediated through decreased cAMP, since the forskolin-enhanced cAMP level was not influenced by IL-2. Human recombinant (from 0.06 to 60pmol/l, equal to 0.001—1ou/l) IL-1β, with or without hCG, did not show any effect on progesterone production during either 24 or 48h of cell culture. It is concluded that IL-2 significantly inhibits progesterone production stimulated by hCG in human granulosa-luteal cells; IL-2 also had a marked inhibitory effect on forskolin-induced progesterone release, but did not influence the increased cAMP level stimulated by forskolin; the inhibitory influence of IL-2 on progesterone synthesis may be downstream in the signal transduction pathway from cAMP activation and HPL-CM and MLA-CM produced inhibitory and stimulatory effects, respectively, on both basal and hCG-stimulated progesterone levels as well as on granulosa-luteal cell proliferation. These activities cannot be completely attributed to IL-2, and other mediators of leukocyte origin may therefore exist.

Human recombinant TNFα (from 6 to 6000pmol/l) showed a dose-dependently stimulated proliferation of cultured granulosa-luteal cells as measured by incorporation of 3H-thymidine, but did not have any effect on the basal level of hCG-stimulated progesterone accumulation during culture periods of up to 72h in either serum-containing or serum-free medium. However, the accumulation of both PGE2
and PGF₂α was dose-dependently increased by TNFα during a 48h incubation period. Time course studies revealed that maximum levels of both PGE₂ and PGF₂α were reached within 12h of culture. It is concluded that TNFα may have a physiological role in stimulating proliferation of follicular cells and PG production at the time of ovulation and formation of the corpus luteum.

The cellular composition in primary culture of human granulosa-lutein cells with or without IL-2 and TNFα was examined by immunochemistry with monoclonal or polyclonal antibodies. Cells collected from follicular fluid were washed and separated by a Percoll gradient followed by nylon mesh filtration to obtain single cells. Cultures were performed in glass-slide chambers for periods up to 144h. After 48h of culture the majority of the cells were shown to be granulosa-lutein cells exhibiting positive staining against 3β-hydroxysteroid dehydrogenase (3β-HSD), oestradiol and vitamin D. About 35% of the total cell population stained positive with monoclonal antibodies against specific antigen sites on cells of bone marrow lineage. The majority of these cells were leukocyte common antigen (LCA) positive (about 20% of the total population). In the group of LCA positive cells, the major cellular components were lymphocytes (1/4 helper T-cells and 1/4 cytotoxic T cells) > natural killer (NK) cells. Most of the T-lymphocytes were activated as judged by expression of the IL-2 receptor. There were also a considerable number of monocytes/macrophages (about 15% of the total cell population). After 48h in culture, the cytokines IL-2 (2500pmol) and TNFα (6000pmol) significantly (p<0.05) reduced the percentage of granulosa-lutein cells and in a dose-dependent manner (IL-2 from 25 to 2500pmol/l and TNFα from 60 to 6000pmol/l) increased the proportion of LCA positive cells and monocytes. At the end of 144h of culture, LCA positive cells and monocytes were found, but with decreasing numbers. These results indicate that white blood cells are present in primary cultures of human granulosa-lutein cells. The main target for the proliferative effects of IL-2 and TNFα in this culture system, during the period of 48h seems to be the leukocytes rather than
the steroid-producing cells. Thus, any observed effects by added cytokines in this culture system may be due to a direct or indirect effect from cytokine-activated resident leukocytes on granulosa-lutein cells.