Lactoferrin Assessment In Follicular Fluid And Embryo Quality And Pregnancy Rate During In Vitro Fertilization Cycles

Thesis

Submitted For Fulfillment Of

Master Degree In Obstetrics &Gynecology

By

Nahla Nazieh EL Sheikh

M.B., B.CH. (2008)

Misr University For Science &Technology

Under The Supervision Of

DR. Mohammed Sayed Ali

Professor of Obstetrics and Gynecology

Faculty of Medicine, Ain Shams University

DR. Mohammed Abdel Hameed Abdel Hafeez

Lecturer of Obstetrics and Gynecology

Faculty of Medicine, Ain Shams University

Faculty Of Medicine

Ain Shams University

(2015)

Acknowledgements

First, I immensely thank ***Allah*** for giving me strength and ability to complete this study.

I would like to express my sincere gratitude to ***Dr. Mohammed Sayed Ali*** Professor of Obstetrics and Gynecology, Faculty of medicine, Ain shams university for the continuous support of my thesis, for his patience, motivation, enthusiasm, and immense knowledge.

I would like to express my special appreciation and thanks to ***Dr. Mohammed abdel hameed abdel hafeez*** Lecturer of Obstetrics and Gynecology, Faculty of medicine, Ain shams university for his insights related to the thesis and providing useful suggestions.

I thank ***Dr. Azza Awad Abdul Razik*** supervisor embryologist in the laboratory of assisted reproduction unit, ain shams university maternity hospital and **the members of the laboratory** for their assistance with follicular fluid collection.

I wish to thank ***Dr. Nahla Mohamed Zakaria Yousef*** Professor of Clinical Pathology Department, Faculty of medicine, Ain shams university, and **the members of the laboratory** of the ain shams maternity hospital for their technical assistance with lactoferrin assay.

I wish to thank ***my family*** for their support and encouragement.

List Of Contents

List Of Figures

Subject **Page**

List Of Tables

Subject **Page**

Introduction

In today's society, assisted reproductive techniques (ART) account for the birth of more than 3 million babies worldwide, and the number of in vitro fertilization (IVF) cycles performed increases every year. However, only 32% of IVF cycles result in a pregnancy ***(De Mouzon et al., 2010);*** therefore, multiple embryos are replaced per treatment cycle to increase pregnancy rates. This has resulted in a multiple gestation rate of 25%, which in turn has led to a greater incidence of many medical complications ***(Pandian et al., 2009).*** Morphological and microscopic applied criteria to grade the most competent oocyte/embryo are subjective and inadequately related to successful pregnancy rate ***(L. Bianchi et al., 2013).***

A positive outcome in an IVF procedure requires the successful occurrence of several events: folliculogenesis, oocyte maturation, fertilization, embryo implantation and normal development ***(*Agne et al., 2013).***

Oocyte growth and development largely depend on the nurturing environment of the follicle, and the attainment of developmental competence by the oocyte is crucial to the formation of viable embryos. Thus, analysis of the follicular environment is a logical step in the development of improved noninvasive selection methods for oocytes and embryos. As follicular fluid is a byproduct of routine oocyte retrieval, it provides the ideal sample for such studies ***(Martina et al., 2012).***

Actually, the FF is the microenvironment in which the oocyte develops and undergoes maturation, and it has been reasonably thought, and to some extent proven, to affect oocyte quality, fertilization and, may be, embryo development ***(Revelli et al., 2009).***

Investigation of follicular fluid composition as a possible predictor of both oocyte developmental competence and embryo viability has increased in recent years ***(Wallace et al., 2012).***

FF is a complex mixture of proteins, metabolites, and ionic compounds reflecting follicular metabolism and comparable to blood serum ***(Hanrieder et al., 2009).***

Lactoferrin (LF), a key component of mammalian innate immunity ***(Legrand and*** **Mazurier,** ***2010),*** is a cationic monomeric 80- kilo Daltons (kDa) glycoprotein of the transferrin superfamily ***(Gonzalez et al., 2009).*** It is secreted by epithelial cells into most mucosal secretions and body fluids. It is also a major component of the secondary granules of neutrophils released on activation ***(Puddu et al., 2010).***

The number of neutrophils increases markedly in the thecal layer of the follicle just before ovulation ***(Yanaihara et al., 2007).***

Lf is a multifunctional protein with a wide range of biological activities including regulation of iron absorption, immune response, antioxidant, anticarcinogenic, anti-inflammatory properties, and antimicrobial activity ***(Puddu et al., 2009).*** It is one of a multitude of host defense proteins and peptides (HDPPs) ***(Wang et al., 2009)*** that constitute a vital first line defense against invading microorganisms.

Lactoferrin can migrate into the oocyte from the serum, as well as being produced by theca cells. In fact, the concentrations of LF in serum and FF are almost identical ***(Kelver et al., 1996).***

It was recently reported that gamma aminobutyric acid-B receptor 2 was down regulated by LF, and LF may modulate the level of intracellular cyclic adenosine 3=:5=monophosphate (cAMP) ***(Tamura et al., 2005).*** It is known that oocyte maturation is influenced by decreasing cAMP levels in the oocyte after ovulation ***(Sato and Koide, 1987).*** Lactoferrin may be involved in that process via the control of the cAMP level ***(Yanaihara et al., 2007).***

In addition, in vitro studies demonstrated that Lf stimulates the growth of lymphocytes ***(Mazurier et al., 1989),*** natural killer activity (NK-cells) ***(Nishiya and Horwitz, 1982)*** and the release of interleukin-8 (IL-8) from neutrophils ***(Shinoda et al., 1996).*** Lf also up regulate the number of phagocytosis and cytotoxicity of neutrophils ***(Gahr et al., 1991)*** and macrophages ***(Lima and Kierszenbaum, 1985).*** Furthermore, Lf down regulates granulocyte/macrophage colony stimulating factor production by

macrophages, the release of IL-1, IL-2, and tumor necrosis factor (TNF-α) from leukocytes, or complement activation ***(Fornili et al., 2010).***

Lactoferrin activates macrophages and induces inflammatory cytokines such as IL-8 ***(Sorimachi et al., 1997),*** and the correlation of LF production with IL-8 was reported ***(Gessler et al., 2004).*** According to previous studies, IL-8, a neutrophil chemoattractant and activating factor, was also found to increase in FF, and the investigators felt that it may have an effect on follicular maturation ***(Belayet et al., 2000).*** IL-8 is thought to be important in the developing follicle for the inflammatory events that occur at the time of ovulation and luteolysis ***(Zeineh et al., 2003).*** In granulosa cells, IL-8 is induced by IL-1ß, and may have a role in follicular maturation ***(Fujii et al., 2003).*** Lactoferrin may interact with IL-8 to affect embryo quality ***(Yanaihara et al., 2007).***

Tumor necrosis factor-α (TNF-α) inhibits progesterone (P) and estradiol (E2) production from granulosa cells ***(Sakumoto et al., 2003),*** and TNF-α concentrations in FF were significantly higher in poor-quality oocytes ***(Kuhara et al., 2000).*** Furthermore, LF-inhibited TNF-alpha was reported ***(Haversen et al., 2002).***

Thus, LF may affect granulosa cells in order to regulate of cytokine releasing such as IL-8 and TNF-α. Therefore, LF in the FF may be one of the biological makers to select the embryos at the time of ET ***(Yanaihara et al., 2007).***

Research Question

Does high lactoferrin concentrations in follicular fluid correlate with good embryo quality and higher clinical pregnancy rate?

Research Hypothesis

High lactoferrin concentrations in follicular fluid correlate with a good embryo quality and a higher clinical pregnancy rate.

Aim Of The Work (Objectives)

The aim of the study is to determine the correlation between follicular fluid lactoferrin concentrations, embryo quality, and clinical pregnancy rate during ICSI cycles.

Follicular fluid

Introduction

During ovarian follicle development, a cavity filled with fluid is formed which called follicular fluid.

Follicular fluid (FF) comprises the preconception microenvironment in which the oocyte develops ***(Gerard et al., 2002).***

The human ovarian follicular fluid (HFF) prevalently results from granulosa and theca cell secretion and from capillary diffusion ***(Rodgers and Irving, 2010).***

During folliculogenesis, the blood-follicle barrier becomes more permeable to plasma molecular components and the follicular fluid (FF) acquires a consistent similarity to the serum ***(Gérard et al., 2002).***

FF is composed of plasma exudates through the follicular epithelium and secreted products of the follicle, especially granulosa cells ***(Yanaihara et al., 2007).*** So, it provides a very important microenvironment in which the oocyte matures and granulosa cells differentiate ***(Fahiminiya and Gerard, 2010).***

Moreover, granulosa cells could play an active role in altering the FF by selectively filtering and perhaps transforming some of the metabolites. There is increasing interest in determining whether the metabolic composition of the FF can provide information about the quality of the oocytes and embryo viability ***(Bokal et al., 2006).***

Variations in concentration of follicular fluid components can also affect the oocyte quality ***(Von Wald et al., 2010).***

Main origins of human follicular fluid are circulating blood, which diffuses through the follicular wall into an antrum of a follicle, and follicular secretions, which are predominantly products of metabolic processes within an oocyte ***(Fortune, 1994).***

Follicular fluid is a useful body fluid for discovery of biomarkers for assessment of oocyte quality, pregnancy outcomes and ovarian disorders ***(Jarkovska et al., 2011).***

Follicular fluid (FF) perhaps has the most potential to reveal information about the impact of menstrual cycle metabolic fluctuations on the oocyte. It is a plasma transudate that fills the follicle antrum and whose composition is in part determined by local follicular metabolic processes. The follicle wall acts as a coarse molecular sieve, allowing small metabolites to pass through while restricting the access of molecules >100 kilo Daltons (kDa). FF supports oocyte maturation and thus its composition may have a direct influence on the oocyte, in both its ability to mature and its quality ***(Revelli et al., 2009).***

The composition of follicular fluid is similar to serum with respect to low-molecular-weight components, with most electrolytes being at the same concentrations in fluid and serum ***(Gosden et al., 1988).***

Physicochemical features of FF

Apart from biochemical characteristics, the physicochemical properties of FF have been studied and correlated with the outcome of the corresponding oocytes ***(Revelli et al., 2009).***

The spectrophotometric absorbance of human FF in the visible spectrum shows two distinct peaks at 415 and 455 nanometer wavelength; it has been observed that oocytes that subsequently fertilized were more frequently associated with FFs having significantly higher absorbances at these two peaks ***(Fisch et al., 1990).***

Metabolomics of FF

Metabolomics studies the small molecules (amino acids, lipids, nucleotides, signalling molecules, etc.) found in biological fluids that are produced through the action of different proteins ***(Revelli et al., 2009).***

Metabolomics has been proven to be a consistent and informative technology for pattern recognition analysis of several biological systems, and is presently being applied to the study of human embryos ***(Botros et al., 2008)*** and oocytes ***(Singh and Sinclair, 2007).*** Aim of metabolomic analysis is to identify and quantify all the metabolites in a biological fluid (e.g. FF) under given physiological conditions at a certain time point. The major difficulties in metabolomics are essentially three: a) many metabolites are of labile nature, chemically complex and have a widely dynamic production pattern; b) methods to amplify metabolites (e.g. as may be done with DNA) and increase sensitivity are lacking; c) metabolomic analysis deals at the same

time with several classes of molecules with different chemical properties ***(Revelli et al., 2009).***

It has emerged that the metabolic profiling of FF collected from large antral follicles is more homogeneous that the one obtained with fluids collected from small follicles, reflecting differences in the biochemical profile linked to oocyte maturational stage ***(Thomas et al., 2000).*** In one of these studies, it has been observed that oocytes able to absorb larger amounts of glucose and actively convert it into lactate show the highest fertilization potential ***(Preis et al., 2005).*** Other studies have focused on the evaluation of oocyte metabolism through the measurement of energy substrates ***(Roberts et al., 2004)*** or of oxygen consumption ***(Scott et al., 2008)*** in culture media.

Qualitative and quantitative alterations in the composition of the FF could affect the quality of the oocyte and hence affect the potential fecundity. These alterations in the FF could be due to metabolic changes in the serum ***(Leroy et al., 2004).***

The major components of follicular fluid are steroid hormones, metabolites, polysaccharides, proteins, reactive oxygen species (ROS) as well as antioxidant enzymes which primarily aid in the growth and maturation of oocyte and follicular cells in addition to protecting them from physical damage and oxidative stress. Follicular fluid also serves as a medium for communication between oocyte and follicular cells, which is required for the acquisition of developmental as well as fertilization competence by the oocyte ***(Edwards, 1974).***