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Association Between Follicular-Serum Levels of Inflammatory Cytokines and Oocyte Quality

Association entre les taux folliculo-sériques des cytokines de l'inflammation et la qualité ovocytaire

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Article original

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ABSTRACT

Background: Oocyte maturity is a significant concern in assisted reproductive technology, impacting oocyte quality and fertility outcomes. This study aimed at investigating the association between follicular and serum levels of inflammatory cytokines and oocyte quality in women undergoing assisted reproductive technology.
Methods: A prospective cross-sectional study was conducted over a period of nine months at the Center for Research and Application in Endoscopic Surgery and Human Reproduction, Yaoundé. Sixty-one women eligible for assisted reproductive technology were enrolled. Comprehensive data collection included sociodemographic details, medical history, and biological samples. Cytokine levels (IL-10, G-CSF, IFN-γ) were measured using sandwich ELISA.
Results: The findings revealed higher follicular concentrations of IL-10 (20.86 pg/ml) and G-CSF (48.04 pg/ml) compared to serum levels (13.91 pg/ml and 29.16 pg/ml, respectively), with significant differences noted ($p < 0.05$). A significant association was found between serum G-CSF levels and the percentage of matured oocytes ($p < 0.001$). In contrast, no significant correlation was observed between IL-10 levels and oocyte maturity.
Conclusion: Understanding cytokine profiles in follicular fluid and in blood could inform therapeutic strategies to improve oocyte quality and fertility outcomes in women undergoing assisted reproductive technology.

RESUME

Introduction : La maturité ovocytaire est d'une grande importance en assistance médicale à la procréation, elle impacte la qualité ovocytaire et le rendement des fécondation in vitro. Le but de ce travail était d'étudier l'association entre les taux folliculaire et sériques des cytokines de l'inflammation et la qualité des ovocytes chez les femmes en Fécondation in-vitro.
Méthodes : Une étude transversale prospective a été menée sur neuf mois au Centre de Recherche et d'Application en Chirurgie Endoscopique et en Reproduction Humaine de Yaoundé. Soixante et une femmes admises en assistance médicale à la procréation étaient incluses. Les données sociodémographiques, les antécédents médicaux et les échantillons biologiques ont été collectées. Les taux de cytokines (IL-10, G-CSF, IFN-γ) ont été mesurés par ELISA sandwich.
Résultats : Les concentrations folliculaires d'IL-10 (20,86 pg/ml) et G-CSF (48,04 pg/ml) étaient significativement plus élevées que leurs concentrations sériques (13,91 pg/ml et 29,16 pg/ml respectivement), $p < 0,05$). Il y avait une association significative entre les taux sériques de G-CSF et la maturité ovocytaire ($p < 0,001$).
Conclusion : L'étude des taux cytokiniques folliculaire pourrait permettre d'élaborer des stratégies thérapeutiques en matière de FIV

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Introduction

The development of ovarian follicles is vital for oocyte competence, with follicular fluid (FF) playing a key role in providing a supportive microenvironment for oocyte growth and maturation. FF, produced by granulosa and theca cells, contains various components such as lipids, cytokines, and growth factors that reflect the surrounding follicular conditions [1]. IL-10 functions as an anti-inflammatory cytokine that helps downregulate the expression of pro-inflammatory cytokines [2]. An imbalance characterized by increased levels of IL-6 and IL-8, along with reduced IL-10, can disrupt the delicate equilibrium between pro- and anti-inflammatory cytokines, leading to alterations in steroidogenesis, delayed follicular maturation, and ovarian dysfunction [3]. The concentrations of inflammatory factors in follicular fluid are crucial for processes like oocyte maturation, follicular rupture, fertilization, and the development of early embryos [4]. These cytokine profiles can vary significantly based on reproductive pathologies, such as polycystic ovary syndrome. Recent studies have shown associations between specific cytokine levels in follicular fluid and oocyte quality, embryo development, and outcomes in assisted reproductive technologies. Understanding these relationships is vital, as they highlight the complex interplay of inflammation, oxidative stress, and ovarian function, which could inform therapeutic strategies for improving fertility in affected individuals [1,5,6].

The ovaries serve dual functions: folliculogenesis and oogenesis, which lead to the production of mature oocytes. These processes are closely interconnected, and their precise regulation relies on numerous exocrine, endocrine, and paracrine factors [7]. The goals of medical assisted reproduction are to recreate the necessary balance for oocyte maturation, fertilization, and the development and implantation of a competent embryo [8]. Multiple factors contribute to the success of in vitro fertilization, especially oocyte quality. With the advancement of intracytoplasmic sperm injection, oocyte quality can be accurately assessed through both nuclear maturity and morphological structure [9]. The follicular fluid within the ovarian follicle's antrum provides the microenvironment essential for oocyte growth and maturation. Intra-follicular paracrine communication facilitates and regulates the microenvironment of the cumulus-oocyte complex, supporting the developmental and meiotic competencies of the oocyte. Previous studies have reported variations in cytokine concentrations in patients with poor responses to IVF stimulation and in women over 40 [10-12]. This study aims at determining the effect of non-invasive markers of oocyte quality for use in women undergoing medically assisted reproduction after ovarian stimulation.

Méthodologie

A prospective cross-sectional analytical study was conducted among women undergoing Assisted Reproductive Technology (ART) at the Center for Research and Application in Endoscopic Surgery and Human Reproduction of Yaoundé (CRAESHRY) over a 9-month period from January to September 2023. Sample collection and biological analyses were carried out at the laboratory service of CRAESHRY. Our study included all women eligible for ART according to the eligibility criteria in force in Cameroon and who agreed to participate in the study. All women on immunosuppressive therapy and those who withdrew consent were excluded from the study.
The data collection tool was a questionnaire administered to each woman included in our study who gave her consent after signing the informed consent form. A file was created for each patient containing the following information: sociodemographic characteristics, medical and surgical history and lifestyle habits, infertility evaluation, ovarian stimulation (only for women undergoing oocyte pick-up during the collection period) and oocyte/embryo quality.

A patient code was generated for increased confidentiality and recorded in each file. Samples were collected from the 61 women participating in this study. These samples were labeled with the necessary patient details for analysis purposes. Blood collected in dry tubes was centrifuged and the serum obtained was immediately frozen. Follicular fluid was also stored in the freezer after microscopy for cytological description of oocytes. The Quantikine® QuicKitTM ELISA kits <<Human G-CSF, Human IFN-γ and Human IL-10>> (R&D Systems, Inc, Minneapolis, USA) were used to analyze the 3 cytokines (G-CSF, IFN-γ and IL-10) in serum and follicular fluid according to the manufacturer's protocol.

The tests can be described as sandwich immunoassays (ELISA) measuring multiple cytokines in a single small volume sample and have been validated according to the principles described in "Fit-for-purpose method development and validation for successful biomarker development" by (Lee et al., 2006). Briefly, after dilution of plasma, serum or cell culture

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supernatant samples in a suitable buffer, 100 µl were deposited in triplicate in each well along with an increasing concentration standard antigen curve. After one hour of incubation at room temperature, the wells were washed 3 times with wash buffer. The biotinylated detection antibody specific for the antigen of interest was then added at a concentration of X µg/ml in each well and incubated for 20 minutes. Three new washes were performed before adding the horseradish peroxidase-conjugated streptavidin diluted 1/X. After a 20-minute incubation, the wells were washed 3 times and TMB substrate (Sigma) was added for 10 to 15 minutes. The reaction was stopped by addition of 1M sulfuric acid and the optical density was measured at 450 nm using a microplate reader (Molecular Devices). Antigen concentrations in samples were determined from the standard curve using SoftMax Pro software.

The collected data was verified to ensure completeness and consistency, then entered into CSPro (Census and Survey Processing System) version 7.0 software and transferred to SPSS (Statistical Package for Social Sciences) version 25.0 for statistical analysis. The mean (+/- standard deviation) or median (+ interquartile range) were used to describe quantitative variables. Text and table processing was done using Microsoft Word 2016. Figures were produced using Microsoft Excel 2016. Student's and Wilcoxon's tests were used to compare means, and the Kruskal-Walli's test to compare medians, in order to search for an association between the different variables. The significance level of p was set at 0.05%.

The respect of the interviewed persons was maintained throughout the study, as well as the anonymity of the data. This study was conducted in accordance with the Helsinki Declaration. Before handing over the questionnaire, all interviewed persons were informed of the purpose and objectives of the study by an oral message. Before starting data collection, we obtained ethical clearance N° 0630 / UYI / FMSB / VDRC / DAASR /CSD from the Institutional Ethics Committee for Research (IECR) of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I and the approval of the director of CRAESHRY to carry out the study. The anonymity of the surveyed persons was ensured. In addition, each person expressed their agreement before answering the questions. Participation in the study was therefore entirely voluntary.

Results

Follicular concentrations of IL10 and G-CSF were significantly higher than their serum concentrations, meanwhile there was no statistically significant difference between serum and follicular concentrations of IFN-gamma. There was a statistically significant association between serum G-CSF concentrations and percentages of matured oocytes.

There was no statistically significant association between follicular G-CSF concentration and the percentage of mature oocytes. The percentage of matured oocytes was significantly higher for serum IFN-Gamma concentrations > 5.2pg/ml. There was no significant association between follicular concentration of IFN-Gamma and matured oocytes.

Table I : *distribution of the patients according to sociodemographic*

Variables	Frequency	%
Age group (years)		
< 25	6	9.8
[25,35[	27	44.3
[35,45[	27	44.3
[45,55[	1	1.6
> 55	0	0

Table II: *distribution of serum and follicular concentrations of cytokines*

	Serum concentration (n=61)	Follicular concentration (n=61)	P-value
IL10			
Median (Interquartile range)	13.91 (10.67 - 15.50)	20.86 (11.16-27.17)	**0,02**
Minimum	2.52	0	
Maximum	95	961	
IFN-Gamma			
Median (Interquartile range)	5.20 (2.64 - 6.50)	4.07 (2.90-6.40)	**0,43**
Minimum	1.05	1	
Maximum	198	204	
G-CSF			
Median (Interquartile range)	29.16 (24.70-34.45)	48.04 (31.39-79.17)	**<0,00**
Minimum	19.69	17.60	
Maximum	69.07	1374	

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Table III: *association between oocyte maturity and cytokines (IL10 and G-CSF)*

Parameters	Percentage of mature oocytes			P-value
	<50%	Between 50-90%	> 90%	
Serum IL-10 concentration (pg/ml)				
0 - 17	9 (90)	20 (80)	9 (100)	
18 - 30	1 (10)	1 (4)	0(0)	0.47
>30	0 (0)	4 (16)	0 (0)	
Follicular IL10 concentration (pg/ml)				
0 - 17	3 (30)	10 (52.6)	5 (62.5)	
18 - 30	3 (30)	5 (26.3)	3 (37.5)	0.34
>30	4 (40)	4 (21.1)	0 (0)	
Serum G- CSF concentration (pg/ml)				
0 - 17	0(0)	0(0)	0 (0)	
18 - 30	1 (10)	28 (77.8)	9 (60)	**<0.001**
>30	9 (90)	8 (22.2)	6 (40)	
Follicular G-CSF concentration(pg/ml)				
0 - 17	0 (0)	1 (2.8)	1 (6.7)	
18 - 30	2 (20)	6 (16.7)	5 (33.3)	0.53
>30	8 (80)	29 (80.5)	9 (60)	

Table IV: *association between oocyte maturity and IFN-Gamma*

	Average percentage of mature oocytes (%)	P-value
Serum concentrations (Frequency)		**0.01**
[0- 5,2] (n=32)	61.76 (+/- 28.56)	
] 5,2- 198] (n=29)	77.41 (+/- 16.87)	
Follicular concentrations (Frequency)	**Median percentages (%)**	**0.1**
[0- 4,07] (n=31)	66.67	
] 4,07-204] (n=30)	79.17	

Discussion

88.6% of participants were equally distributed across the age groups of 25-35 years and 35-45 years. These findings are consistent with the work of Nana and al in 2011 [13], who reported an average age of 30.76 years, as well as the study by Meka and al in 2016 [14], who found that 89% of women were aged 25-34 years. Belinga and al [15] in Cameroon in 2021 reported an average age of 31.2 years (±3.46). This observation may be attributed to the fact that our study was conducted in an urban area, where the educational level of women is significantly increasing, and where the age of first motherhood has risen.

There were significantly higher concentrations of IL-10 and G-CSF in follicular fluid compared to serum levels, alongside similar concentrations of IFN-gamma in both compartments. The results suggest that the cumulus luteinizing cells are not merely passive recipients of circulating factors but actively contribute to the follicular microenvironment through localized cytokine synthesis. These findings are consistent with existing literature, which indicates that pro-inflammatory cytokines like IL-10 and G-CSF are synthesized within the ovary and released during critical phases of follicular maturation and ovulation [1,4,6].

The evaluation of the cytokinic environment in both follicular fluid and serum revealed that the presence of G-CSF and IFN-gamma at certain levels was associated with oocyte maturity and their potential competence. Serum concentrations of G-CSF were significantly related to oocyte maturity at optimal levels. These findings align with those of Lédée and al in 2008 [16], which demonstrated that G-CSF at similar concentrations could influence the mRNA content of the oocyte, thereby enhancing its maturation and competence. However, our follicular concentrations of G-CSF were not significantly associated with oocyte quality, differing from the results of Achour-Frydman and al. in 2010 [17], who found that G-CSF could significantly distinguish oocytes that lead to good-quality embryos from those resulting in lower-quality embryos. Our follicular concentrations were comparable to theirs despite the lack of a significant association with oocyte quality in our study. This discrepancy may be attributed to our monocentric methodology and smaller sample size, in contrast to their larger multicenter studies. The serum and follicular concentrations of IFN-gamma in our study were consistent with existing literature, with only serum concentrations significantly associated with oocyte maturity. According to Lédée and al.[16] , IFN-gamma levels were statistically higher in the follicular fluid of women with the best percentages of matured oocytes.

Interestingly, our IL-10 concentrations were generally higher compared to those reported by other authors, suggesting potential variations in the follicular microenvironment across different populations. This aligns with the findings of Revelli et al., 2009 [18], which indicated that while IL-10 contributes to maintaining a specific hormonal microenvironment within the follicle, its association with good oocyte and embryonic quality remains unestablished.

Conclusion

This study highlights the critical role of inflammatory cytokines, particularly G-CSF and IFN-gamma, in influencing oocyte quality among women undergoing assisted reproductive

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technology. The significant association between elevated serum G-CSF levels and increased percentages of matured oocytes suggests that inflammatory markers in follicular fluid may serve as valuable indicators of oocyte competence. Understanding the intricate interplay between cytokine profiles and ovarian function can provide insights into the mechanisms underlying diminished ovarian reserve and its impact on fertility outcomes. Future research should focus on exploring therapeutic interventions targeting these cytokines to enhance oocyte quality and improve ART success rates.

Conflict of interest: None

Authors' contributions
Mounchikpou N.G., Ngoungoure M.A and Voundi V.E. **conceived and designed the study.** Mounchikpou N.G., Ngoungoure M.A. and Voundi V.E **carried out the experiment and, analysed and interpreted the results**. With the help of Ngoungoure M.A; Mounchikpou N.G **drafted the manuscript** and Ndolo K.A, Bilkissou M, Ngaha J, Manga F **revised the manuscript**. Belinga E and Toukam M supervised the work at all stages.

List of abbreviations
IL-10: Interleukin 10
IFN-gamma: Interferon gamma
G-CSF: Granulocyte Colony-Stimulating Factor
ELISA: Enzyme-Linked Immunosorbent Assay

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