

The effect of follicular fluid-related hormones and vascular endothelial factor levels on the formation of high-quality embryos

Lin Liang¹, Xiao Xi Zhao^{2,*}¹The Reproductive Center, Affiliate Hospital of Inner Mongolia Medical University, 010050 Hohhot, Inner Mongolia, China²Department of Gynecology and Obstetrics, Affiliate Hospital of Inner Mongolia Medical University, 010050 Hohhot, Inner Mongolia, China*Correspondence: zhaoxx.3675@163.com (Xiao Xi Zhao)DOI: [10.31083/j.ceog4805178](https://doi.org/10.31083/j.ceog4805178)This is an open access article under the CC BY 4.0 license (<https://creativecommons.org/licenses/by/4.0/>).

Submitted: 1 January 2021 Revised: 11 February 2021 Accepted: 23 March 2021 Published: 15 October 2021

Background: The purpose of this study was to investigate the possible influence of follicular fluid (FF)-related hormones and vascular endothelial factor (VEGF) levels on oocytes, and the resulting effect on formation of high-quality embryos, to provide a scientific basis for improving controlled ovarian stimulation. **Methods:** The levels of hormones and VEGF in FF were determined by performing enzyme-linked immunoassays. According to the number of high-quality embryos obtained, the patients were divided into three groups, and the levels and constitution of hormones and VEGF in FF were compared. Based on the results of correlation analysis, the number of oocytes harvested and the concentration of follicle-stimulating hormone (FSH) in FF were substituted into multiple logistic regression models. **Results:** The number of high-quality embryos was negatively correlated with FSH concentration (correlation coefficient = -0.305 , $p = 0.0001$). The concentration of VEGF, FSH, luteinising hormone, and estradiol in FF was significantly different among the different high-quality embryo number groups. Multiple logistic regression results showed that the FSH concentration in the FF of the group without embryos was significantly higher than that of the other two groups, and the number of eggs obtained in the third group was significantly higher than that of the group without embryos. **Conclusion:** In the late stage of follicular development, FSH concentration in FF and the number of oocytes obtained affects the formation of high-quality embryos.

Keywords

Follicular fluid; Vascular endothelial growth factor; Hormones

1. Introduction

The success of *in vitro* fertilisation and embryo transfer (IVF-ET) is closely related to the formation of high-quality embryos. There are many factors that affect embryo quality, but the developmental potential of oocytes directly affects the quality of embryos as well [1]. The final stage of oocyte development and maturation is in the follicular fluid (FF), which constitutes the micro-environment of its growth and development. The FF contains hormones, cytokines, and various nutrients, which play an important role in the maturation and development of follicles [2, 3]. Some of the hormones in the

FF are synthesised by granulosa cells around the ovum, and some are derived from the circulating blood [4]. The capillary network around the follicle regulates the concentration of pituitary-derived hormones in the FF [5].

The main hormones in the FF include follicle-stimulating hormone (FSH), luteinising hormone (LH), estradiol (E2), anti-Mullerian hormone (AMH), progesterone (P), and testosterone (T). Among these hormones, FSH, LH, E2, and AMH are responsible for the growth and development of oocytes [6].

FSH and LH are synthesised and secreted by the pituitary gland; they enter the FF through blood circulation. During each menstrual cycle, FSH acts to recruit follicles, while LH promotes follicular maturation [7]. E2 is produced by the granulosa cells of the oocytes and plays an important role in the selection of dominant follicles [8]. AMH is produced by the granulosa cells of the antral and preantral follicles, and is closely related to the antral follicle number while being an important indicator of follicle reserve capacity [9]. Vascular endothelial factor (VEGF) is produced from follicular granulosa thecal cells. It plays a central role in the regulation of ovarian angiogenesis, and is critical for ovarian follicle growth. During folliculogenesis, VEGF secretion, which is induced by gonadotropins, determines the formation of the vascular network in the thecal cell layer of the follicle [5].

In order to understand the effect of the concentration of various hormones in the FF on the formation of high-quality embryos, we investigated the correlation between the number of high-quality embryos formed and hormones in the FF, to provide a scientific basis for improving controlled ovarian stimulation.

2. Materials and methods

2.1 Patients

This study was approved by the Ethics Committee of the Affiliate Hospital of Inner Mongolia Medical University (No. YKD2016108). All patients provided written informed consent prior to their inclusion in this study. From February

2017 to December 2018, 118 patients who were receiving IVF treatment for infertility at the reproductive centre of our hospital were recruited into this study. For FF examination, 5 mL of FF was collected from each patient during oocyte (egg) collection.

These 118 patients had the diagnosis of tubal infertility. The semen test result of their partners (according to WHO semen test standards) was within the normal range. Tests done for the patients before the IVF treatment, such as peripheral blood FSH and LH, and gynaecological ultrasonography, showed no significant abnormalities. There was no history of pelvic surgery, but that of hormonal treatment starting three months before admission. Controlled ovarian stimulation with long-term ovulation induction was performed in the luteal phase.

The embryos referred to in this study are blastomere stage embryos cultured for three days, and divided into three grades according to the embryo score standard; grade 1 and 2 embryos are regarded as high-quality embryos. Embryo classification criteria are as follows: for grade 1 embryos, the cell size is uniform, the shape is regular, the zona pellucida is intact, the cytoplasm is uniform and clear, there is no particle phenomenon, and the fragments are between 0–5%; in grade 2 embryos, the cell size is slightly uneven, the shape is slightly irregular, the cytoplasm may be granular, and fragments are between 10–20%; and grade 3 embryos have obvious uneven cell size or even serious unevenness, irregular shape, granular phenomenon, and fragments greater than 20%.

The grouping of patients according to the number of high-quality embryos was as follows: group 1 (no embryos, $n = 18$), group 2 (embryos: 1–2, $n = 58$), and group 3 (embryos ≥ 3 , $n = 41$).

The grouping according to the number of eggs obtained was as follows: group 1 (eggs: 1–3, $n = 52$), group 2 (eggs: 3–10, $n = 57$), and group 3 (eggs ≥ 10 , $n = 9$).

The grouping according to the of content of FSH in FF was as follows: group 1 (FSH: 1.6–6.4, $n = 40$), group 2 (FSH: 6.4–12.64, $n = 40$), and group 3 (>12.64 , $n = 38$).

2.2 Methods

2.2.1 Controlled ovarian stimulation

Starting from the middle of the luteal phase of the previous menstrual cycle, a gonadotropin-releasing hormone agonist (GnRHa) was injected daily for down regulation. Between the third and fifth day of menstruation, when the down regulation standard was reached, gonadotropin (GN) was used to induce ovulation.

A long-term controlled ovarian stimulation during was conducted the luteal phase. In cases with at least one follicle of 18 mm (approximately) or three follicles >16 mm in both ovaries, a dose of 500–1000 recombinant human chorionic gonadotropin (r-hCG) units was injected at night. The eggs were collected after 36 hours.

Table 1. The spearman correlation analysis results of oocytes, embryo, VEGF and FF-related hormones.

		VEGF	FSH	LH	E2	AMH	ET
Oocytes	r	-0.151	-0.157	-0.092	-0.086	0.24	0.654
	Sig.	0.103	0.09	0.32	0.356	0.009	0.000
Embryo	r	-0.14	-0.335	-0.112	-0.234	0.186	0.654
	Sig.	0.13	0.000	0.226	0.011	0.044	0.000
VEGF	r	-0.14	0.341	0.418	-0.018	-0.17	-0.14
	Sig.	0.13	0.000	0.000	0.847	0.066	0.13

Table 2. The average and standard deviation of each group after grouping according to the number of high quality embryos obtained.

	Group 1 (n = 18)	Group 2 (n = 59)	Group 3 (n = 41)	p
Age	33.8 ± 5.4	34.8 ± 4.8	34.1 ± 4.7	0.542
BMI	24.59 ± 1.53	23.80 ± 1.96	24.31 ± 1.89	0.167
Oocyte	3.1 ± 3.1	3.5 ± 2.3	8.0 ± 6.0	<0.0001
Embryos	0	1.3 ± 0.5	4.3 ± 2.2	<0.0001
VEGF	166.0 ± 41.6	157.3 ± 138.8	138.0 ± 43.3	0.046
FSH	14.3 ± 3.7	10.2 ± 7.8	8.0 ± 4.4	<0.0001
LH	25.7 ± 7.3	22.4 ± 17.2	20.4 ± 5.7	0.016
E2	159.7 ± 21.9	126.1 ± 93.0	99.5 ± 75.2	0.017
AMH	1602.4 ± 589.9	1751.5 ± 1272.1	1861.7 ± 712.3	0.263

2.2.2 Preservation of the follicular fluid (FF)

The collected FF samples were centrifuged at 2500 rpm for 10 minutes and stored in a cool tube at -80°C .

2.2.3 Sample analysis

The levels of FSH, LH, E2, AMH, and VEGF in the FF were measured by an enzyme-linked immunosorbent assay (ELISA; Shanghai Jingkang Biological Engineering Company, Shanghai, China).

2.2.4 Observation index

The number of eggs and embryos were enumerated.

2.2.5 Semen analysis

The semen was analysed using the Computer-aided Semen Analysis System (CASA). Normal semen standards were as follows: (1) seminal volume: 2–6 mL; (2) liquefaction time <30 minutes; (3) pH value between 7.2 and 8.0; (4) density >20 million/mL; (5) sperm survival rate $\geq 60\%$; (6) sperm motility A $\geq 25\%$ or A + B $\geq 50\%$; (7) sperm malformation rate $>4\%$.

2.3 Statistical analysis

Data were analysed using the SPSS 19.0 software. First, the normality of the data was tested, and then an analysis was performed using Spearman correlation and the rank-sum test of multiple sample comparisons. p -values < 0.05 were considered statistically significant. Then, the number of oocytes

Table 3. Logical regression model parameter estimation and test results.

GET	B	S.E	Wald	df	Sig.	Exp (B)	95% CI for EXP (B)		
							Lower	Upper	
2	intercept	-0.746	1.484	0.252	1	0.615			
	[GEgg = 1.00]	0.616	1.508	0.167	1	0.683	1.852	0.096	35.601
	[GEgg = 2.00]	2.169	1.59	1.862	1	0.172	8.752	0.388	197.319
	[GEgg = 3.00]	0b	a	a	0	a	a	a	a
	[GFSH = 1.00]	20.018	0.635	993.798	1	0	4.94×10^8	1.42×10^8	1.71×10^9
	[GFSH = 2.00]	1.114	0.645	2.982	1	0.084	3.047	0.86	10.794
	[GFSH = 3.00]	0b	a	a	0	a	a	a	a
3	intercept	0.247	1.249	0.039	1	0.843			
	[GEgg = 1.00]	-3.322	1.356	6.001	1	0.014	0.036	0.003	0.515
	[GEgg = 2.00]	0.405	1.369	0.087	1	0.767	1.499	0.102	21.93
	[GEgg = 3.00]	0b	a	a	0	a	a	a	a
	[GFSH = 1.00]	21.383	0	a	1	a	1.93×10^9	1.93×10^9	1.93×10^9
	[GFSH = 2.00]	2.25	0.818	7.559	1	0.006	9.489	1.908	47.188
	[GFSH = 3.00]	0b	a	a	0	a	a	a	a

a. The reference category is: 1.00.

b. Because this parameter is redundant, it is set to zero.

obtained and the concentration of FSH in FF that correlated with the number of high-quality embryos, were entered into multiple logistic regression models.

3. Results

Table 1 shows the Spearman correlation analysis results between number of oocytes retrieved, the number of high-quality embryos, VEGF in the FF and concentrations of various hormones in the FF. The number of oocytes obtained was positively correlated with ET (correlation coefficient: 0.654, $p = 0.0001$) and AMH (correlation coefficient: 0.24, $p = 0.009$), but not with other hormones. The number of high-quality embryos obtained was positively correlated with the number of oocytes obtained (correlation coefficient = 0.654, $p = 0.0001$). There was a negative correlation with FSH in the FF (correlation coefficient = -0.305 , $p = 0.0001$), a weak positive correlation with AMH (correlation coefficient = 0.179, $p = 0.044$, respectively) and a weak negative correlation with E2 (correlation coefficient = -0.193 , $p = 0.03$). VEGF was positively correlated with FSH and LH (correlation coefficient = 0.317, $p = 0.0001$; 0.395, $p = 0.0001$).

Table 2 shows the average age, number of retrieved oocytes, number of high-quality embryos, and the average concentration and standard deviation of various hormones and VEGF in the FF among the three groups. There were statistically significant differences in the numbers of harvested oocytes, VEGF, FSH, LH, and E2 among the three groups. There was no difference in age among the three groups.

Table 3 shows that multiple logistic regression results. FSH content in the FF of the group without embryos was higher than that of the other two groups (Group 2: OR = 1.42×10^8 – 1.71×10^9 ; $p = 0.0001$; 95% CI: 1.42×10^8 – 1.71×10^9 ; and Group 3: OR = 9.489; $p = 0.006$; 95% CI: 1.908–47.188).

4. Discussion

The factors affecting the formation of high-quality embryos include age [10], ovarian function [11], and the quality of the man's semen [12], among others. In this study, a group of patients who were treated with IVF due to tubal infertility, in whom ovarian function and the spouse's semen examination were not significantly abnormal, were selected as the study group to explore the relationship between various hormones in the FF and the number of high-quality embryos formed. The results showed that the number of high-quality embryos was negatively correlated with levels of FSH and E2 in the FF and weakly positively correlated with AMH. There were significant differences in the levels of VEGF, FSH, LH, and E2 in the FF among the three groups. Multiple logistic regression results show that the number of high-quality embryos was related to the number of retrieved oocytes, and the concentration of FSH in FF was a negative factor for the formation of high-quality embryos.

In IVF-ET, FSH has two sources: production by the pituitary gland and exogenous FSH. In this study, FSH in the FF was negatively correlated with the number of high-quality embryos, while LH showed no such correlation. Previous studies have shown that high concentrations of FSH and LH in the FF can promote oocyte maturation and fertilization, and that they are beneficial to the formation and development of embryos [6]. However, recent studies have shown that increase in FSH in the FF can lead to increase in the number of polar bodies and embryo aneuploidy [13, 14]. Our results also showed that the number of high-quality embryos decreased in the group with a higher FSH level in the FF, thereby indicating that the concentration of FSH in the FF affects the quality of oocytes and embryos. E2 was weakly negatively correlated with the number of high-quality embryos, which may be the result of a synergistic effect of FSH and E2. There

was a weak positive correlation between AMH and the number of high-quality embryos. When AMH in the FF was high, the number of oocytes obtained was higher, and the number of embryos obtained also increased. Our results also showed that VEGF levels were positively correlated with FSH and LH in the FF, but not with E2 and AMH. This result further confirmed that the amount of FSH and LH entering the FF is related to the number of capillaries around the follicle.

In this study, three groups with different numbers of high-quality embryos were compared. The higher the number of high-quality embryos obtained was, the lower the levels of VEGF were. Previous studies have shown that the concentration of VEGF in the FF affects the developmental potential and fertilisation ability of oocytes, resulting in decreases in embryo quality and pregnancy rates [5]. The results of this study are consistent with those of previous studies.

In the early stage of follicular development, FSH and LH promote follicular development, but in the late stage, FSH interferes with the arrangement of oocyte chromosomes on the equatorial plate, resulting in increase in aneuploid oocytes, thus affecting the formation of normal embryos. These results further indicate that lower concentrations of VEGF, FSH, LH, and E2 in the late FF are helpful in improving the developmental potential of oocytes and increasing the number of high-quality embryos.

5. Conclusions

In conclusion, the number of high-quality embryos is directly related to the number of oocytes. The concentration of FSH in FF affects the formation of high-quality embryos; the capillary network around the follicle regulates the concentration of FSH in FF. In the late stage of follicular development, high concentration of FSH affects the development potential of oocytes. Hence, reducing the amount of FSH entering into FF from the blood circulation may increase the number of high-quality embryos.

Author contributions

XXZ designed the research study and analyzed the data. LL responsible for collecting research specimens and performed the research. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the hospital affiliated with Inner Mongolia Medical University. The ethical approval number is YKD2016108. Informed consent was obtained from all subjects involved in the study.

Acknowledgment

We would like to express my gratitude to all those who helped me during the writing of this manuscript.

Funding

This research was funded by the Natural Science Foundation of China, grant number 81660542. This research was funded by the Inner Mongolia Natural Science Fund, grant number 2018MS08091.

Conflict of interest

The authors declare no conflict of interest.

References

- [1] Cimadomo D, Fabozzi G, Vaiarelli A, Ubaldi N, Ubaldi FM, Rienzi L. Impact of maternal age on oocyte and embryo competence. *Frontiers in Endocrinology*. 2018; 9: 327.
- [2] Bulgurcuoglu-Kuran S, Ozsait-Selcuk B, Gungor-Ugurlucan F, Koksak G, Günay C, Faruk B. Higher follicular fluid glycodeclin levels are negatively correlated with embryonic development in assisted reproduction. *JBRA Assisted Reproduction*. 2012; 2: 346–351.
- [3] Dumesic DA, Meldrum DR, Katz-Jaffe MG, Krisher RL, Schoolcraft WB. Oocyte environment: follicular fluid and cumulus cells are critical for oocyte health. *Fertility and Sterility*. 2015; 103: 303–316.
- [4] Revelli A, Piane LD, Casano S, Molinari E, Massobrio M, Rinaldo P. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. *Reproductive Biology and Endocrinology*. 2009; 7: 40.
- [5] Monteleone P, Giovanni Artini P, Simi G, Casarosa E, Cela V, Genazzani AR. Follicular fluid VEGF levels directly correlate with perifollicular blood flow in normoresponder patients undergoing IVF. *Journal of Assisted Reproduction and Genetics*. 2008; 25: 183–186.
- [6] Hennet ML, Combelles CMH. The antral follicle: a microenvironment for oocyte differentiation. *The International Journal of Developmental Biology*. 2012; 56: 819–831.
- [7] Kline JK, Kinney AM, Levin B, Kelly AC, Ferin M, Warburton D. Trisomic pregnancy and elevated FSH: implications for the oocyte pool hypothesis. *Human Reproduction*. 2011; 26: 1537–1550.
- [8] Valbuena D, Martin J, de Pablo JL, Remohí J, Pellicer A, Simón C. Increasing levels of estradiol are deleterious to embryonic implantation because they directly affect the embryo. *Fertility and Sterility*. 2001; 76: 962–968.
- [9] Schenk M, Kröpfl JM, Obermayer-Pietsch B, Feldmeier E, Weiss G. Anti-Mullerian hormone concentrations in individual follicular fluids within one stimulated IVF cycle resemble blood serum values. *Journal of Assisted Reproduction and Genetics*. 2017; 34: 1115–1120.
- [10] Keefe DL. Telomeres, reproductive aging, and genomic instability during early development. *Reproductive Sciences*. 2016; 23: 1612–1615.
- [11] Chang Y, Li J, Li X, Liu H, Liang X. Egg quality and pregnancy outcome in young infertile women with diminished ovarian reserve. *Medical Science Monitor*. 2018; 24: 7279–7284.
- [12] Lazaros L, Vartholomatos G, Pamporaki C, Kosmas I, Takenaka A, Makrydimas G, *et al*. Sperm flow cytometric parameters are associated with ICSI outcome. *Reproductive Biomedicine Online*. 2013; 26: 611–618.
- [13] Hammoud I, Vialard F, Bergere M, Albert M, Gomes DM, Adler M, *et al*. Follicular fluid protein content (FSH, LH, PG4, E2 and AMH) and polar body aneuploidy. *Journal of Assisted Reproduction and Genetics*. 2012; 29: 1123–1134.
- [14] Liang L, Zhao X. Correlation between aneuploidy pregnancy and the concentration of various hormones and vascular endothelial factor in follicular fluid as well as the number of acquired oocytes. *Journal of Perinatal Medicine*. 2019; 48: 40–45.