Impact of follicular fluid glycodelin on the process of fertilization and implantation after the transfer of embryos in the process of in vitro fertilization (IVF)

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Abstract
The aim of the study was to assess the impact of the level of glycodelin isoforms in the follicular fluid on the process of fertilization and implantation potential of the embryos after transfer. Study group consisted of 57 patients undergoing infertility treatment by in vitro fertilization (IVF). First group included 35 patients in whom classical embryo transfer (IVF-ET) procedure was performed, whereas in the second (22 patients) intracytoplasmic sperm injection (IVF-ICSI) procedure was applied. Long stimulation protocol with GnRH agonist and recombinant FSH was applied. Follicular fluid was obtained by transvaginal ultrasound follicular puncture. The level of glycodelin in the follicular liquid was determined by ELISA method. The level of follicular fluid glycodelin in in vitro fertilization procedure is significantly higher in patients with a negative pregnancy test result regardless of the method of fertilization (IVF-ET, ICSI). Conclusions: 1) Higher concentrations of glycodelin in follicular fluid derived from follicles in which oocytes were not fertilized can indicate inhibitory effect of glycodelin on the process of combining sperm with zona pellucida. 2) The results of the study suggest that glycodelin isoforms in follicular fluid may affect the process of fertilization and implantation through various regulatory mechanisms.

Key words: in vitro fertilization, glycodelin, follicular fluid

Introduction

The effectiveness of IVF treatment is measured by the percentage of pregnancies obtained, and the safety of these procedures depend on the number of transferred embryos. Still, reliable biochemical markers that could help in choosing the most promising embryos for transfer, are unknown. Such markers could help in reducing the number of transferred embryos without reduction the chance of getting pregnant. Numerous reports describing the mechanisms regulating the processes of fertilization and implantation highlight the role of a group of glycoproteins known collectively as glycodelins [2, 3, 10, 11].

Glycodelins synthesized primarily in the reproductive tract are present in placental tissue, amniotic fluid, seminal fluid, temporal and follicular fluid. Depending on differences in the glycolysation and place of its production we can distinguished:

- glycodelin A – amniotic fluid, endometrium, decidua, serum [12];
- glycodelin S – vesicles and seminal fluid [2, 3];
- glycodelin F – vesicular fluid and fallopian tubes [2, 3];
- glycodelin C – cumulus oophorus [6].

The impact of glycodelins on the reproductive cycle include its participations in the mechanisms of regulation of the immune system (immunosuppression), creation of the so-called endometrial implantation window, the impact on acrosome reaction and process of capacitation, and in the mechanism of combining sperm with oocyte. Their role in apoptosis was also described [10].

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Materials and methods

Study group consisted of 57 patients undergoing infertility treatment by in vitro fertilization (IVF). The study included women who participated in the IVF procedure for the first time. Depending on the infertility factor patients were qualified for classical IVF method – 35
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patients or ICSI (intracytoplasmic sperm injection) – 22 patients. The average age of patients was 32 ± 2.8 years. In all cases long stimulation protocol with GnRH agonist (Decapeptyl – Fehrring) and recombinant FSH (Gonal – Merck Serono) was applied. The mean duration of stimulation was 10 ± 2 days. 36 hours before the scheduled follicular puncture an injection of follicle-commissioned 10 000 IU chorionic gonadotropin HCG (Pregnyl, Organon) was given.

After the puncture it was frozen and stored at liquid nitrogen temperature: – 196°C until determination of the glycodelin.

Embryo culture was carried out in the commercial media (Vitrolife). Semen for insemination was purified by centrifugation in a discontinuous gradient – SpermGrad (Vitrolife). Verification of fertilization was made after 16-18 hours. The criterion for the normal fertilization was the presence of two pronucleus (2 PN). Grading of embryos was carried out after 48 and 72 hours of culture. Transfer of embryos to the uterine cavity was performed using K-Soft catheter 5000 (Cook) in the third day of culture. Pregnancy was diagnosed, based on the concentration of subunit beta – HCG, 14 days after embryo transfer.

The level of glycodelin in the follicular fluid was determined by ELISA method, using reagents of the Bioserv Diagnostic (Rostock, Germany) company. In this procedure 50 ml of centrifuged follicular fluid was used. Each of the tested samples were analyzed twice, with the final result recognized as the average expressed in ng/ml. Determination procedure was performed according to the producer. Shortly this procedure can be described as follow:

• The 96-well plate coated with an antiGD antibody was covered in duplicate with 50 ml samples of follicular fluid.
• After 90 minutes of incubation and washing, conjugated with horseradish peroxidase (HRP) second antibody antiGD was added and then incubation was performed for another 30 minutes.
• Following that time, the plate was washed and the TMB-substrate was added for color reaction catalyzed by HRP. Color reaction was conducted at room temperature for 15 min. After that time the reaction was stopped using H2SO4 (0.25 mol/l).
• Reading (λ = 450 nm) and calibration of the curve were made using Biotek reader.

The results were statistically analyzed using Mann-Whitney test. The level of statistical significance was recognize at the 0.05 level.

Results

A. Group of IVF-ET

First group included 35 patients in whom classical IVF-ET procedure was performed. A comparison of glycodelin concentration in the follicular fluid in relation to the result of pregnancy test is presented on Fig. 1. Lower concentration of glycodelin was observed in women in whom treatment was successful (positive pregnancy test), compared with a group of patients in whom there has been a failure (a negative pregnancy test) – the difference is statistically significant. Statistically significant lower level of glycodelin was observed in the patients in whom normal development of the healthy embryo was diagnosed (Fig. 2).

Fig. 1. Comparison of the levels of glycodelin in the follicular fluid depending on the outcome of the pregnancy test – IVF-ET group (p < 0.05)

In all figures of his paper the central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. A line extends from the minimum to the maximum value, excluding "outside" and "far out" values which are displayed as separate points.

Fig. 2. Comparison of the levels of glycodelin in the follicular fluid depending on the presence or absence of the embryo – IVF-ET group (p < 0.05)
B. Group of IVF-ICSI
In the group treated by intracytoplasmic sperm injection procedure (ICSI), 22 women were compared by analyzing the concentration of the glycodelin in the follicular fluid in the relation to the biochemical diagnosis of the pregnancy based on βhCG determination in the serum and development of the normal embryo. As in the IVF group, in the ICSI group significantly lower levels of glycodelin in patients with a positive biochemical pregnancy test were observed (Fig. 3). Figure 4 shows a statistically significant difference in glycodelin concentration between the group with present or absent embryo (higher in the presence of embryo). In patients undergoing ICSI results are opposite than in the group of women undergoing classical IVF (IVF-ET).

![Fig. 3. Comparison of the levels of glycodelin in the follicular fluid depending on the outcome of the pregnancy test – IVF-ICSI group (p < 0.05)](image)

![Fig. 4. Comparison of the levels of glycodelin in the follicular fluid depending on the presence or absence of the embryo – IVF-ICSI group (p < 0.05)](image)

**Discussion**

The role of glycodelin in the process of fertilization and implantation is still the subject of many scientific reports [2, 3, 5, 9]. The glycoprotein exists in several isoforms with a common core protein, differing in the carbohydrate part. Previously isoform S (seminal fluid), isoform A (endometrium, amniotic fluid) and isoform F (follicular fluid) were isolated and described. Recently, appeared reports describing a new isoform C present in the cells of the cumulus oophorus [4, 6].

Glycodelin F is a basic isoform present in the follicular fluid [4, 6-8, 13]. It inhibits the merger of sperm with zona pellucida. Additionally, it suppresses acrosomal reaction dependent on progesterone, what may be part of a mechanism preventing its preterm occurrence. After the ovulation part, vesicular fluid is transported to the oviduct with the oocyte-cumulus complex. Exposure of spermatozoa to the action of glycodelin F increases with the approach of this complex. In the last few years there have been reports describing a new isoform present in the extracellular matrix of cumulus, which unlike other isoforms, stimulates the process of sperm connection to zona pellucida.

Hayes et al. [1] evaluated in vitro the impact of glycodelin on the production of VEGF in cells of the cumulus oophorus taken from patients participating in IVF. Patients with normal gonadotropin response showed increased production of VEGF in response to glycodelin, while patients with poor response showed a decrease effect. Researchers suggest important role of glycodelin in the regulation of follicular angiogenesis, which disorder may lead to deterioration in the quality of oocytes and embryos in the IVF procedure.

Our results demonstrate high concentration of glycodelin in the follicular fluid collected during the aspiration of ova in the IVF procedure. In both groups of patients, treated with IVF-ET or ICSI, significantly higher levels of glycodelin were observed in patients with negative pregnancy test. A similar relationship has been demonstrated in IVF-ET group when fertilization of an ovum and development of proper embryo were analyzed. In contrast in the group of ICSI an opposite tendency was observed – higher concentrations of glycodelin positively correlated with development of normal embryos. This differences may be explained by the inhibitory effect of the glycodelin F, present in the cumulus oophorus, on the connection of the spermatozoa with zona pellucida; such connection is omitted in the ICSI procedure. A similar trend in the biochemical pregnancy test result in both groups suggests that glycodelin isoforms in follicular fluid may affect the process of fertilization and implantation by other regulatory mechanisms, which require further studies.
Conclusions

1) The level of follicular fluid glycodelin in patients undergoing *in vitro* fertilization procedure is significantly higher in patients with a negative pregnancy test result regardless of the method of fertilization (IVF-ET, ICSI).

2) Higher concentrations of glycodelin in follicular fluid derived from follicles in which oocytes were not fertilized can indicate inhibitory effect of glycodelin on the process of combining sperm with zona pellucida.

3) Our results suggest that glycodelin isoforms in the follicular fluid may affect the process of fertilization and implantation through various regulatory mechanisms – the precise explanation of this mechanisms requires further study.


References


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