1ST PRIZE

THE CLINICAL VALUE OF OVOSICARE® ProFIV IN AN *IN VITRO* FERTILISATION CYCLE: A CASE REPORT

Dr Luis Ignacio Devesa Otero

Hospital Vithas, Vigo and Centro Médico Vithas, Pontevedra

ABSTRACT

The aim of this case study is to evaluate the impact of using the inositol and probiotic compound (Ovosicare® ProFIV) prior to IVF in a couple who achieved pregnancy after previous failed attempts.

KEY WORDS

3.6:1 Myo-inositol to D-chiro-inositol ratio. Probiotics. Microbiota. Oocyte quality. Embryo quality.

MEDICAL HISTORY AND ANAMNESIS

Heteroparental couple, both cisgender, who attended their first consultation in 2022. The female is currently 36 years old and the male is 45. They have a history of several years of reproductive dysfunction and have undergone 3 assisted reproduction treatments at other clinics: 2 cycles of artificial insemination (AIH) and one cycle of *in vitro* fertilisation (IVF). In their first IVF cycle, 3 embryos were obtained on day 3 but did not result in pregnancy.

The woman's personal history included allergy to trimethoprim-sulfamethoxazole and no diseases other than uncomplicated urinary tract infections (UTIs). She had undergone a tonsillectomy, has had no previous pregnancies and menarche occurred at the age of 14. She has had regular cycles. There are no further relevant comorbidities in the medical history of their families. The male partner had only chronic hepatitis C (HCV) infection, with an undetectable viral load, and had undergone an appendectomy. No other relevant medical history. No previous children.

PHYSICAL EXAMINATION AND DIFFERENTIAL DIAGNOSIS

At the first consultation, we performed a thorough physical examination of the patient, including a complete gynaecological assessment, and did not detect any apparent alterations. A follicular count at the limit of normality (AFC 4/6) was observed, with no evidence of anatomical or structural Müllerian anomalies.

Previous insemination cycles were

performed with human menopausal gonadotropin (hMG) and letrozole.

The previous IVF cycle was performed with a combined protocol of recombinant follicle stimulating hormone (FSH) and hMG. The result was 12 oocytes retrieved, 8 fertilised and 3 embryos at D+3 of development, which were transferred, and the transfer did not result in pregnancy. We can, therefore, infer the presence of a mixed factor, even though we do not have data on blastocyst formation since the culture was stopped on day 3.

Different studies are carried out in order to refine the specific diagnosis.

Additional tests carried out on the female patient:

- Complete blood and serological tests did not reveal any relevant alterations. Immune to Rubella.
- Vaginal and endocervical cultures: *Candida albicans* treated locally
- Blood karyotype: 46, XX (normal).
- Vaginal-cervical-endocervical smear: negative for intraepithelial lesion and malignancy.
- Follicular reserve test. Antral follicle count: 6 (right ovary) + 4 (left ovary) and anti-Müllerian hormone (AMH) measurement of 3.0 ng/ml.
- Blood group: A positive.
- Urine culture: positive for *Escherichia coli*, treated with amoxicillin (875 mg) + clavulanic acid (125 mg). Post-treatment urine culture was confirmed negative.

Additional tests carried out on the male patient:

- Complete semen analysis: diagnosis of asthenozoospermia (total motility 10%).
- Karyotype: 46, XY (normal).
- Infectious serology: Positive for HCV antibodies. Undetectable viral load. All other serology tests negative.
- Blood group: A positive.

TREATMENTS AND EVOLUTION

After assessing the case, we decided to recommend IVF treatment with the patient's own eggs, adding the use of annexin columns with the intention of improving the fertilisation rate. We also recommended embryo culture up to the blastocyst stage. Controlled ovarian stimulation treatment for IVF was performed in March 2022 as follows:

- Pre-treatment: none.
- Short stimulation protocol with gonadotropin-releasing hormone (GnRH) antagonists.
- Gonadotropin therapy consisted of a combination of rFSH and rLH, initiated on day 2 of the cycle at a daily dose of 262.5 IU, and continued for a total duration of 9 days.
- Oocyte retrieval: 16 oocytes were obtained, of which 14 were in metaphase II stage (MII), 1 in metaphase I stage (MI) and 1 in germinal vesicle stage (GV).
- Oocyte quality (see Table I and Annex 1).
- Laboratory techniques used: intracytoplasmic sperm injection (ICSI) and application of annexin columns.
- Fertilisation Rate: 85.7% (12 fertilised out of 14 MII microinjected).

TABLE I. Final results of the qualitative evaluation of IVF cycles.

	Total oocyte score	Successful fertilisation	Fertilisation score	Reaches D+5/+6	PGT-A	Normally progressing pregnancy
Start of cycle	0.86	85.71%	58.30%	25%	ND	No
Cycle after pre-treatment	0.9	80%	75%	50%	1BT	Yes

- Embryonic development was carried out up to the blastocyst stage, achieving vitrification of 3 embryos on day +5 (see Table I and Annex 1).

All the embryos generated were transferred but pregnancy was not achieved. In this situation, we diagnosed the patient with implantation failure according to current criteria[1] and recommended additional studies in search of an aetiological diagnosis for this condition[2]:

- Endometrial receptivity test in a substituted cycle[3,4] (with 120 hours of progesterone): her endometrium was receptive.
- Endometrial microbiome assessment test: dominance of *Lactobacillus* (99.51%).
- Test for pathogens causing chronic endometritis: not detected. No intervention was required.
- Diagnostic hysteroscopy: no anomalies were observed.
- Thrombophilia test: negative.
- Basic test for autoimmune diseases: negative.
- In case of a new cycle, we recommended preimplantation genetic testing for aneuploidy (PGT-A).

Once the additional studies had been completed, the patients decided to undergo another IVF cycle with their own gametes in October 2022. Case history:

- Pre-treatment: vitamin complex based on inositols and probiotics (Ovosicare® ProFIV) for a period of 31 days prior to stimulation and for 3 months in total (including stimulation).
- Short stimulation protocol with GnRH antagonist as pituitary suppression.
- Gonadotropin therapy consisted of a combination of rFSH and rLH, initiated on day 2 of the cycle at a daily dose of 262.5 IU, and continued for a total duration of 8 days.
- Oocyte retrieval: 6 oocytes (5 MII and 1 GV) were retrieved.
- Oocyte quality (see Table I and Annex 1).
- Laboratory techniques used: ICSI and application of annexin columns.
- Fertilisation rate: 80% (4 fertilised out of 5 MII microinjected).
- Embryo development was carried out up to the blastocyst stage, achieving vitrification of 2 embryos on day +5 (see Table I and Annex 1).
- PGT-A analysis of the embryos obtained: one euploid embryo and one aneuploid embryo.

IVF cycle 1 start 28/03/2022														
Trigger ≥16	Oocytes retrieved	MII	Uniform ZP (yes/no)	Uniform cytoplasm (yes/no)	Polar body of adequate size (yes/no)	Presence of inclusions in perivitelline space (yes/no)	Vacuolisation (yes/no)	SER (yes/no)	Fertilisation	Cytoplasmic halo (yes/no)	Early division	Blastocyst	PGT-A result	Pregnancy
10	16	14												
		1	Yes	Yes	Yes	no	no	no	Yes	no	no	no		
		2	Yes	Yes	Yes	no	no	no	NF	no	no	no		
		3	no	Yes	Yes	no	no	no	Yes	Yes	no	no		
		4	Yes	Yes	Yes	Yes	no	no	Yes	Yes	no	no		
		5	Yes	Yes	Yes	Yes	no	no	Yes	Yes	no	no		
		6	Yes	Yes	Yes	no	no	no	Yes	Yes	no	no		
		7	Yes	Yes	Yes	Yes	no	no	Yes	Yes	no	D+5, BB		No
		8	Yes	Yes	no	Yes	no	no	NF	no	no	no		
		9	Yes	Yes	Yes	Yes	no	no	Yes	Yes	no	no		
		10	no	Yes	Yes	no	no	no	Yes	no	no	D+5, BB		No
		11	no	Yes	Yes	no	no	no	Yes	no	no	no		
		12	no	Yes	Yes	no	no	no	Yes	no	no	no		
		13	Yes	no	Yes	Yes	no	no	Yes	Yes	no	D+5, BB		No
		14	Yes	Yes	Yes	no	no	no	Yes	no	no	no		

IVF cycle 2+PGT-A start 01/11/2022														
Trigger ≥16	Oocytes retrieved	MII	Uniform ZP (yes/no)	Uniform cytoplasm (yes/no)	Polar body of adequate size (yes/no)	Presence of inclusions in perivitelline space (yes/no)	Vacuolisation (yes/no)	SER (yes/no)	Fertilisation	Cytoplasmic halo (yes/no)	Early division	Blastocyst	PGT-A result	Pregnancy
6	6	5												
		1	Yes	Yes	Yes	Yes	no	no	Yes	Yes	no	no		
		2	Yes	Yes	Yes	no	no	no	Yes	no	no	no		
		3	Yes	Yes	Yes	no	no	no	no	no	no	no		
		4	no	Yes	Yes	no	no	no	Yes	Yes	no	D+6 BB	Euploid	beta-hCG positive-ongoing pregnancy
		5	Yes	Yes	Yes	Yes	no	no	Yes	Yes	no	D+6 BB	Aneuploid	

ANNEX 1.

Qualitative assessment system for successive cycles based on ESHRE[5] criteria

a) Oocyte quality

The following prognostic parameters were assessed for each MII oocyte retrieved in the stimulation cycles: uniform *zona pellucida*, homogeneous cytoplasm and adequately sized polar body.

- Signs of poor prognosis: presence of cytoplasmic inclusions in the perivitelline space, presence of vacuoles and smooth endoplasmic reticulum.

Positive values scored "1" and negative values "0", giving a total score for each oocyte (see Annex 1). In this way, a value was obtained for each of the oocytes in the cycle and then the average of all the oocytes belonging to the same cycle was taken.

b) Fertilisation

The successful fertilisation (or the lack thereof) of each of the retrieved oocytes was assessed, and the percentage of successfully fertilised oocytes per cycle was calculated.

In parallel to the assessment of oocyte quality, the presence of a cytoplasmic halo and the possible existence of early division were taken into account when assessing successful fertilisation.

The scoring system was also similar, with a value of "1" for positive parameters and a value of "0" for negative parameters.

c) Embryo culture

To evaluate this part of the cycle, we took into account the embryos reaching blastocyst stage, considering as "n" the total number of successfully fertilised oocytes on D+1 of embryo culture.

Results of the qualitative assessment of successive cycles

a) Oocyte quality

In the IVF cycle without pre-treatment with inositols and probiotics, the mean oocyte score calculated according to the above-described system was 0.86. Calculating the same mean score in the IVF cycle in which pre-treatment with inositols and probiotics (Ovosicare® ProFIV) was performed, we observed that the calculated value was 0.9. These results suggest the possible beneficial effect of inositols and probiotics as a pre-cycle treatment, although this improvement is likely to be small.

b) Fertilisation

Regarding the cycle without pre-treatment with inositols and probiotics, we observed successful fertilisation in 12 of the 14 oocytes microinjected, which represents a percentage of 85.71%. In relation to the cycle with pre-treatment with inositols and probiotics, we identified successful fertilisation of 4 of the 5 microinjected oocytes (80% fertilisation rate). With regard to the score for establishing the qualitative assessment, we found that in the first treatment cycle, 58.3% of the oocytes had a good prognosis parameter, a percentage that increased to 75% after pre-treatment with inositols and probiotics (Ovosicare® ProFIV). Thus, we can infer that successful fertilisation is slightly reduced in the second treatment, while good prognostic parameters for embryo development and cycle progression are more frequent in oocytes from the cycle with pre-treatment with inositols and probiotics.

c) Embryo culture

At the end of embryo culture during the first IVF cycle, 3 blastocysts were obtained from 12 successfully fertilised oocytes on D+1, i.e. a rate of 25% of these oocytes reached this stage. In the second stimulation cycle, 2 embryos were obtained at the blastocyst stage from 4 successfully fertilised oocytes on D+1, with a rate of 50% reaching D+5/+6. In the comparative evaluation of this parameter, we observed a clear improvement in the rate of embryos generated reaching D+5/6 in the cycle with pre-treatment with inositols and probiotics (from 25 to 50%).

FINAL RESULT

In the first of the IVF cycles, 2 of the 3 embryos obtained were transferred, both in a modified natural cycle. Pregnancy was not achieved. In the second IVF cycle, we performed a euploid embryo transfer in a modified natural cycle, achieving a positive beta-hCG result 11 days after the transfer.

Subsequent ultrasound examinations confirmed that the pregnancy was progressing normally. Currently, the patient is in her 31st week of gestation, with ultrasound and analytical studies showing normal evolution of the gestation.

FINAL DIAGNOSES

1) Primary Mixed Factor Reproductive Dysfunction.
2) Previous failures of assisted reproductive techniques.
4) Implantation failure without identifiable aetiology.
5) Normally progressing pregnancy.

DISCUSSION AND DESCRIPTION OF THE SIGNIFICANCE OF THE CASE

It should be noted that the analysis we have presented with the intention of establishing the clinical value of taking inositol and probiotic preparations prior to an IVF cycle has clear methodological limitations.

On the one hand, with regard to follicular recruitment, which clearly decreased in the second treatment cycle, it is difficult to pinpoint the cause, as there are certain factors, such as environmental factors, a possible decrease in ovarian reserve due to the time lag between one cycle and the next, or even physiological inter-cyclical variability in terms of follicular recruitment, which may account for these differences. It is not possible to establish the relationship between using the above-mentioned preparations and this detrimental effect on the number of oocytes.

On the other hand, oocyte quality, fertilisation and, above all, reaching the blastocyst stage seem to present more favourable parameters with the use of the preparations under study, with the exception of a slightly lower rate of successful fertilisation, which could be related to low oocyte retrieval rather due to the impact of the product we used

It should also be noted that, although the morphological and clinical criteria for assessing oocyte and embryo quality are obtained from the prognostic parameters indicated by the Istanbul Consensus[5] (2011), the scoring system we have estab-

lished is arbitrary and its sole purpose is to provide a quantitative approach with which to assess oocyte and embryo quality parameters.

Finally, regarding the differences between cycles, it should be noted that in the second cycle, a preimplantation genetic study was performed on the entire embryo cohort, with an aneuploidy rate of 50%, so the transferred embryo had a higher expected success rate than the embryos generated in the first cycle for each transfer. Taking into account the expected aneuploidy rate by age,[6] there is a high probability that some of the embryos transferred in the first cycle were euploid, but this cannot be confirmed with the data available.

In conclusion, it should be highlighted that it is difficult to assess the efficacy of an inositol and probiotic-based compound in the face of two cycles in the same patient, as other factors may influence the response and the evolution of the treatment. Despite our favourable findings, further studies are needed to establish the suitability of these preparations in clinical practice.

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