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ASPECTS ON SOCIAL EGG FREEZING – CURRENT STATE IN BULGARIA

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Abstract. Problem statement: *Oocyte cryopreservation for non-medical reasons has been widely promoted among young women who tend to delay their reproduction. As the methodology has proved its safety and efficiency in the field of assisted reproduction technology and the embryo laboratory, the interest in oocyte cryopreservation has increased highly. However, do social freezers ever come back and use these oocytes, or they keep them as a safety boat with no real near-future plans for reproduction?* ***Methods:*** *The following study has been performed as a retrospective analysis of 296 women who had oocyte freezing procedure for non-medical (social) reasons, medical conditions (oncological treatment) and oocyte donation from January 2013 to June 2023 at Medical Complex Ob/Gyn "Dr Shterev" – Sofia, Bulgaria.* ***Results:*** *Throughout the observed period, 190 women with 221 procedures vitrified their oocytes for future use. Only 9.47% of them had medical conditions and 7.36% were hindered to use their reproductive gametes at the day of the oocyte retrieval (absence of spermatozoa, sickness). The average age of the women in this group was 35.59 ± 1.5 years and the mean number of cryopreserved oocytes was 5.63 ± 1.4 per women. Compared to social cryopreserves, and bound to the Bulgarian legislation, the 106 women who donated their oocytes were younger (28.86 ± 1.5 years ($p < 0.05$)) with 7.34 ± 1.7 ($p < 0.05$) cryopreserved oocytes per donor. As the clinic has well represented program for oocyte donation 82.1% of the donors were used. In result, there were 37 clinical pregnancies with 47 children born. In the same period, only 46 (24.2%) women who stored their own gametes in our cryobank came back and claimed them for assisted reproduction. Nine clinical pregnancies had been registered and 10 children were born. An intriguing fact we can point from the medical history of the clear social freezers (SF) is the record of previous procedure(s) for abortion on demand. It was reported for 12% of the women in this group.* ***Conclusion:*** *According to the results we observed as evident that patients need more clarity towards the procedures for oocyte cryopreservation and consequent fertility treatment. We should rise more awareness to the most preferable age for cryopreservation and number of oocytes to be stored.*

Key words: *social freezing, oocyte cryopreservation, postpone reproduction, assisted reproduction*

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INTRODUCTION

Oocyte cryopreservation (OC) has been inseparable branch of assisted reproduction technology (ART) ever since the first report for live birth after thawing and insemination of cryopreserved oocytes in 1986 was published [1]. Through the years, the methodology for oocyte freezing has been constantly improving and, in the present, it is considered a standard and safe procedure in the IVF lab. Different commercial media available for direct use facilitate the cryopreservation performance. Although well-developed and widely applied OC is still an expensive procedure – concerning both freezing and cryostorage period taxes. In contrast to patients who choose to preserve their fertility for medical reasons, where numerous countries provide government reimbursement, for social freezers (SF) such is not offered [2]. In Bulgaria there is no financial support for SF. The public Fund "Assisted Reproduction Center" (ARC), supports financially not only couples with reproductive issues, but also women under 34 years and diagnosed with cancer who wish to preserve oocytes prior therapy.

Oocyte freezing and storage is strongly indicated for certain patient groups (diagnosed with malignancy and need of consequent chemo or x-ray therapy; established high risk of premature ovarian failure; patients with hyperstimulation or others who tend to collect single oocytes in non-stimulated cycles for further treatment; gamete donation). It is recommended in cases where ethical and legal issues could arise and although for numerous reasons it is not to be considered straightforwardly as fertility insurance it gives a safe time window to postpone reproduction [3, 4, 5]. Despite its high-cost OC is highly effective and could benefit a wide range of patients if offered timely, cautiously, and submitted with adequate information.

The following study investigates all cases of OC performed at Medical Complex Ob/Gyn "Dr Shterev" ever since it was firstly performed, thoroughly listed and followed up from January 2013 up until June 2023.

MATERIALS AND METHODS

This research was performed as a retrospective analysis of 296 women who had oocyte freezing procedure for non-medical (social) reasons, medical conditions (oncological treatment) and oocyte donation. The women who went through oocyte freezing were 190, as 171 of them vitrified their oocytes for future use, and 20 for certain medical conditions or need of malignancy treatment. Through the observed period, from January 2013 to June 2023, the oocytes of 106 women included in the clinic donation program, were also frozen and stored. The study was conducted at Medical Complex Ob/Gyn "Dr Shterev" – Sofia, Bulgaria.

Two hours after the ovarian puncture the cumulus cells were removed and only oocytes at metaphase II (MII) were frozen via ultra-rapid vitrification. The oocytes were vitrified with the use of Kitazato vitrification media and the Cryotop® Method. For the thawing procedure Kitazato thaw media was preferred.

Comparison of age and number of cryopreserved oocytes in the different groups was conducted. Review of utilization rate and survival rate (SR) after oocyte thawing were also investigated. Embryo quality (EQ) at the day of embryo transfer (ET) was investigated as well as clinical pregnancy rate (CPR) and live birth rate (LBR).

All of the data needed for the study was extracted from the hospital computer software JOYSTICK healthcare management system (certified with ISO/IEC 27001:2017 and ISO 9001:2015 "Smart Software Systems").

The statistical significance of association was tested by Student's t-test for continuous data. $P < 0.05$ was considered to be statistically significant.

RESULTS

Throughout the years the number of women who decided to freeze gametes strongly varied, with a positive tendency with each subsequent year. The summarized distribution of patients who willingly cryopreserve own oocytes to indicated for medical condition freezing (MCF) and donor oocyte cryopreservation for each of the analyzed years is represented in Table 1.

Overall, 190 women with 221 procedures for egg retrieval in the investigated period froze their oocytes voluntarily as only a small portion consisted of women diagnosed with malignancy (9.47% of the patients). The average age of the women freezing own gametes (both SF and MCF) was 35.59 ± 1.5 years (36.3 ± 1.6 for MCF) and the mean number of cryopreserved oocytes was 5.63 ± 1.4 (4.42 ± 1.2 for MCF) per women.

It should be clearly stated that there are numerous differences between healthy women cryopreserving gametes and women diagnosed with malignancy. In relation to the small number of the latter, the low usage of gametes and for the purpose of the present study, the authors agreed to represent the following data conjoined along for both subgroups freezing own oocytes.

Table 1. Summarized data distribution of social freezers (SF) to patients indicated for medical condition freezing (MCF) and donor oocyte cryopreservation through the surveyed years

Year	SF	MCF	AVG № of frozen oocytes	AVG AGE	Donor program freezing	AVG № of frozen oocytes	AVG AGE
2013	4	1	3.6	33.8	1	11	26
2014	5	0	5.2	36.2	2	5.5	29
2015	6	0	7.66	37.83	4	6.25	30.25
2016	7	0	4.25	37.5	12	6.76	27.16
2013	15	1	7	33.93	3	4.3	29
2018	9	1	5.5	33.5	15	8.57	28.68
2019	14	1	5.06	34.46	18	8	28.38
2020	15	2	6.83	35.44	19	7.22	29.52
2021	21	8	4.22	35.64	9	7.9	28.4
2022	33	3	7.32	36.21	10	7.54	28.7
June 2023	42	3	5.33	33.88	10	7.8	31.9

Donor oocytes were regularly frozen for future use in donation cycles when excess number of gametes was collected or in cases when further synchronization with the recipient was needed. Compared to social cryopreserves, and bound to the Bulgarian legislation, the 106 women who donated their oocytes were younger (28.86 ± 1.5 years ($p < 0.05$)) with 7.34 ± 1.7 ($p < 0.05$) frozen oocytes per donor.

The data indicates higher number of retrieved MII oocytes for cryopreservation in donors compared to SF and MCF who froze own gametes. It is of no doubt that the age difference in the groups is in direct relation not only to the number of the collected oocytes, but their quality and overall clinical pregnancy rate (CPR) [6, 7].

Utilization rate of the cryopreserved gametes was investigated.

According to the National Statistical Institute (https://www.nsi.bg/) and Eurostat (https://ec.europa.eu/) the average age of the population in Europe for 2020 was 44 years. Data for Bulgaria shows increase in average age from 39.3 in 2011 to 42.2 in 2010 and as high as 44.8 in 2020. It is evident that with such tendency and in relation to postponed reproduction increasing number of women would be indicated to use donor oocytes.

Clinical registry data indicates that the utilization rate for donor oocytes is 82.07%. As already mentioned, the demand for donor oocytes is regularly rising, and for most of the procedures (75%) the donated oocytes were thawed at the same or the following year past freezing.

After generating pre-implantation embryos and performance of fresh or frozen embryo transfer (ET) there were 37 clinical pregnancies with 47 children born. In the same period, from January 2013 to June 2023, only 46 (24.21%) women who had stored gametes in our cryobank came back and claimed them for ART treatment. Only two of these 46 women were of the MCF women. As a result, 9 clinical pregnancies were registered and 10 children were born, all of them from social freezing patients. At the moment, in our clinic, the usage rate of frozen own oocytes is relatively low. The distribution of oocyte thaw in the time after freezing strongly depends on the initial intention of the patient to undergo such procedure. For those who collected oocytes in natural cycles or cycles with mild stimulation (16 women) the usage was done in the same or the following year after vitrification. For the other 30 women the thaw and fertilization were conducted in longer time window, as the maximal was after seven years of cryopreservation.

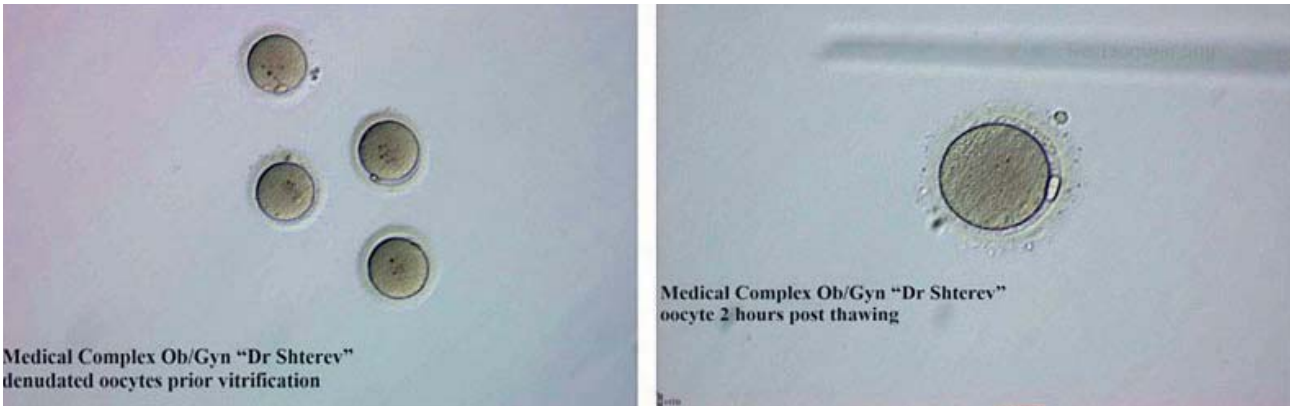
It should be considered that the storage periods in this procedure tend to be longer and the utilization is yet to be performed and calculated.

We further compared data in order to establish differences and similarities between patients who used their own frozen oocytes for fertility treatment and the results we obtain for the donor thaw gametes.

The average age of the donors that we thawed and fertilize oocytes from was 27.8 ± 1.3 years compared to 36.4 ± 1.9 for patients with own cryopreserved oocytes.

Cryosurvival rate (CSR) was followed up. In comparison to donor oocytes, where 86.04% of the vitrified oocytes survived and were consequently subject to intracytoplasmic sperm injection (ICSI), in women with own cryopreserved gametes 79.68% were vital after thawing (example of denudated oocytes prior vitrification and visualization of oocytes after thawing is represented in Picture 1).

For both groups day 3 of the embryo development was preferred day for ET. It was performed for 62.5% in SF and MCF compared to 65.75% in donor cycles (Figure 1).



Picture 1. Example of denudated oocytes prior vitrification and visualization of oocytes after thawing

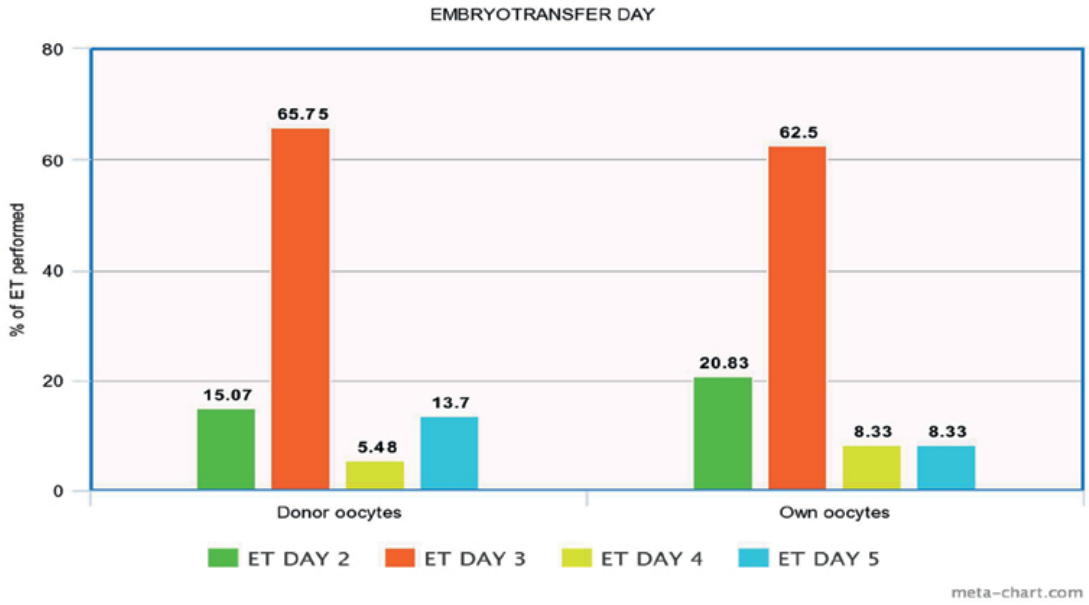


Fig. 1. Comparison of ET day distribution for pre-implantation embryos generated from thaw donor or own oocytes

We looked into embryo quality (EQ) development and particularly the quality of the embryos that were transferred. Embryos were classified as follows:

Top quality embryos

day 2-4 even blastomeres; no or less than 5% fragmentation

day 3-6 to10 even blastomeres; no or less than 5% fragmentation

day 4 – fully compact morulae (FCM); no or less than 5% fragmentation

day 5 – blastocyst stage, preferable BL 4AA; BL5 AA;

Good quality embryos

day 2-2 to 4 uneven blastomeres, 10-25% fragmentation

day 3-6 to10 uneven blastomeres; 10-25% fragmentation

day 4 – partially compacted morulae (PCM); 10-25% fragmentation

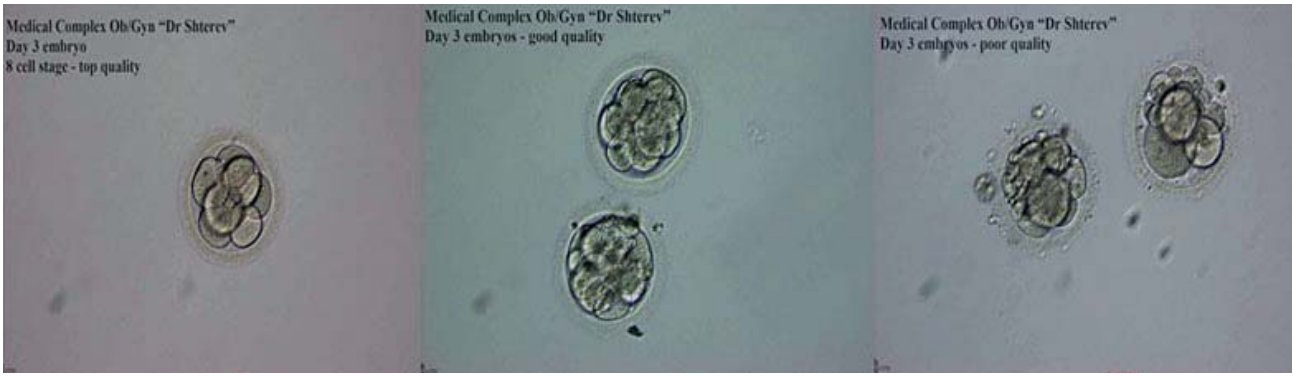
day 5 – early blastocyst stage

Poor embryo quality: embryos with slower cleavage timing for the chosen for ET day, fragmentation above 25%.

Examples of embryo quality are represented in Pictures 2 and 3.

Top quality embryos were chosen for ET preferably. When no such were available pre-implantation embryos of good quality were transferred. Poor quality embryos were exceptionally transferred. The procedure was performed after thorough consultation regarding chances to achieve pregnancy with the patients and their informed consent.

The distribution according to quality of the embryos prepared for embryo transfer is summarized in Table 2.



Picture 2. Difference of embryo quality on day 3

Picture 3. Difference of embryo quality on day 5

Table 2. Quality of the embryos prepared for ET

EQ	Donor oocytes	Own oocytes
Top quality	82.19%	54.17%
Good quality	13.7%	29.17%
Poor quality	4.11%	16.67%

At the preferred day of ET for 82.19% of the cases when donor oocytes were thawed, we had embryos of top quality to be transferred. For patients who had ET with own cryopreserved oocytes comparably lower percentage of the transfers – 54.17% were with embryos of top quality.

The average number of embryos to be transferred when own cryopreserved oocytes were utilized (SF and MCF) was 1.92 ± 0.7 compared to 2.39 ± 0.7 ($p < 0.05$) in donor oocyte cycles.

Prior ET an artificial hatching of the embryo via thinning of the zona pellucida (ZP) of the selected embryos was performed. Laser with 1.48 mm infrared diode, Zilos-tk® Hamilton Thorne was applied, and embryos were placed in medium containing hyaluronan – EmbryoGlue®.

In result 37 clinical pregnancies with the use of vitrified donated oocytes were followed up and 47 children were born. Thaw and fertilization of own cryopreserved oocytes resulted in 9 pregnancies and 10 born children, all born to clear social freezing.

In the process of assembling data for the study, a side note from the medical history record of the patients attracted our attention. For 12% of the women classified as clear social freezers record of previous procedure(s) for abortion on demand was evident.

In that context and connected to the low percentage of utilization of own stored oocytes we can speculate that for certain number of women parenting a child is not part of their near-future plan.

DISCUSSION

Gamete cryopreservation has been greatly developed and incorporated, and is inseparable branch of modern assisted reproduction worldwide.

Fertility preservation through gamete cryobanking is proved, reliable and accessible procedure that gives women opportunity to parent their own biological child. It is highly recommended for patients diagnosed with malignancy or any other medical condition or treatment with established adverse effect on fertility. Gamete cryopreservation offers remarkable advance to women who have decided to postpone their reproduction and conceive later in life.

Medical centers conducting fertility treatment usually provide fertility preservation counseling and have on site cryobanks. Network structures for gamete and tissue storage have also been developed. Some of the affirmed ones would be the Danish network (www.rigshospitalet.dk), FertiPROTECT® (www.fertiprotect.com), German-Austrian-Swiss centralized and decentralized network between the countries, Oncofertility® Concortium (www.oncofertility.northwestern.edu) for knowledge exchange in the field of fertility preservation [8, 9].

In Bulgaria, gamete cryopreservation for non-medical reasons is still not enough known and practiced among young women who have consciously decided to have children later in life. According to research it is evident that women have positive attitude, find a benefit and would consider fertility preservation if informed adequately [10, 11].

Since reproductive age worldwide is rising and the replacement fold of 2.1 live birth rates per women have declined [12] more awareness of fertility issues and women's age, as well as fertility preservation should be raised.

One of the main concerns to fertility preservation for women is the adequate timing to take advantage from this method. In the field of assisted reproduction, it is well defined that women's fertility declines with age, oocyte quantity decreases, and aneuploidy rates increase [13]. From the perspective of the accumulated knowledge and in order to increase the chance to conceive when postpone childbirth, it is recommended to freeze at least 10-15 oocytes. The number of the cryopreserved gametes should be individualized in women of advanced maternal age – 36 and above years [14, 15].

Another aspect to social freezing at this point is its high cost. Depending on the number of the retrieved MII oocytes for freezing, the sum for vitrification (comparable for most fertility centers in Bulgaria) would vary between 250 euros (for 3 oocytes) and around 700 euros (for more than 9 oocytes). Additionally, the patient would be charged for cryostorage periods. Depending on the requested time for preservation the cost would vary between 150 to 750 euros (6 or 9 months and 5 years respectively). The thawing of the eggs is also charged and would contribute to the overall price of the following IVF procedures when the patient decides to use the gametes.

The total financial burden of the procedure for social freezing rises some reasonable additional questions. Usually, the fertility specialists' community, strongly advises preserving "young" oocytes. For most young women (still studying or at the beginning of their career) such expenditure is beyond their means. Another point of view would be that social egg freezing is not imperative and urgent medical procedure that necessarily needs to be financially supported. But still, in respect to that matter, partial government reimbursement or preferential loan could be developed and available for younger women preserving fertility.

Another piece of the significant information concerning oocyte cryopreservation would be the destiny of the collected and stored gametes in case of patients' death. Unfortunately, in higher relevance to cancer patients, there's always such probability. According to the Bulgarian legislation and the regulations on assisted reproductive activities in our country, if the patient has passed away, his or her gametes could not be claimed, stored or used by their partner or any other relative. If embryos are stored, and there are informed consents and expressed written will while alive, the living partner could store and use the frozen embryos. Surrogacy in Bulgaria is strictly forbidden.

From the current study, observing ten-year period of the ART laboratory practice of our clinic, it is evident that fertility preservation is not well known among Bulgarian women. The average age of the women in the group where own oocytes were frozen was 35.59 ± 1.50 years and the mean number of cryopreserved oocytes was 5.63 ± 1.37 per women. Both age and number of preserved gametes are strongly unsatisfactory in order to secure reasonable success rates of 40-70% clinical birth rate (CLBR) [14].

The small number of women (24.21%), who had cryopreservation of own oocytes, and used them in consequent ART treatment, confirms other studies reporting low utilization rate of frozen gametes [16]. Although the recommended period for usage of the cryopreserved gametes would be up to five years after freezing, the patients are allowed and could store and use their gametes after longer periods, without any proven changes to their quality or potential to result in healthy pregnancy. The initial idea of the procedure concerning social freezing suggests longer storage periods. That is relevant especially for younger women in their 20s who tend to use their gametes in their 30s and even 40s. That would be one of the explanations behind the low usage rate of own cryopreserved oocytes and that longer periods to study and estimate the true value and utilization rate after vitrification are needed. Some studies indicate, that for some women, spontaneous pregnancy was reported, so they never claimed their gametes [17, 18].

Another issue concerning the use of frozen oocytes postmortem should also be explained. According to the Bulgarian legislation the ownership of the gametes – spermatozoa or oocytes is respectively assigned to the male or female patient respectively. Embryos are owned by both partners of a couple. Surrogacy is strictly forbidden.

In comparison, as high as 82.07% of the donors with frozen eggs were used for women who were in need of gamete beneficence. Since gamete cryopreservation in order to postpone childbirth has been well developed in the last decades, if high percentage of women have reached to the point to be in need of donor oocytes due to advanced maternal age and depleted ovarian reserve, that would only prove the lack of enough and easily accessible information on fertility preservation.

Freezing via vitrification has been proven to be safe and highly efficient method with CSR of 84% to 99% [19, 20]. Comparison of CSR for donor to own oocytes on thawing in our clinic shows adequate CSR of 86.04% per frozen donated eggs vs relatively lower CSR of 79.68% when own oocytes were thawed. The lower survival rate and the overall low oocyte retrieval of 5.63 ± 1.37 eggs per woman compared to the recommended 10-15 oocytes initially reduces the chances of parent biological child.

Embryo transfer day was chosen based on the number and the quality of the pre-implantation embryos, and with reference to previous medical history of the patients. If possible, ET at day 5 and blastocyst stage was preferred, with the transfer of a single best embryo. When only embryos of poor quality were available, the embryologist team provided thorough consultation and discussion with the couple, in order to take their informed decision and consent on whether to transfer the embryos [21, 22].

Analysis of EQ at ET day indicates notable difference in the percentage of embryos with good quality characteristics between the ones originated from donor compared to own oocytes (82.19% vs. 57.17%). Also the number of embryos approved for ET were significantly higher in the donor group 2.39 ± 0.67 compared to 1.92 ± 0.72 ($p < 0.05$) when own (SF and MCF) cryopreserved oocytes were utilized. These results are only coming to confirm, that the lower number of oocytes retrieved at the follicular puncture have negative correlation to embryo quality and an optimum of 6-15 retrieved oocytes should be perceived as objective [23].

Research indicates that cryoprotectant exposure is associated with major drop in endoplasmic reticulum Ca^{2+} store content [24]. The comparatively small number of retrieved oocytes and the overall AMA of the patients cryopreserving own oocytes could suggest incorporation of rich in Ca^{2+} medium after intracytoplasmic injection in order to improve the outcome results [25].

CONCLUSIONS

As different personal and social aspects of modern-day society slowly displace the time point in women's life to conceive and deliver children, the biological laws stay unaffected. Women's fertility potential, although favored by various lifestyle habits – physical activity, healthy eating, avoiding environmental pollutants and emotional stress, stays at its height in their 20s. Although information on the topic is easily accessible, research suggests that there is considerable amount of misunderstanding to the chances to get pregnant with advancing age [26]. From that perspective, and with sufficient and timely delivered information, the demand for oocyte freezing in the absence of a medical indication will rise exponentially. Unfortunately, most of the social freezing cases are conducted in section to good laboratory practice guidelines for freezing under 30 years of age and number of cryopreserved oocytes in the range of 10 to 15 [27]. In order to increase the results according to CPR and in correspondence to the patients set expectation individualized approach should be considered. There are two crucial problems related to oocyte competence and ovarian aging that need to be surmount by retrieving higher number of oocytes during an IVF treatment. For low prognosis patients, the POSEIDON (acronym for Patient-Oriented Strategies Encompassing Individualized Oocyte Number) concept defines certain approach in order to suggest better tools to increase IVF treatment success rates [28].

Fertility preservation is emerging as a complex and substantial matter in ART today. It generates the necessity for actions in order to raise the awareness in target patients that would benefit of gamete cryopreservation. In order to provide accurate and thorough counseling and treatment close interaction between the patients, reproductive specialists, reproductive biologists, oncologist and other specialists is strongly recommended.

Disclosure summary: *The author(s) declare no conflict of interest(s).*

Abbreviations

ARC – Assisted Reproduction Center
ART – assisted reproduction technology
CLBR – clinical birth rate
CPR – clinical pregnancy rate
ET – embryo transfer
EQ – embryo quality
FCM – fully compacted morulae
ICSI – intracytoplasmic sperm injection
LBR – live birth rate
MCF – medical condition freezing
OC – oocyte cryopreservation
PCM – partially compacted morulae
POSEIDON - acronym for Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number
SF – social freezers
SR – survival rate
ZP – zona pellucida

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