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Oocyte quality in women with thalassaemia major: insights from IVF cycles

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ABSTRACT

Background: Women with thalassaemia major typically experience hypogonadotropic hypogonadism because of the toxic effects of iron overload on the anterior pituitary. Moreover, in affected women, serum anti-Mullerian hormone (AMH) and antral follicle count (AFC) are also shown to be reduced, suggesting that the peripheral excess of iron could also harm the ovarian reserve. To date, the detrimental effects of the disease on oocyte quality have not been investigated.

Materials and methods: Women with thalassaemia major who underwent *in vitro* fertilization (IVF) cycles were retrospectively identified over a 9 years period. They were matched (with a 1:5 ratio) by study period and age to a control group of infertile women undergoing IVF. Embriological variables were compared between the two groups. The primary outcome was the rate of top quality embryos.

Results: Twenty-one women with thalassaemia major (exposed group) and 105 controls (unexposed group) were ultimately included. Serum AMH was 0.6 [0.2–1.8] and 1.5 [0.7–3.5] ng/ml, respectively ($p = 0.05$). AFC was 4 (1–7.5) and 11 (5.5–16), respectively ($p < 0.001$). The total dose of gonadotropins used was higher in exposed women but the number of retrieved oocytes and oocytes used did not differ. The fertilization rate was higher in exposed compared to unexposed women, being 100% (76–100%) and 75% (50–100%). respectively ($p = 0.03$). The cleavage rate was also higher, being 75% (39–100%) and 50% (29–64%), respectively ($p = 0.04$). In contrast, the rate of top quality embryos did not differ, being 20% (0–76%) and 25% (5–50%), respectively ($p = 0.98$).

Conclusions: Despite lower ovarian reserve, oocyte quality is not significantly affected in women with thalassaemia major.

Introduction

Thalassaemia is one of the most common monogenic disease worldwide and still poses a serious health problem [1]. From a molecular point of view, it is characterized by the partial or complete deficiency in the synthesis of α or β-globin chains that compose the major adult hemoglobin ($\alpha_2\beta_2$). As a consequence, impaired globin chains precipitate in the cell, causing premature destruction of both red blood cells precursors and mature red blood cells [2]. From a therapeutic point of view, when globin chains deficiency is complete (thalassaemia major, TM), patients require lifelong regular blood transfusions that result in iron overload-related complications such as liver insufficiency, cardiomyopathy and endocrine dysfunction [2].

Progress in the therapeutic approach, including the availability of oral iron chelators, has led to improved patient survival and quality of life [2]. Consequently, among female patients, parenthood has become possible and the desire for pregnancy has grown [3–6]. However, reproductive disorders are still common: iron overload produces a toxic effect on the anterior pituitary, leading to hypogonadotropic hypogonadism, with low gonadotropin secretion, amenorrhea and infertility [3]. In addition, affected women face pregnancy complications such as intrauterine growth restriction, low birth weight and prematurity [6].

Recent studies also revealed that patients with TM have lower levels of anti-Mullerian hormone (AMH) and reduced antral follicular count (AFC), suggesting that the peripheral excess of iron may also be toxic locally to the gonads, harming the ovarian reserve [4,7–11]. In addition, iron excess, in particular

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non-transferrin-bound iron and its redox active form, may actually impact on oocyte quality. Increased levels of redox activity in follicular fluid of women with the disease and an inverse correlation between serum AMH and non-transferrin-bound iron levels have been reported [12]. To note, free iron can generate reactive oxygen species (ROS) through the Fenton reaction, causing oxidative stress, a condition that was shown to negatively affect oocyte developmental competence [10]. However, this potential detrimental effect remains speculative since direct evidence on oocyte quality in women with TM is lacking.

Disentangling whether oocyte quality is hampered in women with TM is a relevant clinical issue. Ovulation induction with exogenous gonadotropins can indeed compensate for the central endocrine disruption but cannot overcome the detrimental effects on the quality of the oocytes. In fact, if a specific and time-related injury to oocyte quality is demonstrated, one may claim oocyte preservation at a young age in affected women. In order to shed light on this topic, we retrospectively collected data on oocyte competence from women with TM who underwent *in vitro* fertilization (IVF) cycles and compared them to a control group of age-matched infertile women.

Materials and methods

Women with TM who were referred to the Infertility Unit of the Fondazione Ca' Granda, Ospedale Maggiore Policlinico of Milan, Italy between January 2009 and December 2017 were retrospectively identified (exposed group). We included women aged 18–42 years who underwent at least one IVF or intracytoplasmatic sperm injection (ICSI) cycle. Women exclusively performing ovarian hyperstimulation were excluded. Controls (unexposed group) were infertile women undergoing IVF-ICSI matched to cases in a 5:1 ratio by age (±6 months) and study period (the following five women fulfilling the criteria for selection and matching). Additional exclusion criteria for both groups were as follows: 1) previous IVF-ICSI cycles, 2) previous ovarian surgery, 3) severe male factor of infertility requiring surgical sperm extraction. Women with abnormal uterine cavity (submucosal fibroids, endometrial polyps, uterine septum), hydrosalpinx and dysthyroidism could be included after surgical or medical correction. Both exposed and unexposed women were included only for the first treatment cycle reaching oocyte retrieval. In our Unit women with FSH $\geq$ 30 IU/L were excluded. The study was approved by the local institutional review board. An informed consent was not required since this is a retrospective study. However, all women referring to our unit signed an informed consent for their data to be used for scientific purposes.

Women with TM referred to our unit for infertility were managed according to a standardized protocol. Firstly, they underwent a pre-pregnancy clinical assessment as reported in details elsewhere [6]. Then, they underwent a diagnostic fertility work-up including transvaginal ultrasound, serum hormonal evaluation (AMH, TSH, prolactin, FSH, LH and estradiol) and tubal patency assessment. The male partner provided two semen samples at least two months apart for fertility evaluation according to WHO criteria [13,14]. Women with an unremarkable work-up were advised to undergo ovulation induction. Otherwise, they were advised to IVF or ICSI. IVF was also considered after six failed cycles of ovulation induction. Given the long-lasting overwhelming therapeutic burden suffered by affected women, an individualized and patient-centred approach was applied and, if requested by the patient, the number of ovulation induction cycles prior to shift to IVF could be reduced. All women were managed by a single physician (R.B.).

Ovarian hyper-stimulation was performed as reported in details elsewhere [15,16]. Briefly, three different protocols could be chosen for unexposed women, *i.e.* the long protocol, the protocol with Gonadotropin Releasing Hormone (GnRH) antagonists and the flare-up protocols. On the contrary, women with TM were not treated with GnRH analogues, giving the documented hypogonadotropic state. Dosages of FSH administered varied between 100 and 450 IU daily and could be modified during treatment. Women with TM systematically received also LH in a 1:2 ratio compared to FSH. Ovarian hyper-stimulation cycles monitoring included serial transvaginal ultrasounds and peripheral assessments of oestrogens and progesterone concentrations. Ovulation trigger was performed when $\geq$3 follicles with a mean diameter $\geq$17 mm were observed. Oocyte retrieval was performed 36 h later. ICSI and IVF were performed in a standard way [15]. Embryos were systematically evaluated at day 2 or 3 and classified according to standardized embryological criteria [17]. Information on embryo quality at cleavage stage was thus always available. Embryo transfer could be done at cleavage stage or blastocyst stage. Supernumerary embryos or, in some cases (retrieval of more than 15 oocytes or progesterone >1.5 ng/ml on the day of ovulation trigger), all available embryos were frozen and transferred in a subsequent cycle.

We chose the rate of top quality embryos as the primary outcome. Secondary outcomes were number of follicles $\geq$11 mm at the end of the stimulation, number of oocytes retrieved, number of suitable oocytes, number and quality of the embryos, fertilization rate, cleavage rate, clinical pregnancy (defined as the US detection of an intrauterine gestational sac and a viable embryo) and live birth rate. Data was analyzed using the SPSS software 18.0 (Chicago, IL). Fisher Exact test, Student *t*-test, unpaired Wilcoxon non parametric test and Spearman correlation were used as appropriate. P values $\leq$ 0.05 were considered statistically significant. The power of the study was calculated setting type I error at 0.05 and expecting the presence of at least one top quality embryo in 80% of cases in unexposed women. The number of women with TM identified in our study (n = 21) consented us to detect as statistically different a drop in the rate of top quality embryos below 50% with a statistical power of 80%.

Results

Thirty-five women with TM referred to our Unit for infertility during the study period. Fourteen (40%) received only ovarian stimulation, of whom six (43%) achieved a live birth. The remaining twenty-one (60%) underwent at least one IVF cycle and were included in the study. All were diagnosed with β-TM, except one case of β-thalassaemia intermedia. This latter patient was included because, from a clinical point of view, she was managed as a TM case. Twelve of them (57%) shifted to IVF after failure of ovarian stimulation cycles, while nine (43%) received IVF straight (in five cases no other concomitant factors of infertility were present).

Serum ferritin of the 21 included women was 1032 ± 828 ng/dl and the duration of transfusional therapy was 33.5 ± 4.1 years. Eighteen women (86%) had concomitant HCV infection and two (10%) had type I diabetes. Primary and secondary amenorrhea was diagnosed in 17 (81%) and 4 (19%) women, respectively.

These 21 exposed women were matched to 105 unexposed controls. Baseline clinical characteristics of the two study groups are shown in Table 1. Affected women had lower serum AMH and AFC. In four of them (19%), a concomitant male cause of infertility was diagnosed.

In women with TM, the total dose of FSH and LH used was 4400 ± 1573 and 2200 ± 786 IU, respectively. Controls were scheduled to a long protocol, a protocol with GnRH-antagonist and a flare-up protocol in 27 (26%), 58 (55%) and 20 (19%) subjects, respectively. The total dose of gonadotropins used was 2428 ± 1225 IU ($p < 0.001$ compared to exposed women). Duration

Table 1
Baseline clinical characteristics of the study groups.

Characteristics	Thalassemia n = 21	Controls n = 105	p
Age (years)	35.1 ± 2.7	35.1 ± 2.6	0.99
BMI (kg/m^2)	23.1 ± 3.5	22.1 ± 4.7	0.36
Previous deliveries	2 (10%)	5 (5%)	0.33
AMH (ng/ml)	0.6 [0.2–1.8]	1.5 [0.7–3.5]	0.05
AFC	4 [1–7.5]	11 [5.5–16]	<0.001
Indication to IVF			<0.001
Unexplained	0 (0%)	32 (31%)	
Endometriosis	0 (0%)	22 (21%)	
Tubal factor	0 (0%)	12 (11%)	
Anovulation	17 (81%)	3 (3%)	
Male factor	0 (0%)	21 (20%)	
Mixed	4 (19%)	15 (14%)	

AFC: Antral Follicle Count.
Data are repoorted as mean ± SD or medain [interquartile range) or number (percentage).

of the stimulation in exposed and unexposed women was 12.3 ± 2.1 and 9.4 ± 2.3, respectively ($p < 0.001$). The other main characteristics of the IVF cycles are presented in Table 2. No statistically significant differences emerged.

Data on the quality of oocytes are presented in Table 3. Fertilization rate and cleavage rate were higher in women with TM. In contrast, the rate of top quality embryos did not differ. Finally, we correlated serum ferritin and the duration of transfusional therapy with oocyte quality outcomes. The correlation indexes between ferritin and fertilization rate, cleavage rate and the rate of top quality embryos was +0.15 ($p = 0.53$), +0.15 ($p = 0.54$) and −0.12 ($p = 0.61$), respectively. For the duration of transfusional therapy, they were +0.18 ($p = 0.44$), +0.12 ($p = 0.69$) and +0.03 ($p = 0.86$), respectively.

Table 2
Characteristics of the IVF cycle.

Characteristics	Thalassemia n = 21	Unaffected n = 105	p
Total number of follicles ≥ 11 mm	9.5 ± 7.2	11.0 ± 7.2	0.39
N. of oocytes retrieved	6.0 ± 6.2	8.4 ± 6.5	0.12
N. suitable oocytes[a]	5.5 ± 6.0	6.1 ± 4.7	0.60
N. of women with no suitable oocytes	1 (5%)	3 (3%)	0.52
Technique[b]			0.79
Classical IVF	5 (25%)	31 (30%)	
ICSI	15 (75%)	71 (70%)	
N. cleavage embryos[b]	2 [1–5]	3 [1–5]	0.51
No viable cleavage embryos[b]	1 (5%)	5 (5%)	1.00
N. top quality embryos[b]	1 [0–4.75]	2 [1–4]	0.72
No top quality embryos[b]	12 (60%)	78 (77%)	0.16
Embryo transfer[c]			0.55
Cleavage stage	14 (74%)	76 (80%)	
Blastocyst stage	5 (26%)	19 (20%)	
Total N. of transfers[c]			0.83
1	13 (68%)	71 (75%)	
2	4 (21%)	15 (16%)	
≥3	2 (11%)	9 (9%)	
Clinical pregnancy[d]	7 (33%)	37 (35%)	1.00
Live birth[d]	4 (19%)	34 (32%)	0.30

Data are repoorted as mean ± SD or medain [interquartile range] or number (percentage).
"Suitable oocytes" include metaphase II oocytes and type 1 cumulus-oocyte complex according to the European Society for Human Reproduction and Embryology Istanbul Consensus Conference, 2011.
Top quality embryo was defined as 4-cells embryo on day 2 or 8-cells embryo on day 3, with a relative degree of fragmentation 10%.
All clinical pregnancies were singletons.
[a] Suitable oocytes refer to metaphase II oocytes and type 1 cumulus-oocyte complex according to the European Society for Human Reproduction and Embryology Istanbul Consensus Conference, 2011.
[b] Data refer to subjects retrieving at least one suitable oocyte (n = 20 and 102 for exposed and unexposed women, respectively).
[c] Data refer to patients performing embryo transfer (95 controls and 19 cases).
[d] Data include both pregnancies obtained with fresh and frozen embryo transfers.

Table 3
Oocytes quality in exposed and unexposed women.

Outcome	Thalassemia n = 20	Unaffected n = 102	p
Fertilization rate	100% (76–100%)	75% (50–100%)	0.03
Cleavage rate	75% (39–100%)	50% (29–64%)	0.04
Rate of top quality embryos	20% (0–76%)	25% (5–50%)	0.98

Discussion

In this study, we did not observe an impairment of oocyte quality in women with TM undergoing IVF. The rate of top quality embryos (our primary outcome) was actually not altered in affected women. Evidence emerging from the secondary outcomes also supports our conclusion since none of the analyzed embryological variables was significantly impaired. Moreover, when we investigated the potential time-related injury of iron overload on oocyte function, we found no correlation between serum ferritin or duration of transfusional therapy and oocyte quality outcomes. The absence of any gradient effect actually confirms the lack of harmful effects of the disease on oocyte quality. To our knowledge, this is the first study reporting on the quality of the oocytes of women with TM.

A surprising result of our study is the observation of a paradoxical improved fertilization rate and cleavage rate in women with TM. This unexpected observation is difficult to explain and a type I error is the most plausible explanation. However, we cannot exclude a different and counter-intuitive explanation, *i.e.* a biological reaction to a somehow partly unfavourable environment. Of interest here is that exposure of oocytes to a sublethal stress was shown to be paradoxically beneficial in some situations, at least in the earlier phases of embryo development in animal models [18,19]. To note, we observed a similar effect in human oocytes accidentally exposed to the content of ovarian endometriomas [20], rich in free iron content [21]. However, it has to be recognized that this intriguing interpretation is highly speculative and more evidence is needed.

Previous studies on ovarian function and TM mainly focused on ovarian reserve and generally supported an impairment: AMH and AFC are consistently lower [4,7–11]. Our data is in agreement with these findings since both biomarkers resulted reduced in exposed women. In this regard, the observation that in our study the number of oocytes retrieved was similar in exposed and unexposed women is not in disagreement and cannot be used to question the detrimental effects on ovarian reserve since the total dose of gonadotropins administered in the two groups radically differed. To note, an histologic study in female thalassaemic adolescents documented reduced number of primordial follicles [22].

Overall, one may conclude from our findings and from the available literature that women with TM could be exposed to an accelerated depletion of the ovarian reserve but not to a significant insult to oocyte quality. On one hand, this information is reassuring since oocyte quality is more important than the residual ovarian reserve on the chances of natural pregnancy [23,24]. On the other hand, one might speculate that a complete exhaustion of the ovarian reserve may occur prematurely, thus potentially reducing the duration of the reproductive period and causing childlessness in some women. Unfortunately, data on the rate of premature ovarian insufficiency (POI) in women with TM is lacking in the literature and this possible concern remains speculative.

To note, only a minority of affected women in our cohort underwent a complete program of ovulation induction without IVF: this low adherence to the first line approach may be explained by the long-lasting overwhelming therapeutic burden suffered by these women and their comprehensible preference for more effective treatments (even if more invasive). In our opinion, this clinical observation deserves attention within the debate on the possible role of fertility preservation in affected women. If the majority of women with TM will ultimately undergo IVF, collecting and storing oocytes at young age may actually increase the chances of pregnancy. This consideration remains, however, theoretical. Confirmation of a high rate of IVF use from other independent units and in-depth cost-effectiveness analyses are warranted. To note, previous experiences in different historical periods reported lower rate of IVF use [25]. Finally, it is worth noting that the clinical scenario of TM-related reproduction impairment may change in the next future. New intensive and more effective chelation therapies may reverse some endocrine complications as a result of reduction in total body iron overload: a case of reversal of hypogonadotropic hypogonadism with spontaneous pregnancy has actually been reported in the literature [26].

Some limitations of our study should be acknowledged. Firstly, the study is retrospective and includes a relatively small number of participants. The former limitation is presumably of scant relevance considering that we focused on validated and common outcomes that are routinely recorded. A prospective recruitment would take several years and cannot be expected to markedly improve the quality of the information. Conversely, the small sample size is a significant limitation (albeit inevitable given the rarity of the condition). Of particular relevance here is the unreliability of the data of pregnancy rate, the most valid mean to measure oocyte quality: our study was underpowered for meaningful conclusions on this outcome. Secondly, affected women with POI could not be included, thus exposing our findings to a possible selection bias. In this regard, however, it has to be reminded that our aim was to provide evidence on the quality of oocytes, not on the amount of ovarian reserve. Thirdly, one may also argue that the inclusion of some women with endometriosis in the unexposed group could have diluted the differences because of the possible detrimental effect of iron also in this condition [21,27]. To rule out the effect of this possible confounder, all the analyses were repeated excluding women with endometriosis (n = 22) but results were mainly similar (data not shown).

In conclusion, oocyte quality does not seem to be affected in women with TM. However, more comprehensive evidence and additional contributions from independent groups are needed for more robust conclusions on the possible role of fertility preservation in women with TM.

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Conflict of interests

None.

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