Trying for a second chance: Iranian infertile couples' experiences after failed ART

Samira Ebrahimzadeh Zagami

Mashhad University of Medical Sciences

Robab Latifnejad Roudsari (✉ latifnejadr@mums.ac.ir)

Mashhad University of Medical Sciences

Roksana Janghorban

Shiraz University of Medical Sciences

Helen T Allan

Middlesex university

Research

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Abstract

Background: Despite significant advances, only 35% infertile couples conceive after ART. If IVF is unsuccessful, couples will need to decide whether to proceed again with assisted conception. The aim of this study was to explore Iranian infertile couples' experiences after failed ART to continue treatment.

Methods: In this qualitative study participants were selected using purposeful sampling method. Data were collected using 36 semi-structured face-to-face in-depth interviews at a regional Infertility Center in Iran from April 2016 to June 2017. All interviews were recorded, transcribed verbatim, and analyzed with conventional content analysis method using MAXQDA software.

Results: Our findings suggest that couples' decisions to continue treatment after unsuccessful ART is shaped by their social, emotional and financial circumstances. We have constructed two themes to describe their experiences: support to continue and trying for a second chance.

Conclusions: Infertile couples rely on support from different sources in their lives to decide to have a further cycle after an unsuccessful ART. Our findings suggest that good marital and family support networks can support infertile couples during this period of decision making. Many of the couples found the financial burden of another cycle challenging. Considering the depression and anxiety caused by failed ARTs, which itself could affect the success rate of any further ARTs, the clinical team should effectively assess psychological readiness of couples who decide to continue with another ART after unsuccessful treatment.

Plain English Summary

Only one-third infertile couples conceive after infertility treatment. After failure treatment, infertile couples will need to decide whether to proceed again with assisted conception. We studied Iranian infertile couples' experiences after failed assisted conception to continue treatment. We interviewed with 36 people at a regional Infertility. Our findings suggest that couples' decisions to continue treatment after unsuccessful treatment is shaped by their social, emotional and financial circumstances. We have constructed two themes to describe their experiences: support to continue and trying for a second chance. So, infertile couples rely on support from different sources in their lives to decide to have a further cycle after an unsuccessful assisted conception. Our findings suggest that good marital and family support networks can support infertile couples during this period of decision making. Many of the couples found the financial burden of another cycle challenging.

Introduction

Existing data suggests that at least 50 million couples experience infertility worldwide (1). One in every four couples in developing countries are affected by infertility (2). The prevalence of primary infertility throughout a couple's lifetime is estimated to be between 17.3% (3), 20.2% (4), and 21.1% (5) in Iranian couples, which is higher than international prevalence (3, 4). Infertility is experienced as a life crisis in

many cultures globally (6–11). And in Iran, as a Muslim country, fertility and procreation especially for women are notably valued (12). Indeed, infertility may lead to separation, polygamy, and eventually divorce in societies where the purpose of marriage is childbearing, because children are very important in these societies. They are the ones who preserve the family name and lead to the continuity of generations (13). Rapheal Leff calls infertility a generative loss and this is supposed to be cross culturally felt in infertile couples(8).

A range of treatment options are available for infertile couples depending on their age and diagnosis(14), ability to self-fund(15) and access to specialist fertility clinics(16). Recent advances diagnostics and in assisted reproductive technologies raise hopes among infertile couples and at the same time present challenges (17). A diagnosis of infertility and ARTs also imposes higher levels of stress on infertile couples, as they manage any physical conditions, medical interventions and their psychological responses to medical procedures (18). Despite significant advances in ARTs, only 35% of couples attain live birth after each cycle(19). However, after 12 treatment cycles, the cumulative live-birth rate is expected to reach 85% in the long run(20). It is therefore unsurprising that Daibes et al. (2018) showed that continued ART treatment was a common response among women to failed ART cycle (21).

However, continuing treatment cycles is not without risks. If the ART cycle is not successful, couples will face new decisions such as tolerance or intolerance of the second or third in vitro fertilization (IVF) cycle. Infertility and its treatment is a stressful process, and many women undergoing such treatment experience extreme emotional stress (22) Men feel embarrassed and angry(23) and they show negative psychological response after unsuccessful treatment (24). Financial, emotional, and physical considerations are taken into account in these decisions (15). Even couples who have a good prognosis and high motivation discontinue treatment in up to 60% of cases due to the psychological burden of ART, which may be due to fear of failure and lack of awareness (25). Tabong & Adongo (2013) found that infertile couples have different experiences after unsuccessful treatment in different family settings. Some couples receive support from their families, whereas some blame their families for their distress (26). A literature review showed that few studies have focused on the experience of infertile couples following unsuccessful ART treatment(27). Those studies which have focused on unsuccessful treatment, have studied the rate and causes of treatment discontinuation as well as the factors associated with unsuccessful IVF such as the cause of infertility, the number of embryos transferred, and the number of oocytes retrieved using quantitative methodologies (27–29). Other studies have measured the effect of psychological interventions to facilitate coping of couples during ART treatment cycles (18, 30–32). However, there have been no studies on the decision-making of infertile couples about retreatment following unsuccessful AR treatment cycles.

Method

This study was a qualitative interview study using content analysis. Content analysis is a standard approach to health, treatment, and social sciences, which uses a set of methods to produce valid results from textual data (33). In this approach, categories are extracted directly from textual data, and the

researcher achieves a deeper understanding of a phenomenon (34). The reason for using the content analysis approach in this study was to achieve the discovery of the meanings, priorities, and decision-making of infertile couples about retreatment after unsuccessful ART treatment.

The present qualitative study, which was conducted from April 2016 to June 2017 at a regional Infertility Center in Iran, was part of a grounded theory study exploring infertile couples' experiences of unsuccessful ART. The setting of the study is affiliated to the Mashhad University of Medical Sciences, Mashhad, Iran and is considered as the referral center in eastern part of the country, which admits patients belonging to different social classes and with various causes of infertility. There were 36 participants including nine couples, nine women, two men, two companions (sisters-in-law) and five medical staff. Maximum diversity in terms of demographics such as age, level of education, place of residence, cause and duration of infertility, and the number of ARTs was considered in selecting infertile couples. Inclusion criteria included Iranian infertile couples with primary infertility that had a history of unsuccessful ARTs treatment with various causes of infertility and at least one failed cycle. Exclusion criterion was unwillingness to participate in the study, secondary infertility, having adopted offspring, history of positive pregnancy test and psychological disorders in infertile couples. Purposeful and snowball sampling were both used to select participants.

Data were collected using semi-structured interviews. The present study was approved by the Regional Ethics Committee of the University with the number of IR.MUMS.REC.1395.120 and permitted to be carried out in the infertility center. Prior to interviews, explanations were given to the subjects about the research objectives, the interviewing procedure, the confidentiality of the information, and the right to participate or to withdraw from the study. Moreover, if they agreed to the interview, a written informed consent was obtained from them. Interviews were conducted in person and recorded after attaining their permission and were implemented at the earliest opportunity. Interviews began with general questions "How did you feel when you realized that your treatment was unsuccessful? What reaction did you show?" and continued with questions such as "What is most helpful or annoying to you in the current situation?" and "Do you have any plans for the future? What choices do you have?" Each interview lasted between 35 and 90 minutes. All interviews were transcribed verbatim. Transcribed text was read several times line by line by the first author so that a general understanding of the content was obtained. The text was then divided and coded into condensed meaning units. The codes were compared, arranged, and then placed in more abstract subcategories and categories by the first two authors. Each interview was conducted after coding the previous interview to be aware of the gaps in the data. The data analysis was carried out using conventional content analysis method in MAXQDA 2010 software. Guba & Lincoln's criteria were used for trustworthiness of the data (33). To this end, a number of coded texts were given to the participants to verify the researchers' perceptions. All the study process was prepared in a thorough, accurate, and written manner and the text of the interviews, codes, and categories emerged was reviewed by two experts in the qualitative research, who were also members of the research team.

Findings:

In this study, the age range of women and men was 21–46 and 30–46, years, respectively. A total of 29 interviews were conducted with infertile patients. The infertile patients' level of education ranged from reading and writing literacy to a Masters degree. The duration of couples' marriages was between 2–21 years and the duration of the treatment varied from 10 months to 18 years. The causes of infertility were as follows: male and female infertility (n = 7 cases), female infertility (n = 7 cases), male infertility (n = 7 cases), and unknown causes (n = 8 cases).

There were two main themes identified in the data analysis: support to continue and trying for a second chance (Table 1).

Support To Continue

Support from a number of sources was important as the failed ART cycle was a significant set-back for the couples. Participants stated that they continue treatment because not to blame themselves in the future:

"Just now, I mainly go for the treatment so that not to blame myself in the future and say if I went there, I got pregnant" (Interview 5, female, 12, female and male infertility).

To manage continuing with treatment, their support came from their spouse, their families and their peers.

Marital support

Some women said their husbands gave them hope and said that they would eventually have a successful treatment. A participant stated:

"He (my husband) says you will bear a child, eventually" (Interview 1, female, 18 months, female and male factor).

"Now, you do it (continuing treatment) firstly. If God wants, you will become pregnant" (Interview 5, female, 12 years, female and male factor).

Some participants also stated that their husbands consoled them after every unsuccessful treatment and encouraged them for retreatment.

"When my husband returned home from his office, we ate lunch and then I told him: "You know what happened dear?" Yes, The result of my pregnancy test was negative) and he said:

" No problem, it's not important, now, you can try it once or twice, nothing happened" Interview 2, female, 10 months, female and male factor).

Another participant stated that her husband approached her with empathy and pointed out to the God will and wanted her to continue her treatment:

"My husband reassured me and said that there is certainly a God will that the treatment was unsuccessful. We would try it again, don't be upset" (Interview 7, Female, 5 years old, female factor).

Some participants also said that their husbands gave them the option to continue or discontinue their treatment.

"Whatever you want to do, we will go and do it, but I understand that he wants me to go and do it twice" (Interview 3, female, 16 years, female and male factor).

In one interview, an infertile woman said that she has possibly felt pressured into continuing the treatment for the sake of her husband:

"But I'm always busy, if I don't go, this poor man would suffer from the trouble and hardship of the matter" (Interview 3, female, 16 years old, female and for men infertility factor).

"I would like her to go to X center again and continue the treatment and pursue her treatment process. Maybe if she goes there, she will get good results one or two years later. Even if it lasts for a long time, it is no problem if she does not hurt." (Interview 15, male, 3.5 years, female factor).

Some male participants said that after a failed treatment, they do not need a counselor and their wives helped them for continuing treatment. *"I say that I don't need a counselor because my wife helps me a lot"*. (Interview 29, male, 8 years, unknown).

Family and peers' encouragement:

Some participants said their families expressed their agreement over the decision for continuing treatment and provided psychological support for them:

"My family hundred percent agree with me to start treatment again." (Interview 5, female, 12 years, female and male factor).

"They also introduced me gynecologists... saying that new methods are introduced every day." Let's go to the gynecologist" (Interview 10, female, 4.5 years, and a male factor).

A participant also considered having a friend as a factor affecting the continued treatment:

"My friend is there (at the treatment center). I'm very comfortable. It's really one of the reasons I can repeat it so much without being bothered because she is also there and have got my back" (Interview 1, female, 18 months, female and male factor).

Another participant, who was the sister in law of one of the infertile women, stated that their psychological support and hope has a significant effect for the retreatment.

"Now, once, twice, three times, ten times, for example, we repeat that it's not a problem, it is not important.... However, our encouragement made her to continue treatment; otherwise she eventually ended it after the first attempt" (Sister-in-law, 32 years old).

In this study, the majority of participants stated that they will continue the treatment until the treatment is effective and they bear a child. As one of the participants, as an answer to the question of how long you will continue the treatment, said, *"I will continue the treatment until the postmenopausal period."* However, the presences of persuading factors at the onset of retreatment were also very important.

Another participant said:

"My sister-in-law (wife of the brother of my husband) was like me, and they did not bear a child. She used injections, her egg count was good and fertilization happened, she finally had the triplet, and since the triplet were born, I and my husband were more eager to go to the doctor, this treatment center or the other one" (Interview 3, female, 16 years, female and male factor).

Observing successful treatment among peers was a factor which raised hope for couples who were considering another attempt at AR:

"When I saw one of the women became pregnant in the infertility center, I became very happy and more hopeful" (Interview 14, female, 3.5 years, female infertility factor).

Trying For A Second Chance

The second theme was 'trying for a second chance' included sub-themes of saving up to continue treatment, finding another clinic and losing trust in AR.

Saving up to continue treatment

After unsuccessful treatment, most of participants had financial problems and had to save up to pay for the second treatment cycle; they described saving, borrowing, taking out loans, or selling valuable items.

"We borrowed so much. I sold my own gold so that we could do it" (Interview 10, female, 4.5 years, male factor).

A participant who was a member of the treatment staff said:

"The majority of them are saving at least for 5 years old so as to undergo IVF. We have so many such cases or, for example, he sold his car to undergo IVF, which, for example, was the means of her husband's work. .. He has sold his cows so that they get an IVF ... or many of them will get a loan, for example" (Interview 34, 40 years old).

Another participant said she was 'ready to sell everything':

"If I'm sure I'll get good result, I'm ready to sell everything to do it" (Interview 15, male, 3.5 years, female factor).

The decision to continue treatment was not only shaped by whether the couples enough money to pay for further cycles. It was also affected by their trust in the treatment center where they had had a failed treatment cycle.

Amidst the financial costs of another cycle, couples also described losing trust in ART and treatment cycles.

Losing trust in ARTs

Some of the participants, after unsuccessful treatment with ARTs, were thought about using herbal treatments, acupuncture, and even non-scientific tasks such as fortunetelling. One of the participants stated that even a gynecologist had introduced her herbal medicines after her repeated unsuccessful treatments with ARTs:

"While we were talking to the female doctor. A., she told me a few days ago to use herbal medicine, and forget about these chemicals for a while and go to the phase of herbal medicine; for example," she referred me to Dr. T." (Interview 2, female, 10 months, female and male factor).

Another participant stated that while interacting with her peers, she saw the success of the use of acupuncture in the treatment success, and decided to do so after a failed treatment:

"Because one of my distant relatives had used acupuncture and had the IUI in the clinic after 17 years, she first went on three sessions of nerve stimulation, after which they told her that she could do the IUI now, but she warmed and readied her uterine and then went to Mashhad and got promising results, they have now twins" (Interview 4, female, 5 years, female and male factor).

Two participants also stated that they had performed cupping after the previous unsuccessful treatment. Another participant also considered the traditional medicine as the only way to achieve the positive outcome after repeated unsuccessful treatments with ARTs:

"I think we might achieve a positive outcome if we pay attention to the traditional medicine" (Interview 18, male, 3 years, female and male factor).

One situation which gave couples hope was the presence of frozen embryos. They felt it would reduce their costs and have less physical effect:

"There are two embryos packages for me and I hope to use one package without undertaking any drug-related cost and undergoing terrible injections every day. I can still be hopeful to the packages that I have" (Interview 1, female, 18 months, female and male factor).

Finding another clinic

Unfortunately most of the participants had doubts about whether to continue the treatment. Some were skeptical about ovulation induction, the use of donated eggs, or the use of alternative therapies such as herbal medicine, or the change of treatment site or physician. A participant stated that the physician recommended using donated follicles due to her old age after the unsuccessful treatment, which was difficult for her to accept and did not follow up on the subject. She was obsessed with this issue during this time period and was hesitant regarding taking any action in this regard:

"I'm a little doubtful on the issue of donated follicle. She (physician) has introduced it (donated follicle) to me now. It has been 20–25 days since the female doctor me, but I have not yet come to see what the story is like, what are its rules, what are its condition?" (Interview 2, female, 10 months, female and male factor).

The majority of the participants also changed their doctor and treatment center after their failed treatment. While carrying out interviews, we found that infertile couples changed between several treatment centers; particularly when after having several failed treatment cycles with a single specialist and health center. Many described being hesitant in choosing a new treatment center:

"My doctor is very good, but she may say that if you change your fortune this way, go to the N center or Tehran or a better place ... maybe their equipment or their fertilization methods may be different" (Interview 1, female, 18 months, female and male factor).

Some of the participants also decided to continue treatment at a treatment center in a city other than their own, after an unsuccessful treatment, on a gynecologist's recommendation and went on a therapeutic trip. The doctor said:

"you now have one fallopian tube. It is very unlikely to get pregnant. You should go to Tehran or Mashhad or Yazd" (Interview 4, female, 5 years, male and female factor).

Table 1
Emerg ed Themes and subthemes from analysis of data

Main codes	Subtheme	Theme
Continue treatment to prevent self-blame	Being affected by compensatory mechanisms	Support to continue
Non-logical decision-making to continue the treatment		
Continuing treatment for the sake of the spouse		
Giving hope	Marital support	
Understanding		
Accompany		
Welcoming		
Selflessness and dedication		
Encouragement for continued treatment	Family and peers' encouragement:	
Introducing the physician by family members		
Introduction of treatment centers by friends		
Spiritual support of surrounding people		
Insisting of family members to continue treatment		
Positive influence of others' advice on frozen embryo acceptance	The presence of promising conditions for the continuation of treatment	
Hoping for the existence of frozen embryos		
Expecting to have an ovum		
Observing successful treatments in peers	contiguity with peers	
Comparing themselves with peers		
Saving for retreatment	Saving up to continue treatment	Trying for a second chance
Selling valuable staff for retreatment		
Borrowing		

Main codes	Subtheme	Theme
Getting loan	The confusion of mental and emotional fluctuations	
Having a mixed feeling of hope and fear to start retreatment		
Being doubtful to start retreatment		
Fear of getting worse by starting retreatment		
Having mental preoccupation to continue treatment		
Hesitance to continue treatment	Losing trust in ARTs	
Being doubtful to use a donor egg		
Uncertainty about using herbal medicine		
Unconvinced to change physician	Finding another clinic	
Being unsure to change the treatment center		

Discussion

We have presented two themes which describe a small sample of Iranian infertile couples' experiences of deciding whether to continue treatment after unsuccessful ARTs. These are: support to continue and trying for a second chance. Iranian culture is a collectivist culture where infertile couples experience stigma or remain marginalized due to negative psychological consequences as a consequence of the infertility and not being able to fulfil an expected maternal role in society. Iran is in other words, strongly pronatalist; marriage and childbearing are integral parts of social structure. (27). Interwoven with these cultural beliefs, almost all of Iranian population are Muslim (35) and in Islam, it is an honor to be a mother and Muslims believe that heaven is under the feet of mothers (36). Among our sample, a failed ART cycle resulted in a significant set-back for the couples and they sought support to help them adjust to the loss of the cycle and plan for the future. In order to manage continuing with treatment, they sought support from their marriage partner, their families and their peers before deciding to continue with another ART cycle, trying for a second chance; this continued treatment entailed saving up to pay for another cycle, while at the same time losing trust in ART and as a result, changing clinics to manage their loss of trust and build their hope in success in the next ART cycle.

The psychological consequences of failed ART cycles are well known with additional consequences for the success of any future cycles. Depression and anxiety increases after unsuccessful infertility treatment in infertile couples (37, 38). Chan et al. (2016) showed levels of depression and anxiety in the infertility journey: when infertile couples decide to continue or to discontinue treatment, and when they

regret the decision three months later (39). Also resilience decreases in repeated IVF failure (40). Omani-Samani et al. (2018) found that the anxiety disorder increases with increasing infertility treatment length in Iranian patients (41). Stress and anxiety levels have also inverse relationship with pregnancy rate in infertile women (40). The couples in this study also expressed anxiety and emotional turmoil as they recovered from the failed ART cycles. The hope to have a child and the fear of not being able to get pregnant in the future is considered a conflict for all participants, all of whom were Muslim (42). The findings of the present research showed that marital reciprocal support and empathy between couples led to continuing treatment. Among infertile couples, men usually play the role of supportive partner during and after treatment. Men are usually expected to be strong and suppress their feelings in the face of distress that may lead to depression (43). In the present study, this was confirmed as the women stated that they were supported by their spouses after unsuccessful treatment; and the men said that they supported their wives and gave them the hope that they would be successful in the future treatments. Men did not disclose their own feelings but focused instead on supporting their wives. Latifnejad Roudsari et al. in their qualitative study on samples in the UK and Iran found that infertile women interpreted infertility as a joint life project that they have to go through it, cooperatively, with their partners. They found that infertility and ART cycles could have a positive impact on their relationships due to caring about each other's happiness, having a loving relationship as well as a spouse's reassuring and supporting approach. As a consequence couples may become closer to each other through the journey of infertility (44).

Social support also plays a key role in enabling an infertile couple to overcome infertility as a life crisis; support during a life crisis may include partners, family and friends (45). In this study, relatives' encouragement after unsuccessful treatment led couples to continue their treatment. Infertile couples needed support from their family and friends after unsuccessful treatment, and at the same time tried for a second chance to get pregnant. In this study, family support was received in various forms. Families tried to help infertile couples following unsuccessful treatment and encouraged them to continue the treatment with emotional and spiritual support. Lee et al. (2009) showed that friends and colleagues provided emotional support for infertile women following unsuccessful treatment (46). Mosalla Nezhad et al. (2014), found two groups of increasing and decreasing factors of hope in Iranian infertile couples when they engage with treatment issues. The increasing factors of hope included spiritual resources, social interactions, family support, and obtaining information through the media. They emphasized that decreasing factors of hope included the nature of therapy and the negative mind. In this case, couples' encouragement could lead to continuing treatment (47), which was consistent with the findings of the present study.

In this sample of Iranian couples, theme "support to continue" showed that the participants evaluated their ability to continue with further ART cycles. They felt that they had to re-invest their spiritual, emotional and financial resources to attempt another ART cycle. While being prepared to continue treatment, the participants showed their flexibility with measures to produce resources, such as increasing their strength and controlling the fertility experience. This has also been found by Bailey et al. who suggested a model of women's ability to continue ARTs despite frequent treatment problems in the

UK. They suggested three main categories of women's continuing of ART treatment cycles: "appraisal"; "stepping away from treatment", and "building self-up for the next attempt". Participants in Bailey's study demonstrated their ability to recognize their strength, ability to rely on strategies helping them through exploring their negative past experiences and monitoring their treatment (48). The majority of infertile couples in our study stated that they would seek retreatment after unsuccessful treatment.

In our study, some couples lost faith in ARTs treatment after their failed ART cycle and turned to traditional and herbal medicine methods. Consistent with the present study, researchers have found couples search for alternative and/or complementary interventions to enhance their chances of pregnancy after unsuccessful treatment (21). Their loss of faith or hope in ARTs after the failed cycle, led some of them to search out an alternative fertility clinic like participants in other studies among Iranian couples (44). In the present study, another factor which affected their decision to continue with treatment was the cost of further ART cycle. Studies in Iranian infertile couples show that some participants have to wait until they can afford the cost of treatment after an unsuccessful treatment(49), and as McCarthy's (2008) found couples take stock both emotionally and financially, as they shift from worries about infertility and focus on the future. This shift is not just related to the choice of continued treatment, but also a decision on how to do it (42).

These findings have implications for health professionals who are working in fertility clinics. They need to consider all aspects of holistic care including psychosocial needs of infertile couples, when caring for women with fertility problems(50) .

Strengths And Limitations

As not much is known about Iranian or Muslim infertile couples' decisions to continue treatment after unsuccessful treatment, strength of this research is that it was conducted in an Iranian setting with an exclusively Muslim sample. The particular context of interviewing couples and family members is a strength but again particularly because of the setting, i.e. Iran where family plays a significant role in supporting couples in family life. The sample included in this study was a relatively homogeneous sample with similar demographic characteristics and this is one of the limitations of the study.

Conclusion

Couples need support during ARTs especially in failed cycles from their social and family networks, which in turn means greater awareness and disclosure of their infertility issue. The findings of the present study show that in a small Iranian sample of infertile couples confronting treatment failure, couples are encouraged and supported for continuing treatment by family and friends. The findings show how their faith and trust in the possibility of successful conception through ART cycles is threatened and that as well as financial costs, there are also emotional costs to considering further ART cycles after treatment failure. In couples where there is such anxiety/depression, that might affect conception(38), then this might be relevant for the clinics in terms of psychological assessment after failed IVF. So it is

recommended that fertility clinics should effectively assess psychological readiness of couples for ART cycles after treatment failure and implement a supportive and counselling programs for infertile couples who face unsuccessful treatment to support them during failed cycles, as unsuccessful treatment increases depression and anxiety in infertile couples, which itself could affect the success rate of any further ARTs.

Declarations

- **Ethics approval and consent to participate:**

The present study was approved by the Regional Ethics Committee of the University with the number of IR.MUMS.REC.1395.120 and permitted to be carried out in the infertility center.

- **Consent for publication:**

Not applicable

- **Availability of data and materials:**

Not applicable

- **Competing interests:**

The authors declare that they have no competing interests.

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- **Authors' contributions:**

SEZ have made substantial contributions to the conception, design of the work; interpretation of data; and have drafted the work. *RLR* have made substantial contributions to design of the work; interpretation of data; and substantively revised it. *RJ* have made substantial contributions to interpretation of data; and have drafted the work. *HTA* have made substantial contributions to the conception, design of the work; and substantively revised it. All authors read and approved the final manuscript

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