State of the art on oocyte cryopreservation in female cancer patients: a critical review of the literature.

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ABSTRACT

During the last decades, important advances in therapeutic options have led to increased survival rates in cancer patients; however, cancer treatments are associated with several potential adverse effects including infertility in those diagnosed during their reproductive years. A proper discussion about fertility preservation options before the use of therapies with potential gonadotoxicity (i.e. oncofertility counseling) is standard of care and should be offered to all patients of childbearing age. Temporary ovarian suppression with LH-RH analogs, oocyte and embryo cryopreservation are standard strategies for fertility preservation in female cancer patients. Oocyte cryopreservation should be preferred to embryo cryopreservation when this latter is prohibited by law, avoided for ethical or religious issues and in single women refusing sperm donation. Despite the increasing use of this strategy, data are still lacking about the efficacy and safety of the procedure in female cancer patients, with most of the evidence on this regard deriving from infertile non-oncologic women. This article aims at critically review the available evidence about the success of oocyte cryopreservation in female cancer patients with the final goal to further improve the oncofertility counseling of these women.

Highlights:

- Physicians should discuss fertility preservation with women facing gonadotoxic therapies. Oocyte cryopreservation is a standard procedure for fertility preservation in female cancer patients.
- Data about the efficacy of the procedure specifically in cancer patients are still lacking, however the reassuring efficacy and safety results in non-oncologic patients can be reasonably translated in the cancer setting.
- More data about the outcome of thawing/warming cycles in cancer patients are needed. Up to date, a registry of pregnancies in this population does not exist.

Keywords:

Oncofertility, oocyte cryopreservation, gonadotoxic therapies, pregnancy after cancer

Background

Among patients diagnosed with malignant cancers worldwide, 3% to 10% are under 40 years of age. Cancer in reproductive age is twice as frequent in females than in males [1, 2]. Important advances in cancer treatments have led to increased survival rates over the past years; conversely these therapies expose patients to potential long term adverse effects, including infertility [3]. The maintenance of future fertility is considered of great importance by patients. At the time of cancer diagnosis, approximately half of the women are concerned about the potential loss of ovarian function and fertility due to use of cancer treatments [4]; moreover, fertility concerns can also influence their therapeutic decisions and the adherence to the suggested treatment [4, 5]. Cancer patients have a strong desire to have biological children instead of searching for other options such as adoption or egg donation [6]. Moreover, the development of secondary iatrogenic infertility negatively affects many aspects of their life and general well-being. For instance, lower marriage rates are found in these patients [7].

However, many patients do not choose to undergo fertility preservation procedures. This may be explained by the difficult moment they are facing, by the fact that they may not be aware of the potential fertility loss and by the fear of delaying cancer treatment [8]. It has been reported that adequate counseling improves the number of women who choose to undergo fertility preservation treatments [9]. Hence, as endorsed by major international guidelines, it is crucial to counsel all cancer patients in reproductive age about the treatment-related loss of fertility and to assist them in taking decisions on fertility preservation [8, 10].

Oocytes cryopreservation is the preferred choice when embryo cryopreservation is not allowed or refused by the patients. Furthermore, preserving oocytes, and not embryos, gives the patient a reproductive autonomy: this is a relevant point taking into account the high risk of relationship disruption in female cancer survivors [11, 12].

The procedure of mature oocyte cryopreservation requires at least two weeks of controlled ovarian stimulation (COS) to induce multiple follicular growth and oocytes retrieval. Since the success rate per oocyte is low [13, 14], it is crucial to obtain as many oocytes as possible [15].

COS can be considered safe in women with cancer when a 2-week delay in the initiation of cancer treatment is possible [3]. The safety of the procedure has been also demonstrated in women with hormone-sensitive cancers with the addition of letrozole or tamoxifen to the standard protocol for COS [16, 17, 18].

However, despite the endorsement of the major Oncology and Reproductive Medicine societies (Table 1), oocyte cryopreservation has not been widely adopted. As shown by Von Wolff and colleagues, this technique was chosen only by a minority of women in Germany, Austria and Switzerland between 2007 and 2013 [9]. Several challenges, including a lack of knowledge of both patients and physicians on the topic, could explain such limited use of oocyte cryopreservation. This article aims at critically reviewing the available evidence about the success rates of oocyte cryopreservation in female cancer patients with the final goal to further improve the oncofertility counseling.

Procedural challenges for cryopreserving the oocytes

Mature oocytes (metaphase II, MII) are fragile cells and numerous challenges should be overcome for obtaining a successful cryopreservation procedure and subsequent storage. The oocyte is the largest human cell, with subsequent low surface area to volume ratio. The overall effect is that oocytes are likely to retain water during freezing and they are particularly vulnerable to physical injury by intracellular ice formation during the cryopreservation process.

Moreover, addition or dilution of cryoprotective agents may further cause osmotic stress [23]. Cryopreservation can cause zona pellucida thickening, premature cortical granule exocytosis [24, 25], as well as risk of meiotic spindle disruption and aneuploidy [26, 27, 28, 29, 30]. In addition, animal [31, 32, 33, 34] and human [35, 36, 37 ,38] studies have demonstrated that cryopreservation can also impact on gene expression profile of MII oocytes by deregulating genes related to oxidative stress, apoptosis, chromosomal structural maintenance, and cell cycle. The cryopreservation procedure seems to negatively affect proteomics. In fact, exposure of mouse oocytes to 1,2-propanediol cryoprotective agent showed to alter

the maternal proteins stored within the oocyte for fertilization support and early embryo development before the activation of the embryonic proteome [39, 40]. On one hand, a protein efflux due to disruption of the plasma membrane, possibly secondary to free-radical production, may explain a down-regulation of proteins following slow freezing. On the other hand, oocyte exposure to toxic cryoprotectants may determine up-regulation of stress proteins that can exhibit widely varied functions such as induction of apoptosis, impairment of meiotic division, and proteasome-mediated degradation. Overall, cryopreservation procedures may have negative effects on the physiology of the MII oocyte. It has been demonstrated that storage time in liquid nitrogen, if it is performed in a suitable way without any temperature alteration, does not alter the molecular integrity of the oocytes [38]. It follows that the potential damage produced to frozen/thawed oocytes is only due to the cryopreservation procedure *per se* rather than the storage-time. In support of this, prior clinical studies in controlled-rate cryopreserved and vitrified human oocytes [41, 42] demonstrated that long-term cryostorage does not adversely affect the outcome of oocyte thawing cycles. The discovery of no detrimental effects of storage time on the oocyte gene expression profile proves that the developmental potential of frozen/thawed oocytes is independent from the duration of their conservation in liquid nitrogen. The wider implication of this finding is noteworthy for the safety of long-term oocyte banking. This is of crucial importance for cancer patients, for whom several years after diagnosis may be required before trying to achieve a pregnancy with the use of the oocytes cryopreserved before starting treatment.

Different methods for oocyte cryopreservation

The available methods for oocyte cryopreservation are slow freezing (or controlled-rate cryopreservation) and vitrification [43]. In slow freezing cryopreservation, oocytes are slowly cooled to below the freezing point in the presence of low concentrations of cryoprotectant agents (e.g. sugars, propanediol). This allows to reduce intracellular ice formation and structural damage due to solute concentrations and osmotic stress [44, 45]. The term “vitrification” refers to any process resulting in “glass formation”, i.e. the transformation from a liquid to a solid in the absence of crystallization. For this purpose, oocytes are brought into a

medium that has a very high concentration of cryoprotective agents and then they are frozen at high cooling rates to become “vitrified” without forming ice crystals [46, 47, 48, 49].

The most used indicators of the efficacy of these procedures are ongoing pregnancy rate and live birth rate per embryo transfer. However, there is a large heterogeneity in the literature (i.e., not all studies reported live birth rate, sometimes results were reported per oocyte or per thawed cycle, etc) and different studies are not easily comparable. Table 2 summarizes the indicators for the evaluation of the success of fertility-preserving procedure.

A 2014 Cochrane review [50] evaluated the performance of slow freezing and vitrification in two randomized controlled trials (RCT) [51, 52]. One study was carried out in infertile women with supernumerary mature oocytes, while the other study was carried out with oocyte donors. Neither of the two RCTs analyzed the outcome in terms of live birth rate. As compared to slow freezing, vitrification was associated with an increased pregnancy rate (relative effect 3.86, 95% confidence intervals [CI] 1.63 to 9.11, p= 0.002), oocyte survival rate after warming (80-90% vs. 45-67%) and fertilization rate (76-83% vs. 54-68%). The main limitation of both studies was the small sample size. A large cohort study based on the Italian assisted reproductive technology (ART) registry evaluated the performance of oocyte cryopreservation in infertile patients with supernumerary mature oocytes. The study included 14,328 cycles performed from 2007 to 2011 in 146 centers. Vitrification was associated with a statistically significant higher performance in terms of pregnancy rate than slow-freezing (odds ratio [OR] = 1.23, 95% CI: 1.11-1.35, P < 0.001), although results varied largely among the different centers. The pregnancy rate using the slow freezing technique was 12.0% for started thawing cycle and 14.8% considering only the cycles that led to an embryo transfer. Using vitrification the pregnancy rate increased to 14.4% and 18.0% respectively [53, 54].

Of note, cryopreservation techniques are highly operator dependent, vitrification even more than slow-freezing due to the need to complete the procedure within seconds [55]. While slow-cooling was the first technique to be used, in the last decade, vitrification has resulted in a breakthrough in oocyte

cryopreservation and has become the standard technique. A recent systematic review and meta-analysis including 6 RCT and 14 observational studies compared slow-freezing, vitrification, and the use of fresh oocytes. The meta-analysis suggested that vitrification/warming is superior to slow-freezing/thawing in terms of clinical outcome and cryosurvival rates. As for clinical outcome, the quality of evidence was considered low because only one RCT reported the clinical pregnancy rate as outcome. The difference between the two techniques was statistically significant only when comparing ongoing pregnancy rate per cycle of warming (RR = 2.81, 95% CI: 1.05–7.51; P = 0.039), but not ongoing pregnancy per transfer (RR = 1.81, 95% CI: 0.71–4.67; P = 0.214). Vitrification seemed to have better oocyte survival rates than slow-freezing: the meta-analysis of three RCT reported rates of 82.3% vs 66.1% (RR = 1.23, 95% CI: 1.02–1.49; P = 0.031) [51, 56, 57, 58].

Efficacy and safety of oocyte cryopreservation

The efficacy and safety of oocyte cryopreservation can be inferred from available data especially in two populations: infertile women and oocyte donors.

During the first decades of use, overall success of both slow-cooling and vitrification techniques was low compared to in vitro fertilization (IVF) with unfrozen oocytes. In 2006 Oktay et al. reported a live birth rate per oocyte of 1.9% after slow freezing and 2.0% after vitrification [59]. Subsequently, four randomized controlled trials compared the efficacy of fresh vs. cryopreserved oocytes. Cobo et al. obtained similar success rates in recipients of fresh or vitrified donated oocytes [47, 60], and two Italian studies confirmed similar results using fresh and vitrified oocytes in infertile patients [48, 61]. Taking into account the results of these four RCT, in 2013 the American Society of Reproductive Medicine (ASRM) removed the "experimental" designation to oocyte cryopreservation [62].

An year later, in 2014, a systematic review and meta-analysis of 17 studies reported an ongoing pregnancy rate per warmed oocyte of 7% (95% CI: 4-10%) [63], a significantly better result than the 2% reported by Oktay et al. in 2006 [59]. However, large multi-centric observational studies suggested that implantation

and pregnancy rates may be lower with frozen oocytes compared with fresh ones or frozen embryos [64, 65].

Table 3 summarizes the results of the studies reporting the efficacy of oocyte cryopreservation in different settings (infertility, oocyte donation, elective cryopreservation, fertility preservation in cancer patients).

Results reported by different centers showed that women's characteristics affect the success of the procedure. In egg donation programs, with oocytes of young healthy women, the pregnancy rate was as high as 49.1% per embryo transfer [47]. In infertile patients, the results varie according to patients' characteristics and the experience of the center.

The Italian experience is particular in this context. From 2004 to 2009, the Italian law n. 40 banned the use of embryo cryopreservation, urging fertility centers to master oocytes cryopreservation techniques in infertile patients. A retrospective study examined the results from 24.173 cycles with frozen and then warmed oocytes reported in the ART national registry from 2005 to 2013. A total of 80.5% of the thawing cycles led to embryo transfer, but only 15.6% of the embryo transfers led to an ongoing pregnancy [54]. These findings were due to a possible selection bias (the best looking oocytes were used in fresh cycles) but also to differences in performance among centers. Centers with large experience had significantly better results, and, examining results per year, the ongoing pregnancy rate increased with the experience of the centre.

From a safety perspective, thousands of babies have been born worldwide from frozen eggs, and several data are available on the obstetrics and neonatal outcomes of a great number of pregnancies. In 2009, Noyes et al. published a large retrospective study reporting the outcome of 900 babies born from oocytes cryopreserved with the slow freezing technique. This study reported an anomaly rate of 1.3%, not higher than the one expected in the general population [70]. In 2014, Cobo et al. reported the perinatal outcome of 1.027 children born from vitrified oocytes as similar to those obtained with fresh techniques [71]. The Italian ART registry reported a reassuring rate of congenital anomalies equal to 0.9% in 24.173 cycles from 2004 to 2009 [53].

Efficacy of oocyte cryopreservation in cancer patients

Although oocyte cryopreservation is routinely used as a method for fertility preservation, specific data on the success of the procedure in female cancer patients are limited so far. A first reason for this observation is that oocyte cryopreservation has been widely used from no more than 10 years. Second, after oocyte cryopreservation, women need time to treat their oncological disease before attempting to have a pregnancy. Third, not all the women who cryopreserved will decide to use their oocytes: some may not desire motherhood anymore and others may conceive spontaneously. Whereas it is known that only 10% of men who cryopreserved semen before oncological treatment come back to use it [72], the data on the utilization rate of cryopreserved oocytes are not yet available.

The first live birth from frozen/thawed oocytes in a cancer patient was reported by Yang et al. in 2007 [73]. The patient was diagnosed with Hodgkin lymphoma at 27 years and decided to undergo oocyte cryopreservation using the slow-freezing technique. A total of 10 out of 13 MII cryopreserved oocytes (77%) survived after thawing. Three embryo transfers were performed on a gestational carrier and the third one resulted in a single ongoing pregnancy and live birth at term. Since then, few more live births have been reported in the literature mainly as single case reports (Table 4). Two small retrospective studies reported data on the outcomes of cancer patients who underwent oocyte cryopreservation before cancer treatments. Martinez et al. [68], described the outcome of 11 patients. The ongoing pregnancy rate per embryo transfer was 54,5% and 4 patients delivered 5 healthy newborns at term. At the time of the cryopreservation procedure, median age was 35.6 years and the number of cryopreserved MII oocytes ranged from 3 to 10. Of these four patients, three had infiltrating ductal breast carcinoma treated with surgery and chemotherapy (plus tamoxifen in two of three cases) and the fourth had non-Hodgkin lymphoma. This last patient did not receive chemotherapy after oocytes cryopreservation but underwent an intracytoplasmic sperm injection (ICSI) cycle with the cryopreserved oocytes because of a severe male factor. Of note, the oldest patient of this cohort (41 years old) cryopreserved 10 oocytes but obtained no euploid embryos after fertilization and did not undergo embryo transfer. In 2016 Druckenmiller et al. [69] reported a 44% live birth rate per embryo transfer after thawing/warming of oocytes in 10 patients with a

median age of 32 years. Oocyte survival rate and fertilization rate were similar to the ones obtained in patients without cancer at the same institution (live birth rate of 33% in non-cancer patients of less than 43 years). A relatively large number of oocytes (n=7, interquartile range: 6-13) was thawed to guarantee fertilizing in at least two of them.

Five newborns were delivered (3 singleton pregnancies and one twin pregnancy). Three women were breast cancer patients and the fourth had a gynecologic malignancy. In one of the three breast cancer patients, a gestational carrier was used because post treatment pregnancy was not recommended.

The limited available data in cancer patients do not allow to draw significant conclusions about the outcome of oocyte cryopreservation in this setting; hence, the efficacy and safety of the procedure is inferred from the results in the general population. Of note, the oocytes obtained from cancer patients usually remain frozen for a longer period of time as compared with infertile non-oncologic women. This could be another confounding factor to assess the efficacy of the procedure in the oncologic population, even though recent evidence suggested that storage time does not affect the cryopreserved MII oocytes at the gene expression level, if the procedure of cryopreservation is adequately performed [38]. However, as mentioned above, the efficacy of oocyte cryopreservation increases with the number of cryopreserved gametes. Therefore it is important to define if there is a reduced ovarian response to stimulation in cancer patients.

Ovarian response to stimulation in cancer patients

The effect of cancer on patients' ovarian reserve and consequently on the response to COS is a key factor for the success of oocyte cryopreservation.

In 2012, a systematic review and meta-analysis performed by Friedler et al. [80] compared the outcomes of 227 COS cycles in female cancer patients with those of 1258 cycles in infertile patients. The study showed that less oocytes were retrieved in patients with cancer as compared to the infertile non oncologic population (11.7 $\pm$ 7.5 vs.13.5 $\pm$8.4, $P < 0.002$). However the studies considered were not homogeneous, and the dose of gonadotropines used for COS varied significantly between cases and controls. Subsequent

studies [81, 82] did not confirm this result. A recent retrospective cohort study [83] matched 244 women with a recent cancer diagnosis and no history of infertility with 362 non-oncologic women undergoing COS for elective fertility preservation. Cancer diagnosis was not associated with a decreased ovarian reserve, response to COS and number of retrieved oocytes. Another recent study by the same group demonstrated also that the inclusion of letrozole in the protocol for controlled ovarian stimulation did not seem to be associated with a negative impact on cycle outcome or oocyte maturity [84].

The study by Druckenmiller et al. [69] analyzed also the difference in ovarian response to COS according to the type of cancer. Patients with hematologic malignancies had the highest number of cryopreserved oocytes, whereas patients with breast malignancies had the lowest. Ovarian cancer patients cryopreserved a low number of oocytes, probably due to a direct insult of the cancer or the required surgery to the ovarian function. Conversely, uterine cancer patients obtained a high number of oocytes; this was probably a consequence of an underlying polycystic ovarian syndrome.

Overall, the available data shows no significant difference in ovarian response in cancer and non-cancer patients. A possible reduced response to COS may occur in *BRCA* mutated cancer patients, even if available data in this subgroup of women are limited and still controversial to draw solid conclusions [85, 86].

If we consider valid the hypothesis that the cancer *per se* does not reduce the response to COS and the quality of the oocytes, the results in infertile women and oocyte donors can then be generalized to inform patients during the oncofertility counseling about efficacy and safety of the procedure. In this scenario, the main predictive factors for the overall success of the procedure are the expertise of the laboratory (the efficiency of the cryopreservation technique and the learning curve of the operators) and the individual characteristics of the women at the time of the procedure (age, ovarian reserve).

Finding the ideal candidate for oocyte cryopreservation

During oncofertility counseling, when discussing the efficacy of the available methods for fertility preservation, the ability to estimate the success of the proposed technique is essential. Anti-Mullerian

hormone (AMH) and antral follicle count (AFC) are widespread used markers of ovarian reserve in the general population and can be used as predictors of response to COS in ART procedures, also in cancer patients [87]. In the study by Lee et al. including 41 women with breast cancer that underwent ovarian stimulation (with the addition of letrozole) for embryo/oocyte cryopreservation, AMH and AFC were predictive of the ovarian response [88]. Specifically, AMH was the most reliable marker, with a likelihood of low response for values inferior to 1.2 ng/ml.

Besides the markers of ovarian reserve, age remains the strongest determinant of the success of the technique. In a recent study, Doyle et al. [67] analyzed 128 autologous IVF cycles from a total of 1283 vitrified oocytes of infertile non-oncologic women who underwent cryopreservation both electively or for medical reasons. Age was the most reliable predictor of the oocyte-to-live-birth efficiency in this cohort. The age-associated live birth rate per warmed oocyte ranged from 8.7% in women aged <30 years to 1.1% in women aged 43–44 years, with an overall oocyte to child efficiency of 6.7%. Cryopreservation of 15-20 MII oocytes gave women aged <38 years a 70-80% chance of at least one live birth, while for women aged 38-40 the number of MII oocytes needed to give a 65-70% chance of a live birth increased to 25-30. To obtain more oocytes, it has been proposed to perform two ovarian stimulation, one immediately after the other [89]. However, in cancer patients the double stimulation is not always feasible, due to the need to start anticancer therapies. Moreover, in older patients, the probability of having a high number of MII oocytes is low even when a double stimulation is performed.

The FertiPROTEKT network registry, including data from Germany, Austria and Switzerland [9] reported a mean of 12.1 $\pm$ 9.9 cryopreserved oocytes in women with cancer younger than 30 years. The numbers decreased to 8.7 $\pm$ 7.1 in women aged 36-40, and to only 5.0 $\pm$ 8.7 in women over 40 years of age.

These numbers are significantly lower than those indicated by Doyle et al. [67] to guarantee a good chance of live birth. However, the main target of the article by Doyle and colleagues was the evaluation of the cost-effectiveness of cryopreservation for non-medical reason (i.e., social freezing). In order to be cost-effective (both in terms of economic costs and procedure-associated risks), an elective procedure should guarantee a success rate higher than the one expected spontaneously in the same population. If we consider oocyte

cryopreservation in cancer patients, the setting is completely different from elective oocyte preservation. Fertility in these patients will be affected not only by the advancing age, but also by the gonadotoxic effect of therapies; hence, for their future fertility, many cancer patients will have to relay mainly on cryopreserved oocytes.

Counseling in difficult cases: old and very young women

Since the risk of cancer treatment-related infertility increases with age, women with the highest probability of developing permanent amenorrhea are often also the ones in which fertility preservation treatments have the lowest chances of success. In facing these situations, a proper oncofertility counseling by fertility specialists is crucial. Hence, a personalized counseling to provide all the information regarding risks, benefits and success rates is mandatory before subjecting a patient to a known inefficient treatment. Alternatives like egg donation or adoption should be discussed as well, specifically with patients with predictably low chances of success with oocyte cryopreservation. It is not always clear when and where to draw the line to consider feasible the fertility preservation procedure. As discussed above, age is the most reliable predictor; however, there is no agreement on the age limit for offering a cryopreservation technique. Druckenmiller et al. [69] proposed to offer oocyte cryopreservation before the age of 43, based on the data of their center showing a 36% live births in non-oncologic infertile women aged 41-42 years. On the contrary, Doyle et al. [67] reported that the success of the procedure in women over the age of 40 is unlikely. In this setting, age together with markers of ovarian reserve and centre's experience (i.e., success rate in non-cancer patients) should be considered as useful tools to make an informed and shared decision with the patient. In general, it can be stated that oocyte cryopreservation over the age of 40 years should be considered exceptional.

Children and adolescent undergoing gonadotoxic therapies are also difficult to counsel. Prepubertal girls who do not yet ovulate due to an immature hypotalamic-pituitary axis are not candidates to COS and subsequent oocyte cryopreservation. A feasible alternative for these patients is ovarian tissue

cryopreservation with or without *in vitro* maturation of immature oocytes [90]. COS and oocyte cryopreservation are effective options in post-pubertal girls [91]. However, pediatric cancers are frequently more aggressive and sometimes a 2-week delay in treatment is not possible [92]. Moreover, younger girls may find emotionally difficult being subjected to serial transvaginal ultrasounds and transvaginal oocyte retrieval. Both young patients and parents need accurate education and counseling about the safety and effectiveness of the procedure. During the process of informed consent acquisition, adolescents should be treated as subjects with increasing autonomy, both legally and because of their physical and mental level of development [93].

Conclusions

Physicians should offer fertility preservation to women facing gonadotoxic therapies, with an adequate counseling within a multidisciplinary contest that includes a fertility specialist. During oncofertility counseling, all the options, both standard and experimental ones, should be presented with an estimate of their efficacy and safety adjusted on the characteristics and needs of the patient, including a discussion on the possibility of delaying treatment initiation.

Oocyte cryopreservation is a standard fertility preserving procedure that allows to conserve reproductive autonomy. It can be considered an effective and safe method to preserve fertility to be done in centers with the adequate expertise given the technical difficulty of the laboratory phase. Cancer patients should be aware that, despite the paucity of data on pregnancies from cryopreserved oocytes in the oncologic population, the reassuring efficacy and safety resulting from infertile non-oncologic patients can be reasonably translated to them. The informed consent to access the procedure should include an estimate of the chance of success based on both patient's characteristics (age, ovarian reserve) and the statistics of the center. The possibility that no oocytes may survive after thawing/warming should be also discussed.

Conflict of interest

The authors declare no conflict of interest

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Author contribution

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