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Case report

Successful *in vivo* retrieval of oocytes after ovarian stimulation for fertility preservation before oophorectomy by laparotomy for a young patient with ovarian cancer: Case report and review of literature

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ABSTRACT

Objective: To report a case of direct *in vivo* oocytes retrieval for fertility preservation before oophorectomy by open surgery in a young patient with ovarian cancer.
Design: case report and literature review.
Setting: University hospital.
Patients: A 29-year-old nulliparous patient, recently diagnosed with low grade serous ovarian carcinoma. The patient consented to the removal of her remaining ovary but wished to preserve oocytes and declined hysterectomy. Conventional trans-vaginal US-guided oocyte retrieval was contra-indicated because of the risk of malignant cell dissemination to the abdomen and the vaginal puncture sites.
Interventions: Controlled ovarian stimulation with gonadotrophins was realized. Comprehensive surgical staging was performed 35 h after ovulation triggering using rHCG. The oocytes retrieval was performed *in vivo* with ultrasound guidance at time of laparotomy before oophorectomy without any time of ischemia.
Results: Seven mature oocytes were obtained and vitrified.
Conclusions: This case highlights the feasibility of *in vivo* oocytes retrieval of mature oocytes during open surgery for gynecologic cancers. By avoiding transvaginal follicular retrieval, the risk of malignant cell contamination to vaginal and parametrial tissues is reduced, limiting cancer upstaging.

1. Introduction

Although rare among women of reproductive age, 1% of epithelial ovarian cancer patients are under 40 (Lavoue et al., 2019). Treatment often has an impact on future fertility. Surgery and chemotherapy may result in premature ovarian insufficiency and inability to carry a pregnancy. Fertility impairment has an impact on quality of life of cancer survivors and fertility preservation (FP) should be offered (Lavoue et al., 2019). Available techniques such as oocytes vitrification and ovarian tissue cryopreservation (OTC) are the mainstay of FP.

OTC is commonly not recommended owing to the risk of reintroduction of malignant cells with transplantation (Dolmans and Masciangelo, 2018). Oocyte vitrification may be an option but puncture of the ovarian capsule to aspirate follicular fluid, could theoretically spread malignant cells in the peritoneal cavity and to the vaginal wall. In the presence of parametrial or cervical infiltration, transvaginal technique is contra-indicated and oocytes retrieval must be done by laparoscopy or open surgery.

Extracorporeal retrieval (*ex-vivo)* of mature oocytes after ovarian stimulation (OS) has been previously described in oophorectomy specimens, resulting in temporary ischemia with unknown consequences on oocyte competence (Pereira et al., 2017; Fatemi et al., 2011; Bocca et al., 2011; de la Blanca et al., 2018).

Here, we report the first case of *in-vivo* mature oocytes retrieval

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before oophorectomy in a young woman undergoing open surgery for ovarian cancer.

2. Case report

A nulliparous 29-year-old woman was referred for bilateral ovarian cysts with elevated CA 125 blood levels (4420 UI/ml). The MRI image is depicted in Fig. 1. Initial laparoscopy with peritoneal cytology, right salpingo-oophorectomy, left cystectomy and multiple biopsies confirmed a low-grade serous ovarian carcinoma (LGSOC) with bilateral ovarian involvement and positive cytology, FIGO IC3.

A comprehensive surgical staging was proposed (hysterectomy, left adnexectomy, pelvic and para-aortic lymphadenectomy and omentectomy). The patient consented to surgery but wished to preserve her uterus.

Before surgery, she was referred for fertility counseling. The left ovary contained 8 antral follicles and AMH levels was 34.3 pmol/L. Proposed treatment was combined FP technique with ovarian stimulation to cryopreserve mature oocytes as well as ovarian cortex cryopreservation for a possible future *in-vitro* folliculogenesis. Advice was given on hysterectomy after completion of childbearing.

Conventional oocyte retrieval by transvaginal ultrasound-guided was deemed unsafe because of the risk of vaginal dissemination and peritoneal spillage. We considered *in-vivo* follicular aspiration during laparotomy offering atraumatic removal of the follicles without time of ischemia. Informed consent was obtained.

Oral contraception was stopped 4 days before starting the controlled ovarian stimulation (COS) according to an antagonist protocol with hMG (Menopur®, Ferring AG) 400UI/day. An antagonist Ganirelix (Orgalutran®, MSD, 0.25 mg) was added from the 6th day of stimulation. On the 10th day, when $\geq$3 follicles had reached 17 mm, a dose of 250 mcg of recombinant rHCG (Ovitrelle®, Merck, Switzerland) was subcutaneously injected to trigger ovulation. The peak of E2 at triggering was 2935 ng/L. Laparotomy under general anesthesia was scheduled 35 h after rHCG.

Oocyte retrieval was performed with the standard equipment used for transvaginal oocyte aspiration for IVF. For the follicle aspiration, a single lumen needle (Ovum aspiration needle 17GA/30 cm, Cook medical) was used with a negative pressure of −138 mmHg as in normal routine vaginal procedures. The follicular aspiration was performed by directly applying the ultrasound probe on the left ovary in the operative field, while an assistant carefully held the ovary with latex and powder-free gloves. The follicular fluids were collected in tubes, placed in a heating block at 37 °C and immediately given to the embryologist who proceeded to identification of the cumulus-corona complexes (COC) under a dissecting microscope.

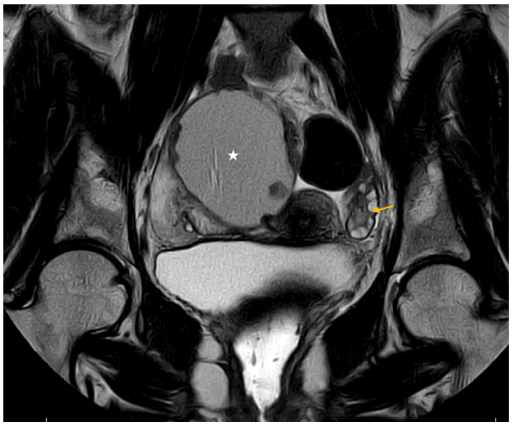

Fig. 1. MRI T2 sequence. Ovarian masses: the right ovary one measured 7 × 6 × 9 cm, and was composed of a uniloculate cyst associated with a solid component with papillary projections (*). The left one presented the same characteristics, though smaller (arrow).

Eight COC were obtained and after removal of their cumulus with hyaluronidase yielding, 7 metaphase II oocytes were obtained and vitrified using an oocyte vitrification method (CRYOTOP Vitrification-KITAZATO) as previously described by Kuwayama et al. (2005).

In accordance with her wishes, left oophorectomy, pelvic and para-aortic lymphadenectomy and omentectomy, sparing the uterus, were then performed with no residual disease at the end of surgery. Stage was established using pathological specimens from both the debulking surgery and the staging procedure, without upstaging after the staging procedure.

A macroscopically normal ovarian fragment was sent for OTC and 48 cortical fragments of 2 mm finally cryopreserved by a slow freezing technique. The abdomen was rinsed with 2 L of distilled water to prevent the dissemination of tumoral cells. No complications occurred. Given the high risk of recurrence, she then received 4 cycles of chemotherapy with Carboplatine and Taxol 175 mg/m^2, following European guidelines (Colombo et al., 2019).

After 17 months follow-up, there is no evidence of recurrence. To date the patient has no planned pregnancy.

3. Discussion

To our knowledge, this is the first publication reporting this retrieval technique after OS in gynecologic cancer.

The procedure was easy, safe and avoided any spillage of malignant cells or time of ischemia.

Cryopreservation of mature oocytes obtained after *in-vitro* maturation with OS represents a efficient and established technique. Vitrification, an ultra-rapid cooling method, allows excellent oocyte survival rates after warming and offers similar outcomes as with fresh or cryopreserved oocytes (Cobo et al., 2016; Practice Committees of American Society for Reproductive Medicine and Society for Assisted Reproductive Technology, 2013).

Oocyte retrieval by traditional transvaginal follicular aspiration is the technique of choice, but this technique may become unsafe in some situations:

- Risk of spillage of tumoral cells in the pelvic cavity after puncture of the ovarian capsule
- Tumoral invasion of vagina and parameters
- Technical difficulties (tumor obstructing the view, follicles near the tumor, tumor between the vagina and the ovary)

There are a number of publications mentioning *ex-vivo* harvesting of oocytes with or without ovarian stimulation beforehand, offering safe oocytes retrieval in oncological situations. The *ex-vivo* retrieval avoids intra-peritoneal tumors cells spillage but increases the time of ischemia from the oophorectomy to oocytes collection.

The *ex-vivo* oocyte retrieval was mainly used in the context of In-Vitro Maturation (IVM) consisting in the harvesting of germinal vesicle (immature oocytes) from antral follicles of unstimulated ovaries then matured *in-vitro* (Park et al., 2016; Segers et al., 2015; Huang et al., 2008; Prasath et al., 2014). IVM is indicated when ovarian stimulation is not possible (prepubertal girl, hormonal-dependent cancer, emergency to initiate treatment). Ovarian tissue oocyte IVM (OTO-IVM) offers an additional oocyte source by performing IVM of immature oocytes recovered from oophorectomy with promising reproductive issues (Segers et al., 2020). Nevertheless, oocyte vitrification after OS is the optimal method of FP since IVM is still considered experimental and offers lower live birth rates (Nogueira et al., 2012).

Only four publications reported *ex-vivo* aspiration of follicles from stimulated ovaries in the context of oophorectomy for borderline ovarian carcinoma or recurrent teratoma (Pereira et al., 2017; Fatemi et al., 2011; Bocca et al., 2011; de la Blanca et al., 2018). The time of ischemia, when noted, varied from 10 to 18 min (Table 1).

Table 1
TH: total hysterectomy; LSO: left salpingo oophorectomy; RSO: right salpingo oophorectomy; BSO: bilateral salpingo oophorectomy; ROC: right ovarian cystectomy; LOC: left ovarian cystectomy; MII: metaphase II; rFSH: recombinant FSH.

Study	Patient's age (y)	Parity	Diagnosis	Surgery	Ovarian stimulation	Fertility preservation technique	Time of ischemia (minutes)	Total Oocytes/ mature oocytes
Bocca et al. (2011)	25	Nulliparous	Borderline serous carcinoma (recurrence)	RSO (history of LSO)	Yes	Ovarian stimulation Ex vivo retrieval	10	22 COC/14 MII
Fatemi et al. (2011)	27		Borderline serous adenocarcinoma in the right ovary (recurrence)	RSO (history of LSO)	Yes	Ex vivo oocytes retrieval	14	11 MII 7 zygotes
Pereira et al. (2017)	37	Nulliparous	BRCA 1 mutation carrier Right ovarian mass Ovarian cancer	BSO laparotomy	Yes luteal phase Gonadotrophin + Letrozole	Ex vivo retrieval	10 (right ovary) and 18 (left ovary)	7MII
de la Blanca et al. (2018)	31	Nulliparous nulligravida	Recurrent teratoma	RSO (history of LSO for mature teratoma) laparoscopy	Yes rFSH antagonist	Ex vivo follicle aspiration with ultrasound guidance	?	6 COC 5 MII
Eskew et al. (2018)	32	MRKH	Bilateral ovarian masses	BSO	Yes	In vivo	0	24 COC 15 MII 5 blastocysts
Kim et al. (2015)	21	nulliparous	Recurrent immature teratoma	LSO laparotomy (history of RSO and LOC) + 4 BEP	Yes Random start	In vivo during laparotomic debulking surgery	0	8 MII
Benard et al. (present article)	29	nulliparous	Ovarian low-grade serous carcinoma with a positive cytology. FIGO stage IC3	Laparotomy LSO, pelvic and para-aortic lymphadenectomy and omentectomy (history of RSO)	Yes antagonist	In vivo oocytes retrieval with ultrasound guidance during laparotomic debulking surgery	0	7 MII
Benard et al. (unpublished)	29	Nulliparous	Endometrioid adenocarcinoma of the uterus Grade 1	TH + BSO and pelvic and para-aortic lymphadenectomy	Yes antagonist	In vivo oocytes retrieval with ultrasound guidance during laparotomic debulking surgery	0	1 MII

Kim et al., first published a case report of a 21-year-old woman who underwent a random-start COS followed by an *in-vivo* oocyte retrieval during open surgery for benign recurrent teratoma. She had a history of right oophorectomy and left ovarian cystectomy for an immature teratoma followed by 3 cycles of adjuvant chemotherapy with bleomycin, etoposide and cisplatin. Eight mature oocytes were obtained and vitrified (Kim et al., 2015).

Eskew et al., reported the case of a young woman with Mayer-Rokitansky-Küster-Hauser syndrome and bilateral ovarian benign masses. Given the inaccessibility of the ovaries transvaginally (too high-located, short vaginal length), the pick-up of the oocytes was realized *in-vivo* during the laparotomy after long agonist stimulation. Twenty-four oocytes were retrieved, 15 mature and 5 blastocysts cryopreserved (Eskew et al., 2018).

The only case of *in-vivo* oocytes retrieval previously reported in gynecologic cancer was realized according to the technique of IVM without stimulation in a 38-year-old woman diagnosed with an ovarian adenocarcinoma with 3 mature oocytes obtained (Fadini et al., 2012).

Data on the impact of ovarian stimulation in the context of ovarian carcinoma are scarce and no recommendations exist (Rousset-Jablonski et al., 2018), but vitrification of *in-vivo* matured oocytes is the FP technique of choice. The definitive pathology confirmed a LGSOC, typically diagnosed in younger patients, with less aggressive behavior and relatively chemoresistant disease. Five year overall survival for LGSOC is 83% (Hannibal et al., 2014). LGSOC express more frequently estrogen and progesterone receptors, as a consequence prudence has been recommended in the case of potentially hormone-sensitive tumors (Rousset-Jablonski et al., 2018). A co-treatment with anti-aromatase could have been discussed. Given the chemotherapy planned, we considered the risk associated with hormonal stimulation as low.

In our case, spillage of tumoral cells in the peritoneal cavity was not an issue because the patient presented with a positive cytology before OS. To decrease the risk of dissemination of tumoral cells, rinsing was carried out at the end of the surgery. The *in-vivo* retrieval was finally decided to prevent vaginal contamination and to avoid time of ischemia. Moreover, ultrasound guidance is easier to perform in the operative field than on the piece of ovariectomy. It improves the aspiration of the follicles allowing an easy and complete oocytes harvesting. Laparotomy was chosen over laparoscopy for oncological reasons (lower risk of tumoral spillage and rupture), as advised by most gynecological oncological societies. In addition, ultrasound guidance was easier and more precise by laparotomy than it would have been if the surgery had been done by laparoscopy.

There is no evidence to conclude on the use of freezing ovarian tissue or the safety of later tissue grafting in women with epithelial ovarian cancer (Lavoue et al., 2019). However, in the future, alternative options might exist such as *in-vitro* culture of primordial follicles or artificial ovary, increasing the chances of young cancer survivors of becoming genetic parents.

4. Conclusions

This is the first case of successful *in-vivo* mature oocytes retrieval after ovarian stimulation in the cours of open surgery for ovarian cancer surgery during first line treatment. This approach should be considered when transvaginal access is contra-indicated. Prior ovarian stimulation allows retrieval of *in-vivo* matured oocytes, and pick-up with direct application of the transvaginal ultrasound probe on the ovary is feasibleand avoids any time of ischemia for gametes.

Conflict of interests

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Julie Benard has no conflict of interest.

Isabelle Streuli has no conflict of interest.
Antonella Biondo has no conflict of interest.
Patrick Petignat has no conflict of interest.
Manuela Undurraga Malinverno has no conflict of interest.

Author contributions

Julie Benard, Manuela Undurraga Malinverno and Isabelle Streuli: writing, data analysis and editing.
Antonella Biondo and Patrick Petignat: editing.

Patient consent

Fully informed consent was obtained from the patient before drafting the case report.

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