Hyperactivation of dormant primordial follicles in ovarian endometrioma patients

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Abstract

Serum anti-Müllerian hormone (AMH) levels decrease after surgical treatment of ovarian endometrioma. This is the main reason that surgery for ovarian endometrioma endometriosis is not recommended before *in vitro* fertilization, unless the patient has severe pain or suspected malignant cysts. Furthermore, it has been suggested that ovarian endometrioma itself damages ovarian reserve. This raises two important challenges: (1) determining how to prevent surgical damage to the ovarian reserve in women with ovarian endometrioma and severe pain requiring surgical treatment and (2) deciding the best treatment for women with ovarian endometrioma without pain, who do not wish to conceive immediately. The mechanisms underlying the decline in ovarian reserve are potentially induced by both ovarian endometrioma and surgical injury but the relative contribution of each process has not been determined. Data obtained from various animal models and human studies suggest that hyperactivation of dormant primordial follicles caused by the local microenvironment of ovarian endometrioma (mechanical and/or chemical cues) is the main factor responsible for the decreased primordial follicle numbers in women with ovarian endometrioma. However, surgical injury also induces hyperactivation of dormant primordial follicles, which may further reduce ovarian reserve after removal of the endometriosis. Although further studies are required to elucidate the mechanisms underlying diminished ovarian reserve in women with ovarian endometrioma, the available data strongly suggests the need to prevent/minimize hyperactivation of dormant primordial follicles, regardless of whether surgery is performed, for better clinical management of ovarian endometrioma.

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Introduction

Endometriosis is defined as the presence of endometrial glandular and stromal tissue outside of the uterine cavity (Giudice & Kao 2004). The most frequently affected organ is the ovary (Giudice & Kao 2004). Ovarian endometriosis typically presents as an ovarian cyst (ovarian endometrioma) (Giudice & Kao 2004). A key concern in the surgical treatment of ovarian endometrioma is whether the surgery has a negative impact on the ‘ovarian reserve’. Ovarian reserve is a poorly defined term that is usually used to refer to the remaining number of dormant primordial follicles in the ovary. However, the term is often used interchangeably with other indices of ovarian function such as antral follicle count (AFC) or serum anti-Müllerian hormone (AMH) levels (Gleicher *et al.* 2011). To avoid confusion caused by the interchangeable use of the term ‘ovarian reserve’, this article will examine how ovarian endometrioma and surgical treatment affect the primordial follicle number, antral follicle count and serum AMH level, considering each parameter as a distinct concept.

To date, many clinical studies have evaluated the impact of surgical treatment of ovarian endometrioma on ovarian reserve. However, as previous systematic reviews (Raffi *et al.* 2012, Somigliana *et al.* 2012) and a meta-analysis (Raffi *et al.* 2012) have shown, heterogeneity between study variables is high, such as mean diameter of cysts (range: 2.5–6.7 cm), length of follow-up (range: 1–12 months) and age (range: 18–46 years old). Furthermore, there were only studies with small sample sizes (less than 70 cases). Despite these limitations of previous clinical studies, there is clear evidence that serum anti-Müllerian hormone (AMH) levels are significantly reduced after surgical treatment of ovarian endometrioma (Raffi *et al.* 2012, Somigliana *et al.* 2012). The serum level of AMH is often considered to be an indicator of ovarian reserve (Practice Committee of the American Society for Reproductive Medicine 2012) and therefore, surgical treatment of ovarian endometrioma is not recommended before *in vitro* fertilization (IVF) unless the patient is experiencing pain that interferes with daily life or an ultrasound detects rapid cyst growth or malignant features (Lessey

et al. 2018). In 2012, *Somigliana et al.* (2012) proposed the need for larger studies aiming to elucidate the mechanisms of surgery-induced damage to the ovarian reserve in patients with endometriosis. However, the mechanisms remain unclear to date. Furthermore, serum AMH levels are reportedly decreased in patients with ovarian endometrioma before surgery (*Muzii et al.* 2018). A meta-analysis showed that the endometrioma-affected ovary had lower AFC both before and after surgery (*Muzii et al.* 2014) suggesting that ovarian endometrioma itself, can alter the number of developing ovarian follicles. Recent systematic reviews and meta-analysis (*Brink Laursen et al.* 2017, *Nickkho-Amiry et al.* 2018), including only one randomized clinical trial (RCT) (*Demiröl et al.* 2006), one prospective and eight retrospective observational studies showed that there is no difference in the odds ratio of live birth between women who underwent surgery for ovarian endometriomas before IVF/intracytoplasmic sperm injection (ICSI) compared with conservative management. Despite the low quality of evidence, both studies concluded that there is no evidence that surgical removal of ovarian endometrioma before IVF/ICSI results in better fertility outcomes. To date, no study has shown that conservative management ovarian endometrioma is superior to surgical treatment before IVF/ICSI in terms of fertility outcomes.

Thus, the major challenges are: (1) determining if surgical treatment of ovarian endometrioma would decrease the primordial follicle reserve, (2) determining how to prevent surgical damage to ovarian function in women with ovarian endometrioma and pain who require surgery, and (3) the best treatment for women with ovarian endometrioma without pain but who do not wish to conceive immediately, considering the harmful effect of surgery on ovarian reserve.

What is ovarian reserve?

Ovarian reserve has been only inadequately defined (*Gleicher et al.* 2011). *Gleicher et al.* (2011) clearly defined that total ovarian reserve mostly consists of non-growing follicles (largely primordial follicles) and to a lesser degree, very slow-growing primary follicles. Because only a limited number of growing follicles survive after recruitment (*Adhikari & Liu* 2009), the still unrecruited primordial follicles represent the true remaining ovarian reserve (*Gleicher et al.* 2011). Follicle stimulating hormone (FSH), AMH and AFCs are used to assess recruited growing follicles but currently, there are no clinical tools to assess numbers of non-growing follicles (*Gleicher et al.* 2011) (Table 1).

Primordial follicle activation signaling pathways

Primordial follicle activation initiates follicle growth and development and this irreversible process is the primary

Table 1 Clinical tools for ovarian reserve assessment.

	Clinical tools
Total ovarian reserve	NA
Non-growing follicles	NA
Recruited growing follicles	FSH
	AMH
	AFCs

mode of primordial follicle depletion (*Adhikari & Liu* 2009). Long-term maintenance of dormant primordial follicles is therefore crucial for sustaining female reproduction throughout life (*McGee & Hsueh* 2000, *Adhikari & Liu* 2009).

The molecular mechanisms underlying the activation of mammalian primordial follicles have not been fully elucidated. The PI3K/PTEN/Akt/FOXO3 and PI3K/Akt/mTOR signaling pathways are the best-characterized regulators of primordial follicle activation during initial recruitment (*Adhikari & Liu* 2009, *Hsueh et al.* 2015). FOXO3 is an essential regulator of follicular quiescence in the dormant oocytes of primordial follicles but FOXO3 phosphorylation by Akt suppresses this function (*Liu et al.* 2007). The PI3K/Akt/mTOR pathway is also critical for primordial follicle activation (*Reddy et al.* 2008, *Adhikari et al.* 2010). Genetic deletion of PTEN or TSC1/TSC2, negative regulators of mTOR, leads to the simultaneous activation of the entire pool of primordial follicles in mice (*Reddy et al.* 2008, *Adhikari et al.* 2010). The activation of primordial follicles can be blocked with mTOR inhibitors (*Goldman et al.* 2017).

The Hippo signaling pathway is an evolutionarily conserved pathway involved in regulating organ size and development (*Yu et al.* 2015). Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ, also known as *Wwtr1*) are major downstream effectors of the Hippo pathway, which also regulates tissue homeostasis, tissue regeneration and tumorigenesis (*Yu et al.* 2015). The core components of the Hippo signaling pathway are present in oocytes, luteal cells, ovarian stroma and thecal cells but the highest level of ovarian expression can be found in granulosa cells in primordial and developing follicles (*Sun et al.* 2015, *Xiang et al.* 2015, *Grosbois & Demeestere* 2018, *Lv et al.* 2019). The Hippo/YAP pathway is a key regulator of granulosa cell proliferation and oocyte maturation, including the process of primordial follicle activation (*Hsueh et al.* 2015).

The Hippo pathway is regulated by intrinsic cellular cues, such as cell–cell contact, cell polarity, the actin cytoskeleton and a wide range of signals, including cellular energy status, mechanical cues and hormonal signals acting via G-protein-coupled receptors (*Aragona et al.* 2013). Extracellular matrix (ECM) stiffness regulates YAP and TAZ subcellular localization via changes in cell geometry and cytoskeleton tension (*Dupont et al.* 2011). YAP and TAZ remain inactive in the cytoplasm

when cells are grown on low-stiffness ECM, whereas high-stiffness ECM induces activation and nuclear translocation in both mouse mammary epithelial cells and human mesenchymal stem cells (Dupont *et al.* 2011). YAP and TAZ activities are also modulated by stretching and edge/curvature contouring of human mammary gland epithelial sheets (Aragona *et al.* 2013). TAZ activity is modulated by cellular compression in zebrafish granulosa cells (Xia *et al.* 2019) and Yorkie, an ortholog of YAP, is activated by stretching in drosophila ovarian follicle cell epithelium (Borreguero-Muñoz *et al.* 2019). The investigation of YAP/TAZ signaling in mammalian ovaries to date, has primarily focused on biochemical manipulation of these pathways rather than mechanical intervention. However, some evidence suggests that hydrogels with different biomechanical properties alter the rate of primordial follicle activation in 3D hydrogel cultures (Kniazeva *et al.* 2015).

The impact of endometrioma on primordial follicle activation

Primordial follicle density is lower in the ovarian cortex surrounding endometriomas than in contralateral, unaffected ovaries (Kitajima *et al.* 2011) and in the tissue surrounding non-endometriotic benign cysts (Schubert *et al.* 2005). The PI3K/PTEN/Akt/FOXO3, PI3K/Akt/mTOR and Hippo/YAP pathways are involved in the pathophysiology of endometriosis (Matsuzaki & Darcha 2015, Song *et al.* 2016, Takeuchi *et al.* 2019) hence, endometrioma may increase rates of primordial follicle activation. Takeuchi *et al.* (2019) demonstrated that accelerated primordial activation also occurs in mouse models of endometriosis but this effect could be inhibited by administration of a PTEN/PI3K/Akt pathway inhibitor (AS101).

Mechanical cues extracellular matrix (ECM) stiffness and stretching

Ovarian endometrioma is characterized by dense fibrosis (Matsuzaki *et al.* 1999), and one of the hallmarks of fibrosis is tissue stiffening (Wells 2013). Persistent stretching of adjacent ovarian tissue by endometriomas has been hypothesized to diminish ovarian reserve (Somigliana *et al.* 2012).

A growing endometrioma may affect the mechanical cues capable of activating YAP and TAZ in the surrounding ovarian cortex. Persistent stretching of adjacent ovarian tissue by non-endometriotic benign cysts also occurs. However, studies have not identified decreased serum AMH levels in patients with non-endometriotic benign cysts before surgery (Muzii *et al.* 2018). Theoretically, larger-sized ovarian cysts stretch adjacent ovarian tissues to a greater degree. Although only a few studies have evaluated this, there is no clear evidence of a relationship

between the preoperative serum AMH level and ovarian cyst size, refuting a negative correlation between the size of ovarian endometriomas or non-endometriotic benign cysts and the serum AMH level (Raffi *et al.* 2012, Mohamed *et al.* 2016). Thus, these findings do not support the notion that persistent stretching of ovarian cyst tissue decreases ovarian reserve. However, the additive effects of different mechanical stimuli on mechanotransduction have been identified (Kang *et al.* 2011, Throm Quinlan *et al.* 2011). For example, the mechanical stimulation of cyclic strain combined with ultrasound can enhance the differentiation of pre-osteoblasts more efficiently than can simple stimuli (Kang *et al.* 2011). There are also interrelated effects of different stiffness and stretch levels on cell phenotypes *in vitro* (Throm Quinlan *et al.* 2011). Therefore, it is possible that, in endometriotic cysts, the combined mechanical effects of stretch and stiffness induce mechanotransduction of both outside-in and inside-out signaling and consequently activate YAP/TAZ.

Recent studies suggest that the Hippo signaling pathway is a key effector of Akt in the regulation of tissue growth (Grosbois & Demeestere 2018, Borreguero-Muñoz *et al.* 2019, Hu *et al.* 2019), alongside classical inhibition of FOXO and TSC1/2 to activate TORC1 (Chen *et al.* 2010). Importantly, activation of Akt alone is not sufficient to activate drosophila Yorkie (an ortholog of YAP and TAZ), because some type of mechanical stimulus is also necessary (Borreguero-Muñoz *et al.* 2019). In mice, Hippo/YAP1 regulates primordial follicle activation, which is mediated by Akt signaling (Hu *et al.* 2019). An *in vitro* study of human follicular activation showed that parallel transient decreases in the mRNA levels of TSC1 and LATS1, which are negative regulators of PI3K/Akt/mTOR and Hippo signaling, respectively, triggered spontaneous follicular activation via synchronized mechanisms (Grosbois & Demeestere 2018). This suggests that PI3K/Akt/mTOR and Hippo/YAP signaling synergistically accelerate primordial follicular recruitment.

Chemical cues growth factors, cytokines and oxidative stress

Chemical cues emanating from the endometrioma may also influence primordial follicle activation but this has not been studied in detail. There are numerous cytokines, chemokines and growth factors that can activate PI3K/Akt pathways in physiological and pathological settings (Yu & Cui 2016, Hoxhaj & Manning 2020). Among potential factors that may regulate the intraoocyte PI3K/Akt pathways activation (Adhikari & Liu 2009), several factors such as vascular endothelial growth factor (VEGF) (Donnez *et al.* 1998) are also involved in pathophysiology of ovarian endometriosis. Increased levels of proinflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF- α) have been shown in endometriotic tissues, endometriotic

cyst fluid and/or the peritoneal fluid of patients with endometriosis (Harada *et al.* 2001). *In vitro* experiments showed that IL-16 (Feeney *et al.* 2014) and IL-1 β (Passos *et al.* 2016) promote activation of primordial follicles in whole neonatal rat ovaries and bovine ovarian tissue, respectively. However, it is not known if the potential factors that activate primordial follicles are produced in substantial quantities, nor if these factors can diffuse into cortical stroma in sufficient concentrations to activate primordial follicles.

Reactive oxygen species (ROS) is increased in the endometriotic cyst wall as well as in the cyst fluid (Sanchez *et al.* 2014). ROS permeates into the surrounding cells or is formed in the healthy tissue as a response to the presence of a cyst (Sanchez *et al.* 2014). The normal ovarian cortex surrounding endometrioma is severely affected by oxidative stress (Matsuzaki & Schubert 2010). A marker of DNA damage (8-OHdG) was 10-fold higher in the ovarian cortex surrounding endometrioma than non-endometriotic benign cysts (Matsuzaki & Schubert 2010). Excessive oxidative stress activates PI3K/Akt signaling mainly by inhibiting phosphatases such as PTEN or directly activating oncogenes such as Akt in cancers (Koundouros & Poulogiannis 2018). Oxidative stress triggers intracellular signals that stimulate fibroproliferation and expression of pro-fibrotic factors, resulting in fibrosis (tissue stiffening) (Ramirez *et al.* 2007). Thus, oxidative stress can activate both YAP and PI3K/Akt signaling in the ovarian cortex surrounding ovarian endometrioma and induces hyperactivation of primordial follicles. Oxidative stress can also induce apoptosis in granulosa cells (Yang *et al.* 2017). IL-1 α may play a critical role in the age-related exhaustion of the ovarian reserve in mice by enhancing the expression of pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α and promoting apoptotic pathways (Uri-Belapolsky *et al.* 2014). Therefore, the possibility that primordial follicles near endometriomas are lost through mechanisms other than follicle activation should be investigated further. Kitajima *et al.* (2011) examined atresia for each follicle based on histological criteria (Gougeon & Chainy 1987) and observed higher rates of atresia in small follicles in the ovarian cortex surrounding endometrioma, which adds plausibility to this concept.

The impact of surgery on primordial follicle activation

The impact of surgery on primordial follicle activation is unclear. A recent study in mice showed that surgical injury to the ovary can activate dormant primordial follicles near the surgical incision through the mTOR signaling pathway (He *et al.* 2017). mTOR is activated in multiple tissue injuries, such as neurons, muscles, the liver and the intestine (Wei *et al.* 2019). An *in vitro* experiment showed that human ovarian fragmentation results in immediate translocation of the Hippo-

effector YAP into the nucleus of granulosa cells, as well as increased follicular growth near the cutting site (Grosbois & Demeestere 2018). The Hippo pathway is regulated by intrinsic cell machineries, such as cell-cell contact, cell polarity and the actin cytoskeleton (Dupont *et al.* 2011, Aragona *et al.* 2013). Cell injury triggers actin polymerization (Aihara *et al.* 2018), which in turn activates YAP (Dupont *et al.* 2011).

The impact and the type of surgical injury to the ovary in patients with ovarian endometrioma may differ from that of either mouse ovarian surgery or *in vitro* fragmentation of human ovary. However, surgical treatment of ovarian endometrioma even by experienced surgeons using meticulous surgical techniques may inevitably lead to cell injury within the ovary, resulting in the Hippo/YAP signaling pathway activation. Further *in vivo* studies are required to investigate whether surgical injury could activate dormant primordial follicles near the injury site of ovary through the activation of the Hippo/YAP signaling pathway.

The possibility that surgery-induced local inflammation could activate primordial follicles and/or affect ovarian reserve have not been examined in detail. However, studies suggested that uncontrolled inflammation may adversely affect ovarian reserve (Bromfield & Sheldon 2013, Uri-Belapolsky *et al.* 2014, Passos *et al.* 2016). A mouse study showed that lipopolysaccharide (LPS) increases primordial follicle activation through the PI3K/PTEN/Akt/FOXO3 signaling pathway, resulting in diminished ovarian reserve (Bromfield & Sheldon 2013). Elevated levels of IL-1 β and IL-1 α may affect ovarian reserve by promoting the activation of primordial follicles and/or apoptotic pathways within the ovarian follicles (Uri-Belapolsky *et al.* 2014, Passos *et al.* 2016). The ability to conduct experiments in humans examining the effect of ovarian surgery alone is limited and the differences in the anatomy of rodent ovaries restricts extrapolation of experimental findings to humans. Large-animal experiments are now needed to better quantify the consequences of surgery on ovarian reserve using ovarian tissue with similar anatomy to human ovaries.

Serum AMH levels before and after ovarian surgery

In recent years, a number of studies have attempted to examine ovarian reserve by measuring serum AMH levels before and after ovarian surgery. AMH is produced by the granulosa cells of developing follicles (predominantly small antral follicles) and thus provides an estimate of the antral follicle count (Jeppesen *et al.* 2013). The main advantage of serum AMH measurements is that they are relatively easy to perform, whereas AFC is determined by transvaginal ultrasound, a comparatively time-consuming process requiring experienced operators.

In studies measuring serum AMH levels before and after ovarian surgery, decline in AMH can be observed within 1 week of surgery for patients with either ovarian

endometriomas or non-endometriotic benign ovarian cysts (Chang *et al.* 2010, Raffi *et al.* 2012, Mohamed *et al.* 2016). It has been estimated that a recently activated primary follicle requires ≥ 350 days to reach the small antral stage, with further maturation to the preovulatory stage occurring over an additional three ovarian cycles (Gougeon 2010). If the number of primordial follicles is reduced during ovarian surgery, changes in AMH levels would only become apparent after at least 12 months. This is when the first of the primordial follicles activated after the surgery would reach the small antral stage and begin to produce AMH in measurable quantities. Changes in serum AMH in the week after surgery are more likely to reflect altered function in the antral follicle population present at the time of surgery.

Reduced AMH levels are also observed at 3, 6 and 12 months after surgical removal of ovarian endometriomas, with some studies suggesting that the recovery of serum AMH level is delayed in patients with ovarian endometrioma compared with non-endometriotic benign ovarian cysts (Chang *et al.* 2010, Kostrzewa *et al.* 2019). The majority of studies interpret these declines as reduced ovarian reserve, typically referring to reduced primordial follicle numbers caused by the surgical intervention. Again, the timescales are too short to make inferences about changes in primordial follicle loss from serum AMH levels. Longer follow-up periods beyond 12-months post-surgery are required. Few, if any, developing follicles beyond the early primary stage are present in normal ovarian cortex removed from patients with ovarian endometrioma (Muzii *et al.* 2002) suggesting that surgery does not reduce serum AMH by removing the AMH-producing follicles. Interestingly, the post-surgery reductions in serum AMH can occur without substantial changes in AFC (Muzii *et al.* 2014, Kostrzewa *et al.* 2019), which suggest that the changes in AMH relate to subsequent change in the function or survival of the antral follicles, rather than primordial follicles.

Before surgery, women with endometrioma tend to have lower AMH levels than unaffected women or women with non-endometrial ovarian cysts (Goodman *et al.* 2016, Kasapoglu *et al.* 2018, Muzii *et al.* 2018). In a study where blood samples were taken 6-months apart without any surgical intervention in between, AMH levels declined about 3.5-fold faster in women with endometrioma compared to unaffected women (Kasapoglu *et al.* 2018). This adds further evidence to the hypothesis that endometrioma is detrimental to ovarian function in the absence of surgery. However, this further complicates interpretation AMH-based studies because it is not clear how much of the failure of AMH to return to pre-surgical levels are caused by the surgery, compared to the underlying pathology of endometriosis, which is not well understood. However, the AMH-data collected to date suggests that the effects observed

relate to changes in antral follicle function. Studies with appropriate designs to investigate changes in primordial follicle numbers have not yet been conducted.

Limitations in examining in ovarian reserve with serum AMH

Serum AMH levels are often incorrectly extrapolated as measure of the ovarian reserve. AFC, serum AMH levels and primordial follicle numbers all decline with age (Cui *et al.* 2016) and therefore, these variables are all loosely correlated (Hansen *et al.* 2011). However, the correlations are not particularly strong and there is evidence that women with similar numbers of primordial follicles can have different primordial follicle activation rates and thus differences in the size of their developing follicle pools (Richardson *et al.* 1987). The inaccuracy of serum AMH as a biomarker of primordial follicle numbers was confirmed in a recent study utilizing histological counts of ovarian cortical biopsies, the gold-standard for examining ovarian reserve (von Wolff *et al.* 2020). At face-value, these findings appear to be inconsistent with recent reports demonstrating that serum AMH levels can predict the remaining time-to-menopause (Depmann *et al.* 2018). However, these tests were only accurate in the 5–6 years immediately prior to menopause (Depmann *et al.* 2018) providing further evidence that AMH is not a suitable predictor of primordial follicle numbers in young women.

In a clinical setting, serum AMH is primarily used to predict the ovarian response to controlled ovarian stimulation for ART (Dewailly *et al.* 2014). A large number of recruitable small antral follicles should release a large quantity of AMH into serum (Jeppesen *et al.* 2013). Very low serum AMH levels indicate that the chance of obtaining oocytes will be low, whereas high AMH levels indicate the patient is at risk of ovarian hyperstimulation syndrome (Dewailly *et al.* 2014). However, there are also issues with the use of AMH as a marker of the AFC because developing ovarian follicles do not produce AMH at a constant rate. AMH production begins when follicles reach the primary stage, with expression levels increasing to the preantral/small antral stage and slowly declining at subsequent stages (Weenen *et al.* 2004, Jeppesen *et al.* 2013). Controlled ovarian stimulation leads to a rapid decline in serum AMH (Lee *et al.* 2010), presumably because the majority of smaller antral follicles rapidly develop into large antral follicles that then cease AMH production. Pathology can also cause changes in AMH gene-expression, as individual ovarian follicles in women with PCOS produce higher levels of AMH than similar-sized follicles in unaffected women (Pigny *et al.* 2003). The possibility that endometriosis leads to a similar change in the per-follicle output of AMH has not been investigated.

Figure 1 Potential impacts of local environment of the ovarian cortex surrounding ovarian endometrioma on ovarian reserve. The local microenvironment of ovarian endometrioma (mechanical cues and/or chemical cues) dysregulates maintenance of dormant primordial follicle by over-recruitment of primordial follicles through hyperactivation of dormant primordial follicles, resulting in diminished ovarian reserve over time. AMH, anti-Müllerian hormone.

The AFC is determined by ultrasound which detects follicles with a fluid-filled antrum but cannot differentiate between healthy follicles and those in the early stages of atresia. AMH is not expressed in follicles undergoing atresia (Weenen *et al.* 2004). Therefore, two women with identical numbers of antral follicles, but different rates of atresia or differences in the composition of small and large follicles, would be expected to have different serum AMH levels. Therefore, we recommend caution in the interpretation that changes in serum AMH levels before and after ovarian surgery are caused by changes in the ovarian reserve. Primordial follicle numbers are not the only determinant of serum AMH levels.

Figure 2 Potential impacts of surgical injury on ovarian reserve. Surgical injury induces apoptosis (atresia) in AMH-producing growing follicles, resulting in an immediate decrease in the serum AMH level after surgery. Surgical injury also induces hyperactivation of dormant primordial follicles, which reduces the true remaining total ovarian reserve after surgery. AMH, anti-Müllerian hormone.

Summary

Data from animals and humans suggest that hyperactivation of dormant primordial follicles caused by the local microenvironment of ovarian endometrioma (mechanical and/or chemical cues) may be the main cause of decreased primordial follicle numbers in patients with ovarian endometrioma (Fig. 1). Thus, conservative management of ovarian endometrioma might decrease ovarian reserve over time (Fig. 1). Surgical injury may induce apoptosis (atresia) in AMH-producing follicles, resulting in an immediate decrease in serum AMH levels (Fig. 2), but no significant effect on AFC, in patients with ovarian endometrioma. Furthermore, hyperactivation of dormant primordial follicles by surgical injury may further diminish ovarian reserve after removal of the endometriosis (Fig. 2). Thus, surgical injury may not be the main cause of the decreased primordial follicle numbers in endometrioma patients but might accelerate follicle activation. Potential signaling pathways of hyperactivation of dormant primordial follicles in patients with ovarian endometrioma are summarized in Table 2.

Perspective

Although further studies are required to elucidate the mechanisms underlying the reduced ovarian reserve in patients with endometriosis, previous studies strongly suggest the importance of preventing/minimizing hyperactivation of dormant primordial follicles, regardless of whether surgery is performed, for better clinical management of patients with ovarian endometrioma. A recent study in mice showed that AMH completely blocked primordial follicle activation, representing a reversible contraceptive agent that mitigates damage to the ovarian reserve associated with gonadotoxic chemotherapy (Kano *et al.* 2017). Another mouse study showed that inhibition of the

Table 2 Potential signaling pathways of hyperactivation of dormant primordial follicles in patients with ovarian endometrioma.

	Pathway(s)	Reference(s)
Local environment of ovarian endometrioma		
Mechanical cues	PI3K/PTEN/Akt/FOXO3	Dupont <i>et al.</i> (2011), Aragona <i>et al.</i> (2013), Borreguero-Muñoz <i>et al.</i> (2019)
Matrix stiffness	PI3K/Akt/mTOR	
Stretching	Hippo/YAP	
Chemical cues	PI3K/Akt	Adhikari & Liu (2009), Koundouros & Poulogiannis (2018)
Growth factors	Hippo/YAP	
Cytokines		
Oxidative stress		
Ovarian surgery		
Surgery-induced	PI3K/Akt/mTOR	Dupont <i>et al.</i> (2011), Bromfield & Sheldon (2013), He <i>et al.</i> (2017), Grosbois & Demeestere (2018)
Injury	PI3K/PTEN/Akt/FOXO3	
Inflammation	Hippo/YAP	

mTOR complex or mTORC1/2 prevents activation of primordial follicles, while preserving ovarian function and normal fertility, during genotoxic chemotherapy (Goldman *et al.* 2017). Rapamycin, a mTOR inhibitor, completely blocked the injury-induced activation of primordial follicles (He *et al.* 2017).

For patients with ovarian endometrioma without pain and with no desire to conceive immediately, treatment with drugs that reversibly block primordial follicle activation without any significant side effects on female health, could be an attractive strategy in patients with ovarian endometrioma, although to date no such drug has yet been developed. For patients with ovarian endometrioma with pain, pre- and post-operative treatment with such drugs may also be effective for preventing hyperactivation of primordial follicles induced by surgical injury.

Further research should focus on the mechanisms underlying hyperactivation of dormant primordial follicles in the local microenvironment of an ovarian endometrioma, and how to prevent such hyperactivation in patients with ovarian endometrioma. The study that demonstrated primordial follicles near endometriomas are also lost through atresia (Kitajima *et al.* 2011) is convincing but additional studies that investigate the biomechanical and chemical mechanisms are still needed. To date, there is no long-term clinical study that evaluated the impacts of either ovarian endometrioma itself or surgical injury on ovarian reserve. Because there are no clinical tools to assess numbers of non-growing follicles, the true remaining ovarian reserve (Gleicher *et al.* 2011), long-term clinical studies are required to investigate whether conservative management and/or surgical treatment of ovarian endometrioma might significantly decrease either serum AMH or AFC over time. Development of clinical tools to assess 'numbers of non-growing follicles' could significantly improve our clinical management of patients with ovarian endometrioma.

Caution is needed in the interpretation of changes in AMH levels following surgery and there is a need to be wary of any study claiming that a decline in AMH level indicates a decline in 'ovarian reserve'.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

S M and M W P were involved in concept, acquisition of data, analysis, interpretation of data, drafting the article and critical revision of the article. All authors read and approved the final version of the paper.

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