The impact of laparoscopic ovarian drilling on AMH & ovarian reserve: a meta-analysis

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Abstract

Laparoscopic ovarian drilling (LOD) has been widely utilised as an effective treatment in anovulatory women with polycystic ovarian syndrome (PCOS). However, there has been a growing concern over a possible damaging effect of this procedure on ovarian reserve. The objective of this study was to investigate the hypothesis that LOD compromises ovarian reserve as measured by post-operative changes in circulating anti-Müllerian hormone (AMH). This meta-analysis included all cohort studies as well as randomised controlled trials investigating serum AMH concentrations and other ovarian reserve markers in PCOS women undergoing LOD. Various databases were searched including MEDLINE, EMBASE, Dynamed Plus, ScienceDirect, TRIP database, ClinicalTrials.gov and Cochrane Library from January 2000 to December 2016. Sixty studies were identified, of which seven were deemed eligible for this review. AMH data were extracted from each study and entered into RevMan software to calculate the weighted mean difference (WMD) between pre- and post-operative values. Pooled analysis of all studies (n=442) revealed a statistically significant decline in serum AMH concentration after LOD (WMD -2.13ng/ml; 95% confidence interval (CI) -2.97 to -1.30). Subgroup analysis based on duration of follow-up, AMH kit, laterality of surgery and amount of energy applied during LOD consistently showed a statistically significant fall in serum AMH concentration. In conclusion**,** although LOD seems to markedly reduce circulating AMH, it remains uncertain whether this reflects a real damage to ovarian reserve or normalisation of the high preoperative serum AMH levels. Further long-term studies on ovarian reserve after LOD are required to address this uncertainty.

Key words: Anti-Müllerian hormone, Laparoscopic ovarian drilling, ovarian diathermy, ovarian electrocautery, ovarian reserve, polycystic ovarian syndrome

Introduction

Polycystic ovarian syndrome (PCOS) is a very common ovarian endocrinopathy with a prevalence of 6-20% amongst women of reproductive age (Yildiz *et al.* 2012) and about 90% amongst women with anovulatory of infertility (Hull 1987). It is characterized by a varied combination of clinical (anovulation and hyperandrogenism), biochemical (excess serum luteinizing hormone and androgen concentrations) and ovarian morphological (polycystic ovaries) features.

For PCOS women with anovulatory infertility, laparoscopic ovarian drilling (LOD) has been well-established as a successful second line treatment for ovulation induction after failure of clomiphene citrate (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2008, Farquhar *et al.* 2012). In addition to being as effective as gonadotrophin ovarian stimulation, LOD, offers several advantages over this treatment such as avoiding ovarian hyperstimulation syndrome and multiple pregnancies, reducing costs and negating the need for complex monitoring (Bayram *et al.* 2004, Farquhar *et al.* 2012). Furthermore, with LOD, a single treatment leads to repeated physiological ovulatory cycles and potentially repeated pregnancies without the need for repeated courses of medical treatment. Moreover, several follow-up studies provided evidence of long-term reproductive and endocrinological benefits of LOD (Gjønnæss 1998, Amer *et al.* 2002, Nahuis *et al.* 2012). We have previously reported long-term improvement in menstrual cycles and reproductive performance in about a third of PCOS women undergoing LOD for up to nine years (Amer *et al.* 2002). Similarly, Nahuis *et al.* (2012) followed PCOS women for up to 12 years after LOD reporting high pregnancy rate (61% conception of a second child) with long-term improvement of menstrual cycles in 44% of cases.

Despite its proven efficacy, there has been a growing concern over the possible damaging effect of LOD on ovarian reserve. Our group and several other researchers have previously reported a significant reduction in serum anti-Müllerian hormone after LOD (Weerakiet *et al.* 2007, Amer *et al.* 2009, Elmashad 2011, Farzadi *et al.* 2012, Syam *et al.* 2014, Sunj *et al.* 2014a, Rezk *et al.* 2016, Giampaolino *et al.* 2016). However, given the relatively small numbers of patients included in these studies, further evidence is required to allow a firm conclusion.

Anti-Müllerian hormone (AMH) is exclusively secreted by granulosa cells of growing follicles including primary, pre-antral, small antral (4-6 mm) and to less extent larger antral follicles (7-9 mm) (Weenen *et al.* 2004, Anderson *et al.* 2010). Thus, circulating AMH is now widely accepted as a reliable marker for ovarian reserve (Coccia and Rizzello, 2008; Robertson, 2008; Andersen *et al.* 2010). Furthermore, serum AMH concentration is generally stable with minimal inter- and intra-cycle fluctuations making it an ideal candidate for detecting small changes in ovarian reserve following LOD (Lambert-Messerlian *et al.* 2016).

Based on the above, we have designed this systematic review and meta-analysis aiming to investigate the impact of LOD on ovarian reserve as determined mainly by serum AMH levels.

Materials and Methods:

This meta-analysis was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (Liberati *et al.* 2009) and was registered in PROSPERO (CRD42016039687).

Inclusion criteria

This meta-analysis included all published cohort studies as well as randomized controlled trials (RCTs) that investigated changes in serum anti-Müllerian hormone (AMH) concentration in anovulatory women with PCOS undergoing LOD.

Outcome measures

Primary measure:

This included postoperative changes in serum AMH concentration.

Secondary measures:

These included postoperative changes in serum follicle stimulating hormone (FSH) concentration and antral follicle count (AFC) on ultrasound scan.

Search strategy

A detailed electronic search was conducted using numerous databases from January 2000 to June

2016 to identify studies investigating the effect of laparoscopic ovarian drilling on circulating AMH levels and other markers of ovarian reserve. Databases included MEDLINE (31 studies), Embase (29 studies), Dynamed (0), ScienceDirect (0), TRIP database (0), ClinicalTrials.gov (0) and the Cochrane Library (0). Medical Subject Headings (MeSH) terms used included: laparoscopy, polycystic ovary syndrome, ovarian drilling, ovarian diathermy, ovarian electrocautery, ovarian reserve, anti-Müllerian hormone and antral follicle count. Search was limited to the English Language, adult Females of reproductive age. Three co-authors (AM, TE and AY) conducted the searches and then an accredited clinical librarian (CJ) independently repeated the search using the same criteria. All identified articles were retrieved, and their reference lists were manually checked for further relevant studies. Published conference abstracts, which could be identified from ScienceDirect database, were also considered for the analysis.

Study selection

Three investigators (AM, TE and AY) independently screened the title and abstract of all identified articles to assess relevance to our meta-analysis. In case of disagreement, the full text was retrieved and reviewed independently by a senior author (SA) for a final decision.

All identified articles were evaluated according to a standardized format including study design, methods, participant characteristics, intervention, and results. Three investigators scored the studies and collected the information independently (AM, TE, AY). In case of discrepancies in scoring between the three investigators, a consensus was reached after discussion or after involvement of the senior investigator (SA).

In two studies, the mean±SD of serum AMH was missing as data were presented as median and range (Amer *et al.* 2009, Sunj *et al.* 2014a). The mean±SD was also missing for AFC in two studies (Farzadi *et al.* 2012, Sunj *et al.* 2014a) and for FSH in four studies (Weerakiet *et al.* 2007, Sunj *et al.* 2014a, Giampaolino, et al, 2016, Rezk *et al.* 2016,). We obtained the mean±SD of serum AMH and FSH levels from the original data of our previous study (Amer *et al.* 2009). Authors of the other studies were contacted and three (Sunj *et al.* 2014, Giampaolino *et al.* 2016, Rezk *et al.* 2016) responded providing the missing data.

Quality of included studies and risk of bias assessment

Modified Newcastle-Ottawa scale was utilised for assessing the quality and risk of bias of the included studies (Raffi *et al.* 2012, Mohamed *et al.* 2016). Each article was scored according to three categories including selection (maximum three stars), comparability (four stars), and outcomes (two stars). Selection was rated according to recruitment bias, selection of consecutive patients and power calculation. Comparability was assessed based on adjustment of analysis for four confounders including patients' age (<40), baseline serum AMH, laterality of surgery and number of punctures according to estimated ovarian volume, type of instrument and energy used. Outcome was scored according to completeness of at least three-month follow-up after surgery. In the current analysis, we have given more weight to comparability factors and used the cut-off level of six stars with a minimum of three stars in the comparability category (Raffi *et al.* 2012, Mohamed *et al.* 2016). Table 1 shows the results of quality scores of the studies included in this analysis.

Data extraction and analysis

Pre- and post-operative mean±SD serum AMH (ng/ml) and FSH (mIU/ml) concentrations and AFC were extracted from the individual articles and entered into RevMan software (Review Manager, version 5.1, The Cochrane Collaboration, 2011; The Nordic Cochrane Centre, Copenhagen, Denmark) for meta-analysis. The weighted mean difference (WMD) between pre- and post-operative values was calculated. Statistical heterogeneity between studies was assessed by I-squared (I^2) statistics and values of ≥50% were indicative of high heterogeneity (Higgins *et al.* 2003). When heterogeneity was significant, a random-effect model was used for meta-analysis. Fixed effect meta-analysis was used when there was no significant heterogeneity.

Overall analysis of data from all studies was first performed, irrespective of duration of the follow up, laterality of surgery and type of AMH assay kit used. In studies with multiple measurements at different post-operative follow up points, the latest AMH measurement was used for the overall analysis. In order to account for confounding factors, subgroup analyses were performed based on duration of follow-up, AMH kits used, laterality (bilateral and unilateral) of LOD and amount of energy applied during LOD. No sensitivity analysis was performed as all the studies scored high on the Modified Newcastle-Ottawa scale indicating low risk of bias (Table 1).

Results

The electronic database search identified 60 studies. All articles were screened and relevant articles were fully reviewed for eligibility for the study objectives and inclusion criteria. As a result, seven articles were deemed eligible for this meta-analysis (Fig.1).

Excluded studies

Of the 60 identified articles, fifty-one did not use the anti-Müllerian hormone as a marker of ovarian reserve and were therefore excluded from this meta-analysis. Two further studies were excluded, one due to lack of preoperative serum AMH levels (postoperative AMH levels were compared with a control group) (Weerakiet *et al.* 2007), and the other one (Sunj *et al.* 2014b) due to duplication of another study (Sunj *et al.* 2014a), which is included in the meta-analysis.

Included studies

Details of the seven studies are shown in table 2.

Study design

This systematic review included five cohort studies (Amer *et al.* 2009, Elmashad 2011, Farzadi *et al.* 2012, Seyam *et al.* 2014, Sunj *et al.* 2014a) and two RCTs (Giampaolino *et al.* 2016, Rezk *et al.* 2016). The RCT by Rezk *et al.* (2016) compared unilateral versus bilateral LOD. The two arms of this RCT were combined and used as a cohort study in the overall analysis, and then each arm was included separately in subgroup analysis. The other RCT compared laparoscopic versus transvaginal hydro-laparoscopic ovarian drilling (TH-LOD) (Giampaolino *et al.* 2016). Only the LOD group of this RCT was included as a cohort study in the current meta-analysis (Giampaolino *et al.* 2016).

Participants

All studies used appropriate selection criteria and all participants underwent the same surgical techniques of LOD. Inclusion and exclusion criteria were appropriately reported in all studies. All patients were accounted for in all studies.

PCOS diagnosis

All seven studies included in this meta-analysis utilised Rotterdam criteria for the diagnosis of PCOS

(Amer *et al.* 2009, Elmashad 2011, Farzadi *et al.* 2012, Seyam *et al.* 2014, Sunj *et al.* 2014a, Rezk *et al.* 2016, Giampaolino *et al.* 2016).

Laparoscopic ovarian drilling

Laparoscopic ovarian drilling (LOD) was performed using monopolar diathermy needle in six studies (Amer *et al.* 2009, Elmashad 2011, Seyam *et al.* 2014, Sunj *et al.* 2014a, Rezk *et al.* 2016, Giampaolino *et al.* 2016). The remaining study used monopolar hook diathermy for LOD (Farzadi et al, 2012). One study randomised patients to undergo either LOD or TH-LOD, but only the LOD arm was included in the meta-analysis (Giampaolino et al, 2016).

With regards to the number of punctures and amount of energy delivered to the ovary during LOD, two studies reported four punctures per ovary at a power setting of 30W applied for 5 seconds per puncture i.e. 450 joules (J) per ovary (Amer *et al.* 2009, Elmashad 2011). In the two studies comparing bilateral versus unilateral LOD, the authors applied 600 J per ovary (5 punctures x 4s x 30W) in the bilateral group and 60 J per $1cm^3$ of ovarian volume in the unilateral group (delivered as 30W for 4s per puncture), which is equivalent to 627 J applied to a $10cm^3$ ovary (Sunj *et al.* 2014a, Rezk *et al.* 2016). Seyam and co-workers reported 4-6 punctures per ovary at a power of 30 W for 4–5 seconds per puncture i.e. 480 – 900 J per ovary (Seyam *et al.* 2014). Giampaolino *et al*. (2016) applied 3-6 punctures per ovary using 40 W for 4–5 seconds per puncture i.e. 480–1200 J per ovary. One study reported six to seven punctures per ovary, but no details were provided regarding the power setting or the duration of each puncture (Farzadi *et al*. 2012).

Concerning laterality, five studies reported that LOD was carried out bilaterally (Amer *et al.* 2009, Elmashad 2011, Farzadi *et al.* 2012, Seyam *et al.* 2014, Giampaolino *et al.* 2016). The remaining two studies compared unilateral versus bilateral LOD (Sunj *et al.* 2014a, Rezk *et al*. 2016).

Length of follow up after LOD

Six studies completed six-month follow-up after LOD, (Amer *et al.* 2009, Farzadi *et al.* 2012, Seyam *et al.* 2014, Sunj *et al.* 2014a, Rezk *et al.* 2016, Giampaolino *et al.* 2016) whilst the remaining study followed participants for three months (Elmashad 2011). Four studies carried out multiple measurements within one month (Amer *et al.* 2009, Farzadi *et al.* 2012, Seyam *et al.*

2014, Sunj *et al.* 2014a) and three months (Table 2) (Amer *et al.* 2009, Farzadi *et al.* 2012, Seyam *et al.* 2014, Rezk *et al.* 2016).

AMH kits

Four AMH kits were used in different studies (Table 2). Immunotech (IOT) AMH enzyme immunoassay kit (Immunotech, Beckman Coulter, Marseille, France) was used in Four studies (Amer *et al.* 2009, Elmashad 2011, Rezk *et al.* 2016; Seyam *et al.* 2014). The intra- and inter-assay coefficients of variation for this AMH assay are below 12.3% and 14.2%, respectively, with a detection limit of 0.14ng/ml. The modified AMH Gen II enzyme linked immunosorbent assay (ELISA) (Beckman Coulter, Chaska, MN, USA) was used by one study (Sunj *et al.* 2014a). The intra and inter-assay coefficients of variation for this AMH kit are both below 10%, with a detection limit of 0.08ng/ml. Farzadi *et al.* (2012) used AMH enzyme immunoassay (EIA) kit (ELAab & USCNLIFE, Wuhan ELAab Science Co.Ltd). The lowest detection limit of this assay is 0.053ng/ml according to instructions provided in the analysis kit.[11] The last study used DSL active AMH ELISA kit (Diagnostic Systems Laboratories, Webster TX). The intra-assay and interassay coefficients of variation for this kit were 4.6% and 8.0%, respectively, with a detection limit of 0.017ng/ml (Giampaolino *et al.* 2016).

Antral follicle count

Four studies reported the AFC as an outcome measure of ovarian reserve (Elmashad 2011, Farzadi *et al.* 2012, Rezk *et al.* 2016, Seyam *et al.* 2014). The authors of another study provided the AFC data, which were missing from the published article, in response to our communication (Sunj *et al.* 2014a). Elmashad (2011) defined AFC as the number of follicles measuring 2–9 mm in diameter. Seyam and co-workers (2014) defined AFC as the count of all follicles measuring 2-10 mm in diameter. The remaining three studies did not define the size of the follicles used for the AFC, but reported using the Rotterdam definition of polycystic ovaries (>12 follicles measuring 2-9 mm) (Farzadi *et al.* 2012, Rezk *et al.* 2016, Sunj *et al.* 2014a).

Potential source of bias

In all seven studies, selection methods were clearly described and recruitment followed a consecutive fashion. This made it possible to assess selection bias in all studies.

Pooled results

Overall results

Table 3 shows mean±SD serum AMH concentrations before and after LOD in all seven studies. Pooled analysis of all seven studies including 442 participants revealed a statistically significant decline of serum AMH concentration after LOD (WMD -2.13ng/ml; 95% confidence interval (CI) -2.97 to -1.30). Heterogeneity between studies was high (I^2 = 87 %) (Fig. 2) (Amer *et al.* 2009, Elmashad 2011, Farzadi *et al.* 2012, Seyam *et al.* 2014, Sunj *et al.* 2014a, Giampaolino *et al.* 2016, Rezk *et al.* 2016).

Subgroup analysis

Studies using different AMH assays

Pooled results of four studies (n=197) using IOT AMH kit showing a statistically significant drop in serum AMH concentration (WMD -2.80; 95% CI -3.22 to -2.38; I^2=0%) with low heterogeneity between studies (Amer *et al.* 2009, Elmashad 2011, Seyam *et al.* 2014, Rezk *et al.* 2016). Each of the other three AMH assays (Modified Gen II, DSL and Abbott Diagnostic kits) was used by one study and meta-analysis was therefore not possible (Farzadi *et al.* 2012, Sunj *et al.* 2014a, Giampaolino *et al.* 2016).

Studies with different length of follow-up

Analysis of four studies including 195 patients revealed a statistically significant decline in serum AMH concentration one month after LOD (WMD -2.11; 95% CI -2.62 to -1.59; I^2= 17%) (Amer *et al.* 2009, Farzadi *et al.* 2012, Seyam *et al.* 2014, Sunj *et al.* 2014a). Similarly, analysis of data from five studies (n=277) with a three-month follow-up showed a statistically significant fall in serum AMH concentration after surgery (WMD, -2.74; 95% CI -3.16 to -2.33; I^2=0%) (Amer *et al.* 2009, Elmashad 2011, Farzadi *et al.* 2012, Seyam *et al.* 2014, Rezk *et al.* 2016). Analysis of six studies (n=419) showed a statistically significant decline in serum AMH concentration six months after LOD (WMD,

-2.03; 95% CI -2.90 to –1.16; I^2= 88%) (Amer *et al.* 2009, Farzadi *et al.* 2012, Seyam *et al.* 2014, Sunj *et al.* 2014a, Giampaolino *et al.* 2016, Rezk *et al.* 2016).

Laterality of LOD

Bilateral LOD was performed in seven studies including 341 patients (Amer *et al.* 2009, Elmashad 2011, Farzadi *et al.* 2012, Seyam *et al.* 2014, Sunj *et al.* 2014a, Giampaolino *et al.* 2016, Rezk *et al.* 2016). Pooled analysis of these studies revealed a statistically significant drop in circulating serum AMH (WMD -2.31; 95% CI -3.29 to -1.33; I^2= 87%). Analysis of two studies (n=101) measuring serum AMH changes after unilateral LOD showed a statistically significant decline in postoperative serum AMH concentration (WMD -1.59; 95% CI -2.69 to -0.49; I^2= 69%) (Sunj *et al.* 2014a, Rezk *et al.* 2016).

Sub-analysis *According to energy delivered to ovaries during LOD*

Four studies including 253 patients used up to 600 J in ovarian drilling. Pooled analysis of these studies revealed a statistically significant drop in postoperative serum AMH levels (WMD -2.45; 95% CI -3.41 to -1.48; I^2= 72%) (Amer *et al.* 2009, Elmashad 2011, Sunj *et al.* 2014a, Rezk *et al.* 2016). Two studies with 159 patients were identified using up to 900-1200 J in ovarian drilling. Pooled analysis of the results showed a statistically significant decline in postoperative serum AMH concentrations (WMD -1.93; 95% CI -3.72 to -0.14; I^2= 94%) (Seyam *et al.* 2014, Giampaolino *et al,*.2016).

Secondary outcomes:

Table 4 shows serum FSH concentrations before and after LOD in six studies (n=412). Pooled analysis of these studies revealed no change in circulating FSH (WMD 0.03; 95% CI -0.46 to 0.52; I^2= 90%) (Amer *et al.* 2009, Elmashad 2011, Seyam *et al.* 2014, Sunj *et al.* 2014a, Giampaolino *et al.* 2016, Rezk *et al.* 2016).

Five studies measured post-LOD changes in AFC, of which one was excluded due to lacking postoperative mean±SD AFC (Farzadi *et al*. 2012). Table 5 shows AFC results of the included four studies. Pooled data of these studies showed no significant change in AFC (WMD -3.46; 95% CI -10.73 to 3.81; I^2= 99%) (Elmashad 2011, Seyam *et al.* 2014, Sunj *et al.* 2014a, Rezk *et al.* 2016).

Further analysis was carried out to AFC follow-up within three and six months. Follow-up within three months were carried out with four studies including 264 patients. Pooled analysis of the results showed no significant changes in AFC after surgery (WMD -5.51; 95% CI -11.20 to 0.19; I^2= 99%) (Elmashad 2011, Seyam *et al.* 2014, Sunj *et al.* 2014a, Rezk *et al.* 2016). Three studies with 241 patients were identified performing follow-up assessment of AFC at six months. Pooled analysis of these studies revealed no significant change to AFC postoperative (WMD 0.04; 95% CI -5.52 to 5.59; I^2= 98%) (Seyam *et al.* 2014, Sunj *et al.* 2014a, Rezk *et al.* 2016).

Discussion

This is the first systematic review and meta-analysis to investigate the impact of LOD on ovarian reserve as determined by changes in postoperative serum AMH concentration. The overall analysis revealed a marked decline of 2.13 ng/ml, which represents 43% of the cut-off level of serum AMH concentration (4.9ng/ml) in women with PCOS (Dewailly *et al*. 2011). This decline in circulating AMH seems to be sustained for up to six months after LOD. Further subgroup analysis taking into account all possible confounding factors consistently showed a significant decline in postoperative serum AMH. The sub-analysis including studies with one- and three-month follow-up and studies using IOT AMH kit revealed low heterogeneity. This suggests that the high heterogeneity between studies seems to be due to variation in the follow-up periods and in the AMH assay kits used.

The exact mechanism of the post-LOD fall in circulating AMH remains uncertain. A possible explanation could be a decrease in AMH synthesis due to loss of the primary, pre-antral and small antral follicles, which are the main source of AMH, as a result of thermal damage during LOD (Weenen *et al.* 2004, Anderson *et al.* 2010). This hypothesis is further supported by the preliminary finding of an obvious trend towards a decline in AFC after LOD, although this did not reach statistical significance, possibly due to the small numbers involved in that analysis and the high heterogeneity. Furthermore, we have recently reported a similar decline of circulating AMH following ovarian cystectomy (Raffi *et al*. 2012, Mohamed *et al*. 2016). This suggests that any surgical trauma to the ovary is associated with loss of ovarian follicles with subsequent reduction in AMH production. Whether this effect on AFC and AMH is temporary with subsequent recovery remains to be investigated with further long term studies. Interestingly, two studies, which are included in this meta-analysis, reported a significant postoperative decline of AFC, which was sustained for up to six months in one study (Seyam *et al.* 2014), but seemed to have recovered at six-month follow-up in the other study (Rezk *et al.* 2016). These conflicting data may explain the outcome of the pooled analysis, which revealed no significant change in AFC at six-month follow-up. Further adequately designed short, medium and long-term cohort studies are required to address this issue.

It is interesting to see that even unilateral ovarian drilling caused a significant decline in circulating AMH, refuting the hypothesis that unilateral treatment could be less damaging to the ovarian reserve. It is worth mentioning that ovulation and pregnancy rates were higher in women undergoing bilateral versus unilateral ovarian drilling (Rezk *et al*. 2016). It is therefore possible to conclude that limiting the drilling to one ovary may compromise the success rates without any significant benefits to ovarian reserve. It was also interesting to see that despite the wide variation of the amount of energy used in different studies ranging between 450 and 1200 J per ovary, the decline in circulating AMH was more or less similar in all studies. This suggests that the range of energy doses utilised in these studies seem to be relatively safe to ovarian function with no excessive tissue damage with the higher doses.

One of the two RCTs in this meta-analysis compared AMH changes after LOD ***vs.*** transvaginal hydro-laparoscopic ovarian drilling (TH-LOD) (Giampaolino *et al.* 2016). In order to minimise heterogeneity between studies we decided to exclude the group undergoing TH-LOD due to the significant differences in techniques between this approach and the standard LOD used in all included studies. Whilst TH-LOD utilises bipolar diathermy, LOD on the other hand uses monopolar diathermy. Interestingly, there was no difference in the AMH changes after TH-LOD (Preoperative AMH, 5.84±1.16 ***vs.*** postoperative, 4.83±1.10 ng/l, $p<0.0001$) compared to LOD (6.06±1.18 ***vs.*** 5.00±1.29 ng/l, $p<0.0001$) (Giampaolino *et al.* 2016). This suggests that the degree of ovarian tissue damage is similar between the two energy modalities. This is surprising as bipolar diathermy is believed to reduce the risk of excessive ovarian tissue necrosis compared to monopolar energy.

It is well-known that serum AMH levels are generally stable with minimal inter- and intra-cycle variations and with a very gradual decline (5.6% per year) with advancing age (Api 2009). We therefore believe that a 43% decline in AMH level after LOD is a marked drop. However, it is still uncertain whether this reflects a real decline in ovarian reserve or merely reflects normalization of the high preoperative serum AMH levels, which is a characteristic feature of PCOS (Amer *et al*. 2004). The well-established high pregnancy rates as well as the well-documented positive long-term reproductive effects of LOD favours the AMH normalisation hypothesis (Amer *et al*. 2002, Gjønnæss 1998, Api 2009). This is further supported by the lack of any effect of LOD on circulating FSH. However, further long-term studies of ovarian reserve after LOD are required to support one of the above two hypotheses. Based on our findings and until further long-term data become available, clinicians could continue to offer LOD to their PCOS patients after carefully weighing the well-known benefits against the potential risks to ovarian reserve.

The main limitation of this review is the high heterogeneity between studies, possibly due to the variation in the AMH assay and amount of energy delivered to the ovary during LOD. Although, all studies used similar techniques of LOD, there were differences in the amount of energy delivered to the ovary with some studies applying 450 - 600J per ovary (Amer et al, 2009; Elmashad, 2011; Rezk et al, 2016; Seyam et al, 2014) whilst others delivering up to 900 J (Seyam *et al*. 2014) and 1200 J (Giampaolino *et al*. 2016) per ovary.

In conclusion, LOD significantly lowers circulating AMH, but this may not necessarily reflect a real damage to ovarian reserve. Given its proven efficacy and its long-term benefits, LOD should remain as an option in the management of anovulatory PCOS patients.

Declaration of interest

The authors report no conflict of interest

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Figure legends

Figure 1. PRISMA Flow Chart of the study selection process

Figure 2. WMD in serum AMH concentrations after laparoscopic ovarian drilling: pooled results for all seven studies

Abbreviations: AMH, anti-Müllerian hormone; CI, confidence interval; WMD, weighted mean difference.

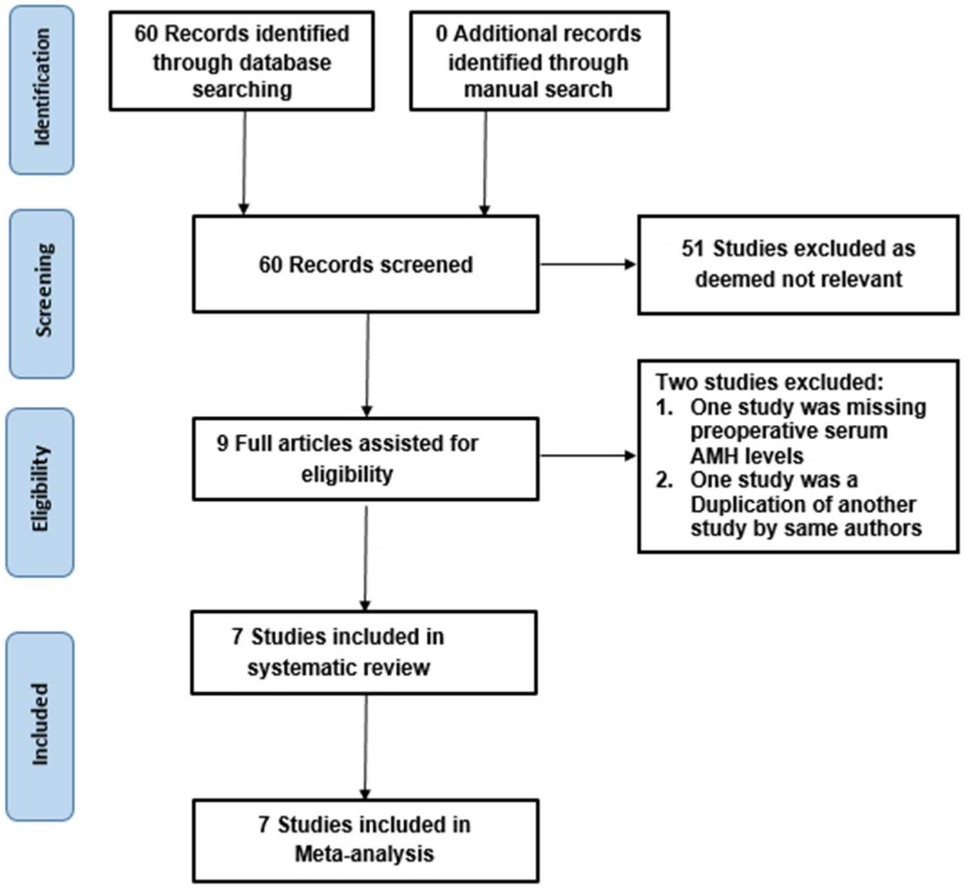

Figure 1. PRISMA Flow Chart of the study selection process

195x180mm (72 x 72 DPI)

Study or Subgroup	Postoperative Mean [ng/ml]	SD [ng/ml]	Total	preoperative Mean [ng/ml]	SD [ng/ml]	Total	Weight	Mean Difference IV, Random, 95% CI [ng/ml]
Amer 2009	3.68	2.5	29	7.19	5	29	9.2%	-3.51 [-5.54, -1.48]
Elmashad 2011	4.2	2.5	23	7.4	4.6	23	8.7%	-3.20 [-5.34, -1.06]
Farzadi 2012	7.7	4.4	30	8.4	4.7	30	8.0%	-0.70 [-3.00, 1.60]
Giampaolino 2016	5	1.29	119	6.06	1.18	119	20.0%	-1.06 [-1.37, -0.75]
Rezk 2016	5.9	1.3	105	8.6	2.3	105	19.1%	-2.70 [-3.21, -2.19]
Seyam 2014	3.1	1.5	40	5.99	2.3	40	16.9%	-2.89 [-3.74, -2.04]
Sunj 2014	5.6	1.8	96	7.03	2.85	96	18.1%	-1.43 [-2.10, -0.76]
Total (95% CI)			442			442	100.0%	-2.13 [-2.97, -1.30]

Heterogeneity: $Tau^2 = 0.88$; $Chi^2 = 44.58$, df = 6 ($P < 0.00001$); $I^2 = 87\%$
Test for overall effect: $Z = 5.02$ ($P < 0.00001$)

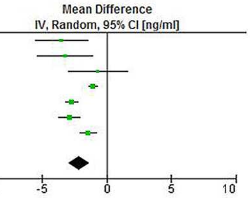

Figure 2. WMD in serum AMH concentrations after laparoscopic ovarian drilling: pooled results for all seven studies
Abbreviations: AMH, anti-Müllerian hormone; CI, confidence interval; WMD, weighted mean difference.

1764x428mm (96 x 96 DPI)

Table 1 Modified Newcastle Ottawa scale for risk of bias and quality assessment of the included studies

Author	Year	Selection (***)	Comparability (****)	Outcome (**)	Overall
Amer *et al.*	2009	**	****	**	8
Elmashad	2011	*	****	**	7
Farzadi *et al.*	2012	**	***	**	7
Seyam *et al.*	2014	**	****	**	8
Sunj *et al.*	2014a	**	****	**	8
Giampaolino *et al.*	2016	**	***	**	7
Rezk *et al.*	2016	**	****	**	8

Table 2 Characteristics of the seven studies included in the meta-analysis

Authors & year	country	Design	n	Age (years) mean±SD	Laterality	Energy per ovary (J)	FU Months	AMH assay	Secondary Outcomes
Amer *et al.* 2009	UK	Prospective cohort	29	28.4±0.9	Bilateral	450	6	IOT	FSH
Elmashad 2011	Kuwait	Prospective cohort	23	28.8±3.1	Bilateral	450	3	IOT	FSH, OV, AFC
Farzadi *et al.* 2012	Iran	Prospective cohort	30	28.4±2.3	Bilateral	***	6	EIA	AFC
Seyam *et al.* 2014	Egypt	Prospective cohort	40	31.6±4.5	Bilateral	480-900	6	IOT	FSH, AFC
Sunj *et al.* 2014a	Croatia	Prospective cohort	96	29.3±3.3 29.3±3.1	Unilateral=49 Bilateral=47	600 ~627¶	6	Gen II	FSH, AFC, OV
Giampaolino *et al.* 2016	Italy	RCT*	119	18-40**	Bilateral	480-1200	6	DSL	FSH
Rezk *et al.* 2016	Egypt	RCT	105	29.7±1.5 29.8±1.4	Unilateral=52 Bilateral=53	600 ~627¶	6	IOT	AFC, FSH

* RCT Arm 1, laparoscopy included in the study; Arm 2, laparotomy excluded
** age range of participants, SD not available
*** 6-7 punctures per ovary, but no data provided on energy
¶ energy delivered as 60 J per 1cm^3 of ovarian volume, which is equivalent to **627** J per a 10cm3 ovary
Abbreviation: **RCT**, randomised controlled trial; **FU**, follow up; **J**, Joules; **OV**, ovarian volume; **IOT**, Immunotech AMH enzyme immunoassay; **EIA**, enzyme immunoassay (ELAab & USCNLIFE); **DSL**, Diagnostic System Laboratories ELISA AMH kit

Table 3 Pre- and Post-operative serum AMH concentrations in all analysed studies

Reference	***n***	**Laterality**	**Serum AMH** (ng/ml), mean±SD			
			Preoperative	Postoperative (1 month)	Postoperative (3 month)	Postoperative (6 month)
Amer *et al.* 2009	29	Bilateral	7.19 ± 5.0	6.75 ± 5.70	5.33 ± 3.90	3.68 ± 2.50
Elmashad 2011	23	Bilateral	7.40 ± 4.60	—	4.20 ± 2.50	—
Farzadi *et al.* 2012	30	Bilateral	8.40 ± 4.70	7.50 ± 4.50	7.00 ± 4.50	7.70 ± 4.40
Seyam *et al.* 2014	40	Bilateral	5.99 ± 2.30	3.40 ± 1.70	3.20 ± 1.70	3.10 ± 1.50
Sunj *et al.* 2014a	49	Unilateral	6.67 ± 2.89	5.02 ± 2.05	—	5.70 ± 2.05
	47	Bilateral	7.42 ± 2.78	4.98 ± 1.68	—	5.60 ± 1.70
	96	Overall	7.03 ± 2.85	5.00 ± 1.80	—	5.60 ± 1.80
Giampaolino *et al.* 2016	119	Bilateral	6.06 ± 1.18	—	—	5.00 ± 1.29
Rezk *et al.* 2016	52	Unilateral	8.60 ± 2.30	—	6.40 ± 1.20	6.50 ± 1.30
	53	Bilateral	8.70 ± 2.40	—	5.20 ± 1.30	5.50 ± 1.10
	105	Overall	8.60 ± 2.30	—	5.79 ± 1.30	5.90 ± 1.30

Table 4 Pre- and Post-operative serum FSH concentrations in all analysed studies.

Reference	***n***	**Laterality**	**Serum FSH** (IU/L), mean±S.D.			
			Preoperative	Postoperative (1 month)	Postoperative (3 month)	Postoperative (6 month)
Amer *et al.* 2009	29	Bilateral	5.3 ± 1.4	4.9±1.6	—	—
Elmashad 2011	23	Bilateral	4.9 ± 1.6	—	4.1 ± 1.4	—
Seyam *et al.* 2014	40	Bilateral	5.4 ± 2.7	5.7 ± 2.3	5.5 ± 2.1	5.45 ± 2.4
Sunj *et al.* 2014a	49	Unilateral	—	—	—	—
	47	Bilateral	—	—	—	—
	96	Overall	5.2 ± 1.17	5.7 ± 1.2	—	6.1 ± 1.2
Rezk *et al.* 2016	52	Unilateral	5.3 ± 1.4	—	5.41 ± 1.3	5.26 ± 1.4
	53	Bilateral	5.5 ± 1.2	—	5.52 ± 1.3	5.49 ± 1.3
	105	Overall	5.4 ± 1.3	—	—	5.3 ± 1.3

Table 5 Pre- and Post-operative antral follicle count (AFC) in all analysed studies.

Reference	***n***	**Laterality**	**AFC**, mean±S.D.			
			Preoperative	Postoperative (1 month)	Postoperative (3 month)	Postoperative (6 month)
Elmashad 2011	23	Bilateral	29.0 ± 2.4	—	15.0 ± 2.2	—
Seyam *et al.* 2014	40	Bilateral	16.75 ± 3.2	14.2 ± 2.8	12.5 ± 2.6	12.2 ± 1.6
Sunj *et al.* 2014a	49	Unilateral	—	—	—	—
	47	Bilateral	—	—	—	—
	96	Overall	14.8 ± 2.7	14.8 ± 4.8	—	21.07 ± 8.2
Rezk *et al.* 2016	52	Unilateral	19.1 ± 5.4	—	15.2 ± 3.3	18.6 ± 3.1
	53	Bilateral	18.9 ± 5.5	—	15.1 ± 3.2	16.4 ± 3.2
	105	Overall	18.9 ± 5.4	—	15.1 ± 3.1	17.4 ± 3.3