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Phytotherapy for menopausal symptoms: recommendations of the Italian Menopause Society (SIM) and the Italian Society of Gynaecology for the Third Age (SIGiTE)

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ABSTRACT

Evidence on the efficacy of phythotherapic remedies for the treatment of menopausal symptoms were reviewed. Evidence of the literature were graded and level of reccomandation defined. A strenght A recommendation was given for isaproponol extract of *Cimicifuga recemosa* (systematic review and metanalysis). Strength B recommendation, due to some inconsistent findings or lower quality studies, was given to the use of soy isoflavones, genistein above 30 mg/day, equol containing product and pollen extract. Strength C reccomendation for very weak evidence of efficacy was given to resveratrol, hops, dioscorea, enotera. No evidence of efficacy on vasomotor symptoms was found for *Hyperycum perforatum*, matcha, ginseng, that find no place in the treatment of vasomotor symptoms.

SOMMARIO

Sono stati valutati gli studi sull'efficacia di rimedi fitotetrapici nel trattamento dei sintomi vasomotori. I livelli di evidenza sono stati definiti in base al tipo e numero di studi presenti e sulla base delle evidenze è stata formulate la forza delle raccomandazioni. Una raccomandazioe di tipo A è stata definita per l'estratto isopropilico della *Cimicifuga recemosa* (sulla base di review e meta-analisi). Una raccomandazione di tipo B, basata su alcuni dati inconsistenti o studi di più bassa qualità, è stata definita per l'uso degli isoflavoni della soia, genisteina a livelli superiori ai 30 mg/die, prodotti contenenti equolo, ed estratto di polline. Una raccomandazione di tipo C, dovuta ad evidenze molto deboli, è stata definita per l'uso del resveratrolo, del luppolo, della dioscorea o dell'olio di enotera. Nessuna evidenza di efficacia è stata trovata per l'*Hyperycum perforatum*, il matcha ed il ginseng che quindi non hanno nessun ruolo nella cura dei sintomi vasomotori della menopausa.

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Phytoestrogens; genistein; daidzein; equol; pollen extract; Cimicifuga racemosa; hot flush.

INTRODUCTION

The use of non-hormonal therapies and treatments for menopausal symptoms is becoming increasingly widespread in conventional healthcare settings, and there are numerous scientific publications dealing with complementary and alternative medicine (CAM). In particular, some CAM therapies are based on substances present in nature and derived from plants of various types in different concentrations and obtained via different extraction methods. Among these, nutraceuticals are chemicals of natural origin that are exploited for their therapeutic and pharmacological potential. For example, nutraceutical supplements and phytopharmaceuticals are often used for the treatment of menopausal vasomotor syndrome (MVS). MVS presents in more than 75% of women; typically, its symptoms last for 5-7 years, but many women present its symptoms for up to 10-15 years. Hormone replacement therapy (HRT) is the most effective treatment available for MVS symptoms, and is therefore the first-line choice. However, other therapeutic options are needed to treat those who are not candidates for HRT due to either contraindications, personal choice or individual preference. In fact, the majority of women would prefer non-hormonal treatments.
Due to the wide range of non-hormonal products available for the treatment of MVS, the Italian Menopause Society (SIM) and the Italian Society of Gynaecology for the Third Age (SIGiTE) think it is timely to emphasize several key aspects of these therapies further to ensuring medical integrity and patient safety. As physicians, we know that the medications we prescribe are substances with well-defined chemical structures whose pharmacokinetics are largely understood, and for which solid and reproducible data is available on their efficacy and safety. However, the purported benefits of phytopharmaceutical products, which are classified as non-hormonal treatments in alternative, complementary or supplementary medicine, are often only supported by small and less than rigorously conducted trials. Although extensive literature has been produced, it is difficult to give a clear and comprehensive assessment of the effectiveness of these products from a scientific perspective. This is mainly due to great differences and flaws in the design of the clinical trials conducted to date, which have often not been either randomized or controlled. Furthermore, efficacy testing in MVS must take into account the placebo effect and non-oestrogenic central nervous system (CNS) effects, as well as oestrogen-like mechanisms of action (selective oestrogen receptor modulator, or SERM, effects).
Neverthless, it is reassuring to know that to be marketed as such in Europe, nutraceutical substances have to undergo controls similar to those typical of drugs. It is good practice to check that such substances are EFSA (European Food Safety Authorities) certified, as they only grant marketing authorization after scientific assessment to demonstrate their efficacy and safety, as well as to verify the quality of raw materials and compliance with good manufacturing practice regulations.

PHYTOTHERAPIES AND NUTRACEUTICALS IN MENOPAUSE: CHARACTERISTICS AND EFFECTIVENESS

Phytoestrogens (isoflavones, lignans and coumestans)

Phytoestrogens are a large group of plant-derived non-steroidal phenol compounds with different chemical structures.

Mechanism of action
The oestrogen-like activities of phytoestrogens are linked to their ability to modulate estrogen receptors (ER). They have both agonistic and antagonistic effects, binding to both ERα and ERβ receptors, and activating ER-dependent gene transcription. Their binding affinity varies greatly from substance to substance, but is generally greater for ERα than ERβ. Functionally, their activity depends not only on their concentration, intestinal absorption and tissue selectivity, but also on individual concentrations of endogenous hormones and intestinal flora profile. Some of these molecules may also have antioxidant and/or antiproliferative activities, linked to their interactions with non-ER-dependent cellular mechanisms (1).
Phytoestrogens can be divided into three main groups:

1. Isoflavones: derived from soy, red clover and other roots, this group includes genistein and daidzein (which are the two compounds of greatest clinical interest), as well as glycitein, biochanin A, and formononetin.
2. Lignans: derived from flaxseeds and other seeds and foods, the lignans include enterodiol and enterolactone.

3. Coumestans: less relevant for the treatment of climacteric symptoms, include coumestrol and 4-methoxycoumestrol.

Genistein and daizein, the most promising active ingredients, are found in high concentrations in soybean and other soy products, but they are also contained in red clover and peanuts. Daizein, which is generally the most abundant substance in various isoflavones, has less intense oestrogen-like activity than genistein; its concentration differs depending on its source, *i.e.*, whether from soy seeds, whole bean or soy-derived proteins, which, in turn, can be extracted in various ways (2). The respective quantities also vary depending on the method of extraction and production of the individual pharmaceutical composition.

Isoflavones from Soybeans

The therapeutic efficacy of the different compounds containing soy extracts on the market changes in relation to several factors. First it depends on the concentrations of the active ingredients, which are essentially genistein and daidzein. These two active ingredients have different affinities for the oestrogen receptor – genistein is much more active than daidzein, and, in turn, the potency of genistein and daizein is 500 to 1000 times lower than that of oestradiol (3). The therapeutic efficacy of these active ingredients also depends very much on the ability of the individual to metabolize them at the intestinal level. They are absorbed in the form of glycosides (binding to a sugar molecule), and the resulting sugar is hydrolysed (and therefore eliminated) by beta-galactosidase intestinal bacteria. Daizein can be transformed into its active metabolite, equol, which, once absorbed, has oestrogenic action similar to that of genistein, being able to bind to both ERα and, with greater affinity, ERβ. However, only 25-30% of the adult female population of Western countries have the ability to metabolize daidzein by transforming it into equol (4). Indeed, equol is produced from daizein by intestinal microorganisms that naturally occur much more frequently in Oriental populations, and inoculation of the Western subjects with S-equol-producing bacteria or through the administration of pre- or probiotics, has thus far been unsuccessful (5).

Finally, it is important to bear in mind that isoflavones may take longer to take effect than the observation periods considered in most trials. In fact, according to studies by Lujin Li *et al*. (6, 7), it takes about 12 weeks of intake for soy isoflavones to achieve half of their maximum effect on vasomotor symptoms, while half the maximum effect of estradiol is reached after three weeks of intake.

Other effects

The positive effects of soy proteins on cardiovascular risk are still the subject of research and debate (8). An initial report by the American society of Cardiology, based on a 1995 meta-analysis, stated that a diet containing at least 25 grams of soy protein per day could reduce total and LDL cholesterol. However, subsequent trials have not confirmed this claim, reporting only modest effects on lipid metabolism (9). Later studies on different soy isoflavones have however, reported different levels of beneficial effects on cardiovascular risk factors, including changes in lipid and carbohydrate metabolism. These findings lend support to the theory that such compounds can reduce cardiovascular risk in the menopausal period (10). Moreover, recent studies have shown that some isoflavones, especially genistein, also exert a protective effect on the endothelium. Evidence from short-term RCTs and observational studies suggest that soy isoflavones, in particular S-equol, are anti-atherogenic, and improve arterial compliance, and may therefore potentially prevent cardiovascular disease and the associated cognitive decline (11, 12). However, many of the possible metabolic benefits of isoflavones may require even longer administration time frames than those needed for MVS therapy.

Isoflavones from Red Clover

Isoflavones found in red clover are biochanine A and formononetin. Both bind to ERα and ERβ, with greater affinity for the latter, but considerably lower than that of oestrogen. In regards to their effectiveness on vasomotor symptoms, the literature reports a fair number of trials, and a specific meta-analysis on red clover extracts (13, 14). However, many meta-analyses and literature reviews consider findings on red clover extract together with those of soybeans, drawing conclusions on the effectiveness of isoflavones as a whole. Hence, their effect on vasomotor symptoms is not entirely clear or definitive. They do not appear to reduce the number of hot flashes in 24 hours significantly (- 3), but a significant improvement in the Kuppermann Index has been reported (**table I**). Some studies have shown that red clover extracts are also effective when administered during the menopausal transition (15). As regards the dura-

tion of therapy and long-term efficacy, the literature only contains a few studies, none of which have reported significant adverse events or side effects.

Lignans from Flaxseed

Flaxseed is considered a rich source of lignans, such as enterodiol and enterolactone, which possess a weak oestrogenic action, capable of modulating oestrogen receptors with an action similar to that of SERMs (16). To date, however, reported effects are not always significant, and there is little evidence to support the use of flaxseeds for MVS therapy (17). That being said, there are other indications for their use in menopause, *i.e.*, for their anti-inflammatory and antioxidant effects, as well as improvements in lipid profile, reducing LDL cholesterol and increasing HDL cholesterol, associated with an improvement in glucose tolerance and a reduction in the risk of metabolic syndrome and atherosclerosis progression. In addition, some experimental evidence

Table I. *Major reviews and meta-analyses on the efficacy of soy isoflavones on vasomotor symptoms.*

Literature	Products	Conclusions	Trial quality	Duration
Nelson *et al*. (2006) (41) Review and meta-analysis of 17 RCTs in Cochrane database on non-hormonal therapies (isoflavones)	a) Red clover (40, 80 or 120 mg) b) Soybean extract (50, 70, or 150 mg)	Red clover does not reduce frequency of hot flashes Results on soy isoflavones are variable*	a) 6 trials (1 good quality, 2 moderate quality) b) 11 trials (8 moderate quality)	12–52 weeks 12–52 weeks
2012 Taku *et al*. (20) Review and meta-analysis of 13 RCTs on soy isoflavones *vs* placebo	54 mg soybean isoflavones (median)	Soy isoflavones reduce the frequency and intensity of hot flashes as compared to placebo	13 good quality studies	6–52 weeks
2013 Lethaby *et al*., Cochrane Review (42) Review of 43 RCTs on phytoestrogens, 39 on isoflavones	a) Genistein b) Soy extract c) Soy-rich diet d) Red clover extract e) Other phytoestrogens	Some trials show significant results for genistein concentrates. Generally non-conclusive evidence on effectiveness in reducing the frequency and severity of hot flashes	a) 4 moderate quality trials b) 11 trials (all sufficient quality) c) 13 trials (6 moderate quality, 1 sufficient) d) 5 trials (2 moderate quality) e) 6 sufficient/poor quality trials	12–104** Weeks
2015 NAMS (43) Position statement based on studies included in the main previous meta-analyses and original studies, predominantly RCTs	Takes into account trials published in previous meta-analyses	Only low-level recommendations for the effectiveness of S-equol (soybean derivative)	Studies with level II evidence (RCTs, systematic reviews with Level II studies or Level I studies with inconsistent results)	12–52 weeks
Chen *et al*. (2015) (44) Meta-analysis and review of 15 RCTs on phytoestrogens	Isoflavones S-equol Red clover	In some study groups meta-analysis has shown that soy isoflavones are effective in reducing the frequency of hot flashes	Differences in outcome measures: - 6/15 trials measured efficacy via KI# - 10/15 trials measured efficacy via hot flash frequency	Parameter not evaluated
Grant *et al*. (2015) (45) Review of comparative efficacy trials on different menopausal symptoms	Generic isoflavones as non-hormonal therapies	Low strength of evidence of efficacy of isoflavone treatment for vasomotor symptoms compared with placebo	5 good quality, 2 moderate, and 28 poor	Parameter not evaluated
2016 Franco *et al*. (46) Review and meta-analysis of 65 RCTs on nutraceutical products, 17 on isoflavones	Soy-rich diet Soy supplements and extracts Red clover extract	Supplementation with different compounds and specific isoflavones significantly associated with modest reductions in the frequency of daytime hot flashes	Does not specify quality assessment of the evidence considered	Parameter not evaluated
2017 Myers and Vigar (14) Meta-analysis of 3 RCTs *vs* placebo	Red clover 80 mg	Red clover significantly reduces hot flashes and KI	Good quality	Parameter not evaluated
2017 Moore *et al*. (47) General review of CAM RCTs and previous reviews (5 years)	Soy beans and soy isoflavones Red clover Phytoestrogens (isoflavones, lignans, coumestans, black cohosh)	Soybeans and soy isoflavones (especially supplements with S-equol) may be recommended for vasomotor symptoms	Does not specify quality assessment of the evidence considered	Parameter not evaluated
2019 DAILY *et al*. (48) Review and meta-analysis of 5 RCTs	S-equol Equol-containing soy isoflavone products	Both products improve hot flash score***	Does not specify quality assessment of the evidence considered	Parameter not evaluated

*Symptomatology improved significantly in 3 of the 7 trials; #Kupperman Index; **1 trial only; ***symptomatology improved in 3 of the 5 trials.

shows that lignans may have anticancer effects, reducing the growth of colon, breast and endometrial tumours; both *in vitro* and *in vivo* studies in mice injected with breast cancer cells show that flaxseeds increase or maintain the effectiveness of tamoxifen in reducing tumor growth and increase the apoptosis of neoplastic cells, while no effect on the activity of aromatase inhibitors has been observed (18).

Evidence on the effectiveness of Isoflavones in Vasomotor Syndrome

The literature on the use of treatments based on soy foods or extracts and the various active metabolites of isoflavones in vasomotor syndrome is very extensive. However, most studies have significant methodological limitations, many not being controlled or being carried out with very different and difficult-to-compare formulations. In addition, trials often have different objectives and evaluate efficacy in different ways, using different scales to assess menopausal symptoms (Kuppermann scale or Greene scale), and/or different criteria for the frequency or intensity of hot flashes and/or night sweats.

Quality of scientific studies

The quality of scientific literature is graded according to the following Levels of Evidence:

- Level 1: high quality randomized controlled trials (RCTs) (Ia) and systematic reviews and meta-analyses (Ib);
- Level II: lower quality RCTs, second-level systematic reviews or level I studies with inconsistent results;
- Level III: non-RCTs, case-control studies, systematic reviews including low-level studies;
- Level IV: case series, small case-control studies.

Strength of recommendations

The strength of recommendations based on the above are classified as follows:

a. Based on level Ia or Ib scientific information
b. Based on level II scientific information
c. Based on level III-IV information

Overall, soy isoflavones have an efficacy comparable to placebo (which in itself has an estimated efficacy of 30-50%), as in many studies their efficacy is not significantly higher. However, the oestrogen-like effect of soy isoflavones does result in a reduction in the frequency and intensity of hot flashes, which in some meta-analyses, mostly from Oriental authors, is statistically significant. Nevertheless, this effect seems to take a long time to reach maximal levels (at least 12 weeks to reach half the maximum effect) and is not always clinically significant (Evidence quality level II). This has led to a type B recommendation regarding the lower effectiveness of treatment of vasomotor symptomatology.

The numerous good quality meta-analyses have often arrived at inconclusive considerations on specific issues, because they take into account different products, dosages, evaluation methods and objectives. However, a number of good individual studies of specific products and dosages have yielded more meaningful conclusions about the effectiveness of these non-hormonal treatments on vasomotor symptoms. In order to clarify these concepts, **table I** provides a detailed overview of the most important and recent literature reviews and meta-analyses, illustrating the relative characteristics of the studies under consideration (**table I** and references).

An important aspect to be assessed with respect to the efficacy of such products is the evaluation of the dose, *i.e.*, the total amount of titrated isoflavones or dose of the specific active ingredient. In general, the dose required for isoflavones to achieve a biological effect is 50-90 mg per day, while the dose of isoflavones considered safe is 100 mg/day. Genistein at a dose of more than 30 mg/d was found to be more effective than placebo (evidence level II), leading to a recommendation strength B on genistein efficacy at a dose of more than 30 mg/day (19).

A meta-analysis by Japanese authors (20) found that, beyond the placebo effect, there is a 20.6% reduction in the frequency of hot flashes in 24 hours, and a 26.2% reduction in their intensity, in studies lasting at least 12 weeks, when at least 18.8 mg/day of genistein was administered. However, it is always necessary to consider the individual intestinal absorption capacity. As mentioned, the majority of the Western population is unable to convert daidzein to equol and therefore provide a satisfactory clinical response. Hence, some manufacturers directly include equol in their formulations, making them more effective even in populations such as Italians unable to activate intestinal conversion (evidence level II, recommendation strength B on the effectiveness of the equol).

Side effects

Based on the numerous studies in the literature, isoflavones are not associated with any major ad-

verse events (evidence level I; recommendation strength A). The most frequent side-effects are gastrointestinal irritation, with flatulence, diarrhoea and a sense of abdominal bloating.

Cancer risk

Due to the oestrogen-like effects of these substances, the risks related to breast cancer and endometrial hyperplasia (21, 22) have also been examined, although there have been few reliable studies in this respect. From the poor-quality and scarse literature that exists, however, it does not appear that phytoestrogens increase the risk of endometrial cancer, and, in fact, in combination with lignans seem to reduce the risk (evidence level III, recommendation strength C).
Few observational studies have been carried out on breast cancer risk, but in those involving a fairly large population overall (23), no increased risk of breast cancer was detected. An RCT on breast biopsies in pre-menopausal and post-menopausal women with early-stage breast cancer given doses of isoflavones of 100 mg/day for 7-30 days found no difference in KI-67 marker expression either pre- *vs* post-treatment or treatment *vs* placebo. However, two genes (FANCC and UGT2A1) were found to be upregulated in the treatment group, and overexpression of 126 genes involved in cell cycle and proliferation was observed in women with high plasma levels of genistein (> 16 ng/ml) *vs* women with low levels (< 6.8 ng/ml) (24). Other isolated studies have shown no histological or mammographic changes following the administration of isoflavones in humans either *in vitro* or *in vivo*. Nonetheless, some *in vitro* studies in animals have shown an increase in the proliferation of breast cancer cells with high doses of the most effective active ingredients, such as genistein (evidence level III, recommendation C on the cancer risk of high-dose genistein). In view of this, and the fact that no specific safety data in populations with breast cancer is available, soy isoflavone supplements are not recommended in such patients.

OTHER PHYTOESTROGENS

Hops

Hop flowers contain a potent flavonoid, 8-prenylnaringenin (8-PN), which is believed to exert more powerful oestrogenic activity than soy-derived isoflavones. In the few individual placebo-controlled and randomized studies we have to date, 8-PN has been shown to reduce the number of hot flashes and improve Greene's score (25) (evidence level III, recommendation strength C).

Resveratrol

Resveratrol is a non-flavonoid diphenol present in different varieties of plants (raspberries, blueberries, peanuts, *Polygonum cuspidatum* roots) and has been extracted in modest concentrations from fermented red grapes with particular fungal contaminations. It has weak phytoestrogenic activity, good bioavailability and good absorption, and exists in free and conjugated forms, but is metabolized very rapidly and consequently has a short half-life. Only a few studies have supported its effectiveness in MVS (evidence level III; recommendation strength C), while its antioxidant properties are much better known and extensively studied (26). Resveratrol is frequently administered in combination with other polyphenols, and in this role also exerts important synergistic activity on hot flashes.

OTHER PLANT-DERIVED NUTRACEUTICALS WITH NON-PHYTOESTROGENIC ACTIVITIES

Black Cohosh (Cimicifuga racemosa or Actaea racemosa)

This has long been used as a medicinal plant by Native Americans, and is currently the most widely used product for menopausal disorders. *Cimicifuga racemosa* (CR) rhizome has several chemical constituents that are extracted in various ways, including nitrogenous alkaloids, phenolic isoflavone compounds such as formononetin, organic acids such as isoferulic acid, and triterpenoids such as cimiracemoside A, 25-O-methyl-cimigenoside, actein, and 23-26-deoxy-actein. Formononetin and triterpenoids are some of its best known bioactive components.
Some initial research reported oestrogen-like effects, but isopropyl alcohol extract, which is the most widely used in current phytotherapy, is devoid of the phytoestrogenic component and therefore cannot exert oestrogen-like effects. Triterpene glucosides are the main bioactive component present in the alcoholic extract, and are responsible for therapeutic effects via central modulation of the se-

rotonin system (27). However, CR does not change circulating levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH) or oestradiol, and does not result in an increase in endometrial thickness; therefore, it has a mechanism of action unrelated to ER activation (28). Although its mechanism of action has not been fully clarified, several recent studies show that the anolic isoproponol extract of *Cimicifuga racemosa* (iCR) has some antioxidant and anti-inflammatory properties, and a mainly central neuromodulatory action (29).
According to the scientific literature, including a major meta-analysis (30), the effectiveness of CR on hot flashes is not higher than that of a placebo, but in this case too the important lack of homogeneity among scientific studies must be taken into account, as pointed out by a subsequent review. In particular, it is necessary to bear in mind the different species of black cohosh used and the different preparations thereof. Numerous individual studies conducted on the isoproponol preparation (iCR) provide significant evidence of its effectiveness in the treatment of MVS, despite a total lack of oestrogenic effects on the levels of circulating hormones. In particular (31), with regard to iCR, one meta-analysis on 28 clinical efficacy studies identified 9 placebo-controlled clinical trials on 9,391 patients which confirmed the efficacy (evidence level Ib, recommendation strength A) and safety (evidence level Ia) of iCR-based medicines.
Several trials have also demonstrated their safety in breast cancer patients, although the duration of observation in these trials was invariably limited (32). Furthermore, iCR can be used without pharmacological interference in patients treated with tamoxifen and aromatase inhibitors, oestrogen and/or progestin, platelet antiaggregants and/or anticoagulants. In general, adverse effects are rare, but hepatotoxicity, pain, abdominal cramps and jaundice have been reported. However, a review of reported cases of hepatotoxicity has made it clear that these effects are not associated with preparations of isopropanol extracts of CR (33).

Pollen extract

Cytoplasmic pollen extract (GC Fem), pistil extract (PI 82), and vitamin E products are also commercially available (34). These extracts are extremely purified, and seem to be safe even for patients prone to allergies. Both *in vitro* and *in vivo* studies demonstrate that these substances neither bind to oestrogen receptors nor exert any oestrogenic effects. Instead, their proposed mechanism of action, in addition to their antioxidant and anti-inflammatory properties, involves modulation of the serotonin system, in particular by inhibiting serotonin re-uptake. This may account for their effectiveness in the treatment of hot flashes, which has been demonstrated by a number of cohort studies and randomized trials reporting a significant reduction in climacteric symptoms (34) (evidence level II, recommendation strength B for vasomotor symptoms). In fact, in a randomized comparative trial (35) pollen extract was not only more effective than placebo, but also it attenuated climacteric symptoms to an extent only slightly lower than oestrogen–progestin therapy. Furthermore, the efficacy and safety of these products have been recently confirmed by other studies, overall showing improvements of women's quality of life (36, 37).

OTHER PLANT DERIVATIVES

Ginseng

Ginseng has no oestrogenic effects on FSH levels, endometrial thickness, vaginal maturation index or vaginal pH. Accordingly, ginseng has no effect on MVS, although some data in the literature show an improvement in depressive syndrome, Kupperman index and Menopause Rating Scale, but not on hot flashes.

Matcha

Matcha, grown mainly in South America, and for its edible root, is recommended as a tonic/adaptogen, against stress and tiredness, Anti-anaemic and aphrodisiac actions have been also reported. The mechanism of action is unknown, but it is has been hypothesized that it may modulate steroid receptors. Matcha contains a weak phytosterol, but the evidence does not support its use for the therapy of vasomotor symptoms.

St. John's Wort

This is a herb derivative (of *Hypericum perforatum*) which has shown effectiveness mainly on depressive symptoms of menopause, and partly on vasomotor symptoms. It may interfere with antidepressant drugs.

Wild Yam* (*Dioscorea*), *Dong Quai* (*Chinese Angelica*), *Evening Primrose* (*Enotera*) *Oil

Only a few low-evidence-level studies are currently available on this group of derivatives. In fact, as yet there is no reliable data on efficacy, minimum effective dose and long-term safety. Due to the limited evidence on efficacy and safety, there is no strong recommendation for their use in the therapy of vasomotor symptoms (38, 39) (evidence level III–IV, recommendation strength C). The few available studies refer to combinations of different substances with non-uniform doses, as often occurs in the Chinese phytotherapy tradition. Bacause of both the paucity of data and the low quality of the published articles (38, 39), it is not possible to give scientifically accurate recommendations regarding these preparations.

CONCLUSIONS

These recommendations have taken into account only some of the many products on the market, considering only those that underwent scientific assessment according to the current criteria for evidence-based medicine. Many of the studies conducted to date have a very limited sample size and period of treatment and do not provide long-term safety and efficacy data. Furthermore, only few studies compared the efficacy of different formulations. As it is the case of drugs, also for these products it is of particular importance to carefully assess contraindications. These may include previous adverse reactions to products of plant origin, or the intake of other medications that may pose a risk of pharmacological interaction. Thus, the choice of therapy will depend on the characteristics and clinical history of the patient, her previous and current use of any other therapeutic agent. The prescription of plant-derived supplements must also take into account the dietary habits of the subject; specifically, Western women tend to have very low dietary intake of soy isoflavones (0.27-1.43 mg/day) but, the diet of vegetarians or women who consume a lot of soy-based products (beverages, yoghurt and tofu, *etc*.) may contain similar amounts to those present in dietary supplements (30-80 mg/day) (40).

MVS generally improves over time, but if patients get some benefits from phytotherapy, there is no need to progressively reduce or stop it abruptly. Conversely, if a patient does not respond to treatment, it would be advisable to reassess her options, bearing in mind that 12 weeks of treatment with soy isoflavones are necessary to reach 50% of their maximum efficacy. In any case, therapy should be regularly re-evaluated, at least every 6-12 months, since the long-term safety and efficacy of these phytopharmaceuticals has not yet been determined.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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Intrahepatic cholestasis of pregnancy: a narrative review of the management

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ABSTRACT

Intrahepatic cholestasis (ICP) is the most common liver disease in pregnancy. It is a multifactorial disease characterized by a supraphysiological rise in bile acid level associated with maternal symptoms. ICP is associated with increased incidence of some fetal complication, such as respiratory distress, premature delivery and stillbirth. For these reasons early recognition, a specific monitoring and appropriate treatment during pregnancy are necessary to improve fetal outcome. However, the optimum management and the best time of delivery still remain unclear. The purpose of this review is to evaluate and compare the most recent definitions and guidelines for intrahepatic cholestasis of pregnancy, trying to provide a global overview improving early diagnosis and adequate management.

SOMMARIO

La colestasi intraepatica (ICP) è la più comune malattia epatica in gravidanza. È una malattia a origine multifattoriale, caratterizzata da un incremento della concentrazione degli acidi biliari associato a sintomi materni. Inoltre, la colestasi gravidica si associa a un'aumentata incidenza di alcune complicanze fetali, come ad esempio la sindrome da distress respiratorio, il parto pretermine e la morte intrauterina. Per questo motivo, una diagnosi precoce, un monitoraggio specifico dei markers biochimici e sintomi caratteristici di questa patologia e un appropriata terapia durante la gravidanza, sono fondamentali per ridurre le complicanze fetali. Tuttavia, il management ottimale di questa malattia e il momento migliore in cui indurre o espletare il parto non sono ancora chiari. L'obiettivo di questa review è analizzare e confrontare tra loro gli studi clinici e linee guida più recenti presenti in letteratura sulla colestasi intraepatica in gravidanza, con l'intento di fornire informazioni più dettagliate per definire i criteri diagnostici utili per la diagnosi precoce e stabilire i più adeguati protocolli diagnostici per curare la colestasi gravidica e ridurre così le sue complicanze materno-fetali.

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Key words
Intrahepatic cholestasis of pregnancy (ICP); bile acid; intrauterine fetal death; Ursodeoxycholic acid; ICP management; pruritus.

INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP), also called obstetric cholestasis, is a relevant and common liver disorder in pregnancy, usually occurring during the second or third trimester (1, 2) however, recent evidence showed that in rare situations ICP could be diagnosed as early as the first trimester. The gestational age at diagnosis does not differ significantly by severity, although severe ICP tended to be diagnosed around a week earlier. ICP is a pregnancy-specific condition with a multifactorial aetiology that includes environmental and hormonal contribution in genetically susceptible women. ICP is characterized by a supraphysiological rise in bile acid levels and pruritus, and requires careful monitoring during pregnancy because of the associated intrauterine fetal death. Fetal death is the worst complication of ICP; the cause of fetal death is not well understood; still, it possibly has a relationship to the toxic effects of bile acids on the fetal heart, causing arrhythmias and chorionic vasospasm causing deprivation of maternal oxygenated blood to the fetus causing asphyxia. For this reason, it is very important to have an active and apprehensive management.
However, clinical symptoms are variable and often unclear, with some patients paucisymptomatic and other symptomatic. Therefore, the early recognition and appropriate treatment of ICP should become a priority for obstetricians. In the last years, there were many attempts to find consensus about the management of ICP to provide an easier and earlier diagnosis and a consequent more accurate management of patients. The purpose of this review is to evaluate the most recent definitions and guidelines for ICP, to provide a global overview and improve early diagnosis and adequate management.

EPIDEMIOLOGY AND ETIOLOGY

The prevalence of ICP is influenced by genetic and environmental factors, varying between 0.7 and 5% in different population around the world (3). Risk factors include advanced maternal age, multiparity, history of oral contraceptive use, history of fertility treatment in women or twin pregnancies and a history of ICP during previous pregnancies (4-8). Interestingly, ICP is more common during colder months, for unknown reasons (4, 6, 9, 10-13). Seasonal variations in ICP have been assumed to be associated with dietary factors related to low maternal levels of selenium, zinc and vitamin D and high levels of copper (6, 14, 15).
The pathogenesis of ICP is poorly understood and is thought to be complex and multifactorial. Genetic susceptibility, hormonal, and environmental factors have been proposed as possible mechanisms. There appears to be a relation between cholestatic properties of reproductive hormones in genetically susceptible women and ICP. The supportive evidence for the genetic susceptibility hypothesis lies in the fact that the disease has been observed more in familiar clustering patterns, first-degree relative, and a higher risk of disease recurrence with subsequent pregnancies (16, 17). Recent studies showed mutations in gene (ABCB4) encoding for hepatobiliary canalicular translocator proteins called multidrug resistance 3 (MDR3) and pedigrees with the mode of inheritance being a sex-limited, dominant pattern (17-19). Other genes probably involved in the development of ICP are ATP8B1 (FIC1), ABCB11 (BSEP), ABCC2, and NR1H4 (FXR) (20-23).
The role of reproductive hormones in developing ICP has also been investigated in different studies. Many studies showed an association between high levels of estrogen conditions such as multiple pregnancy, ovarian hyper-stimulation effect and late second-trimester presentation of ICP (24).
In fact, ICP typically occurs in the late second trimester or in twin pregnancies, when the estrogen levels are the highest level and resolvers after delivery, when sex hormone levels fall down.
ICP shows similar characteristics seen in women taking contraceptive pills high in estrogen quantity. High circulating estrogen levels may induce cholestasis in genetically predisposed women in ICP (25). Physiopathologically, estrogen seems to reduce the expression of nuclear hepatic bile acid receptors and hepatic biliary canalicular transport proteins in genetically susceptible women causing impairment of hepatic bile acid homeostasis and subsequent increased level of bile acids (26). The role of progesterone is less understood; however, recent studies, including some animal model studies, have demonstrated that progesterone sulfated metabolites are partial agonists of farnesoid X receptor FXR (also called bile acid receptor) (26). The progesterone sulfate metabolites alter the hepatobiliary transport system by impairing the functioning of the main hepatic bile acid receptor (27, 28). A previous study showed that ICP may be associated with an increase in 3α-monosulfated and disulfat-

ed progesterone metabolites, compared with normal pregnancies. In fact, ICP symptom severity is correlated to sulfated progesterone metabolite levels in the urine of ICP women (29).
Finally, a number of studies have established that the gut microbiota changes during pregnancy, and this can be associated with the gestional metabolic alterations, including ICP, observed in late pregnancy (29-31).
Some Authors showed that imbalances in the maternal serum cytokine profile may be associated with ICP. In particular, the Authors showed that circulating proinflammatory cytokines are increased, including IL-6, IL-12, IL-17, and TNF-α, while anti-inflammatory cytokine IL-4 is decreased (32, 33). Work in obstructive cholestasis has suggested that bile acids levels associated with ICP cause the release of proinflammatory cytokines into the circulation that accumulate in the liver and can lead to hepatic injury (33).

CLINICAL PRESENTATION

The ICP is an exclusion diagnosis: elevated serum total bile acid levels (≥ 10 μmol/l) and normal or increased serum transaminases with pruritus are the typical characteristic findings of this pregnancy-specific disease (1, 2). ICP can be classified based on serum total bile acid levels in: mild cholestasis (10 < bile acids < 39 μmol/L), moderate cholestasis (40 < bile acids < 99 μmol/L) o severe cholestasis (bile acids > 100 μmol/L). The main symptom of this disease is generalized intense pruritus sine materia (without lesion, rash and other excoriation) that may precede biochemical abnormalities. It often develops after 25 weeks of gestation, with 80% of cases occurring after the 30th week of pregnancy (15).
Pruritus typically predominates on palms and soles of the feet and worsens at night, its location may be discriminatory for ICP diagnosis (4, 6). Other symptoms of cholestasis are nausea, anorexia, fatigue, right upper quadrant pain, dark urine, and pale stool, steatorrhea, malabsorption of fat-soluble vitamins and weight gain. Clinical jaundice is rare but may present in 14 to 25% of patients after 1 to 4 weeks from the onset of pruritus, with dark urine and pale feces (6, 9, 34). Some patients also complain of insomnia secondary to pruritus. Generally, the physical examination is unremarkable except for scratch marks on the skin from pruritus. Generally, ICP is a benign condition for the mother, in fact it has rapid postnatal resolution and pruritus often disappears in the first days following delivery. Symptoms, abnormal liver function and biochemical abnormalities, spontaneously and rapidly (within 6 weeks) revert to normal after delivery with good maternal prognosis. However, liver tests and bile acid concentrations controls are recommended to be performed during pregnancy and 6 to 8 weeks after delivery. According to recent data, ICP seems to be associated with an increased risk of developing other hepatobiliary diseases, such as hepatitis C, cirrhosis, and gallstones. In addition, patients with underlying chronic liver diseases (*e.g.*, hepatitis C or chronic hepatitis of different etiologies) have an increased risk of developing ICP (5, 13).
ICP is also associated with altered maternal lipid profiles: dyslipidaemia (35, 36), increased risk of gestational diabetes mellitus or impaired glucose tolerance (36, 37) and preeclampsia; therefore is important a strict follow-up for these diseases in women with ICP, especially among those with early presentation and twins' gestations. A large Swedish national cohort highlighted that patients with ICP had an OR of 2.62 (95% CI, 2.32-2.78) for preeclampsia (38). Raz at all showed that patients with total bile acid levels > 40 micromol/L have the highest risk of for eclampsia and preeclampsia. They also suggested that the diagnosis of preeclampsia occurs approximately 2-4 weeks after ICP diagnosis, and proteinuria preceded elevated blood pressure (39). Moreover, women with ICP are also at a higher risk for cardiovascular disease morbidity (40). Perhaps ICP is responsible for endothelial injury and atherosclerosis; therefore, blood lipid panel and cholesterol testing may be offered to higher risk women with ICP during the postpartum period, when pregnancy specific hormones lose their effect on plasma lipid profiles. In literature has been reported that postpartum hemorrhage risk is not increased among women with ICP (40).

FETAL RISKS

If on one side, generally, ICP is a benign condition for the mother, on the other side, it may be associated with a higher rate of adverse neonatal outcome (**table I**). ICP increases the risk of spontaneous and iatrogenic preterm labor, fetal distress, sudden intrauterine fetal death and admission to the neonatal unit (4, 9, 12, 41). Some studies have shown that adverse pregnancy outcomes were higher in patients diagnosed to have early onset

ICP compared to the late-onset ICP. The analysis revealed significantly higher rates of fetal distress, intrauterine growth restriction, preterm birth, and low birth weight in the early-onset ICP group (42). Many studies highlight the importance of regular monitoring of serum bile acid levels in women diagnosed with ICP and suggest that women with bile acid levels that do not exceed 40 micromoles/L may not be at an increased risk of fetal complications. In a prospective study in the United Kingdom of women with severe ICP (defined as bile acid levels ≥ 40 μmol/L), Geenes *et al*. (41) have found that women with ICP have an increased risk of preterm birth (25% *vs* 6.5%; aOR 5.39; 95% CI 4.17-6.98), neonatal unit admission (12% *vs* 5.6%; 95% CI 1.97-3.65), and stillbirth (1.5% *vs* 0.5%; aOR 2.58; 95% CI 1.03-6.49). They also found that meconium-stained amniotic fluid was associated with increasing levels of bile acids. Analysis with logistic regression by Otzas *et al*. have revealed that the probability of preterm delivery does not increase until (mean platelet volume) MPV levels exceeded 11.2 fL [odds ratio (OR) = 2.68, 95% confidence interval (CI) = 1.13-6.32, P = 0.025]. Total bilirubin levels exceeded 0.6 mg/dL (OR = 3.13, 95% CI = 1.21-8.09, P = 0.019) if we consider as outcome a low APGAR score, only increased postprandial total bile acid levels of ≥ 51 μmol/L are found to be significantly predictive (OR = 3.02) (43). Although the precise mechanism is not established, it is likely that elevated bile acids influence myometrial contraction leading to the increased pre-term labor rates. This is suggested by *in vivo* and *in vitro* data even if the laboratory findings were thousand times more concentrated than those, we observe in ICP affected women. Indeed, when bile acids are administered to pregnant ewes via an intravenous infusion pump, they have increased rates of preterm delivery, and different experiments demonstrated that the addition of bile acids to the culture medium of cultured uterine myocytes enhanced expression of the oxytocin receptor (1, 9). In addition, elevated bile acid concentrations impair cell membrane permeability and stimulate the release of prostaglandins, thus leading to enhanced uterine reactivity as well as increased sensitivity to oxytocin, which might cause preterm labor (44). Several experimental studies on animals showed that high bile acids levels have a harmful effect on cardiomyocytes (45). In rats, cardiomyocyte exposure to taurocholate provoked arrhythmias and impaired contractility. Therefore, it was hypothesized that the fetal deaths in ICP may be caused by an acute cardiac event caused by raised fetal serum taurocholate concentrations (16, 17). Finally, other recent studies showed that fetal cardiomyocytes express receptors for bile acids known as the nuclear bile acids receptor farnesoid-X receptor (FXR) that have been found to be involved in cardiac injury and cardiomyocyte apoptosis (46). It has been speculated that bile acid salts may also accumulate in the placenta, lead to edema, and accelerate placental cell apoptosis with subsequent alteration of the placental function. Moreover, a vasoconstrictive effect of bile acids on isolated human placental chorionic veins has also been shown, which may explain the occurrence of sudden fetal distress, asphyxia, or death in newborns.

Moreover, elevated maternal and amniotic fluid levels of bile acids can lead to umbilical vessels spasms with resultant reduction in the trans-placental nutrient exchange and fetal oxygenation. The hypothesis to explain increased neonatal morbidity also includes a direct effect of bile acids on neonatal lung, possibly leading to a "bile acid pneumonia" (47, 48). Since bile acids was found in the in bronchoalveolar lavage fluid of neonates affected by RDS, some authors have speculated that bile acids inhibit surfactant activity (48, 49).

The rate of malformations or abortions is not increased in ICP. The prevalence of in utero and perinatal mortality is estimated at 0.5%. Some studies showed that total bile acid concentrations are more highly predictive of stillbirth for singleton pregnancies than the other biomarkers, for example aminotransferase, aspartate aminotransferase and bilirubin. Indeed, a large systematic review demonstrated that the highest risk of stillbirth occurred in women with total bile acids greater than or equal to 100 micomol/L (50).

MANAGEMENT: DIAGNOSIS

The diagnosis of ICP relies on clinical symptoms and biochemical evidence of liver dysfunction, after excluding other causes of hepatobiliary diseases. Clinical symptoms are characterized by generalized itching, mainly localizing to the palms of the hands and the soles of the feet, with a nocturnal predominance (51, 52). It occurs during the second and the third trimester and rarely during the first trimester. There is not any rush over the skin and jaundice is rare and more likely associated with other hepatic disorders (42). The pruritus can precede the rise in serum bile acids by several weeks. Therefore, if symptoms persist and there is no other

explanation, it is necessary to repeat measurement of total bile acid concentration and serum transaminases if the previous values were normally (51).
The serum analysis is important to evaluate the elevated total bile acid concentration whit cut-off values ranging from 10 to 14 μmol/L as abnormal (42, 53, 54). We can consider mild cholestasis (10 < bile acids < 39 μmol/L), moderate cholestasis (40 < bile acids < 99 μmol/L) o severe cholestasis (bile acids > 100 μmol/L). Total bile acids are often reported including cholic, chenodeoxycholic, deoxycholic, ursodeoxy-cholic, lithocholic, and hyodeoxycholic acids. In healthy non-pregnant women, chenodeoxycholic acid levels in serum are higher than cholic acid levels (55). In women with ICP, cholic acid is greatly increased over chenodeoxycholic acid, often in a ratio of 3 to 1.
There are different types of assay available for bile acid test. Mass spectrometry and liquid chromatography can determine total and fractionated bile acid, while enzymatic assay provide only total bile acids. For ICP diagnosis, we usually analyze fasting bile acids; however, sometimes we could also use random bile acids because the differences between random and fasting results are small (56). Liver transaminases aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are also commonly elevated in ICP, possibly preceding the increase in bile acids by 1 to 2 weeks. However, elevated transaminases are not necessary for the diagnosis.
Once pregnant women have itching symptoms bile acid levels should be immediately measured. Regardless of the severity of the disease, total bile acid and liver function need to be checked every 1~2 weeks until delivery. For those with severe degree, the detection interval can be reduced moderately (42). It is important to note that ICP is an exclusion diagnosis: other liver diseases should be considered in case of atypical symptoms, such as abdominal pain, ascites, asterixis, jaundice or tremor. The differential diagnosis includes autoimmune hepatitis, viral hepatitis, acid fatty liver disease, preeclampsia, Hellp's syndrome, alcoholic liver disease, biliary obstruction and others rarest disorders (57). The clinician must evaluate risk factors for non-alcoholic fatty liver disease (such as obesity, type 2 diabetes, or dyslipidaemia), strong personal or familial history of autoimmune diseases, a family history of liver diseases, or exposure to medications or toxins that could cause liver damage. In these cases, alternative diagnosis and referral to a liver specialist should be considered. A suspicion for an alternative diagnosis based on atypical symptoms should suggest additional adjunctive tests such as liver ultrasound, the appearance of the liver on ultrasound should be normal with ICP (58). Other biochemical changes in serum analyses can be found, for instance Oztas E *et al*. have demonstrated that women with ICP have a significantly higher mean platelet volume (MPV) (mean 10.2 ± 1.0 *vs* 11.0 ± 1.3; P < 0.001) and platelet distribution width (PDW) (mean 13.1 ± 2.3 *vs* 14.7 ± 2.8; P < 0.001) values compared to controls (43).

MANAGEMENT: TREATMENT

Different medications have been proposed to treat ICP with the potential goal to reduce both maternal symptoms and the risk for adverse perinatal outcomes. First line treatment is represented by ursodeoxycholic acid (UDCA), with the mechanism of action not completely understood. Still, several studies demonstrated that treatment is associated with a reduction in total serum bile acids in both maternal and umbilical cord serum and a qualitative change in the serum bile acid pool (59, 60, 61). It has been hypothesized that UDCA acid concentrates in hepatocytes and bile, resulting in decreased hepatic cholesterol synthesis, hepatic cholesterol secretion, and intestinal cholesterol reabsorption. The symptoms improvement is usually observed within 1 to 2 weeks after initiation and a decrease in serum bile acids two weeks after. The standard starting dose for UCDA is 10-15 mg/kg/ day, which can be divided into twice ore three-times daily doses. UCDA can be titrated to a maximum dose of 21 mg/kg/day (~1500 mg for a 72 kg patient), usually split into multiple doses.
This therapy seems to improve symptoms and reduce liver dysfunction; however, its beneficial effects are still debated. For instance, the PITCHES trial has demonstrated that ursodeoxycholic acid is not effective in reducing a composite of adverse perinatal outcomes in women with ICP. Indeed, according to this study, although ursodeoxycholic acid appears to be safe, it has no clinically meaningful effect on maternal itch symptoms. In fact, it does not reduce maternal bile acid concentrations, and the reduction in alanine transaminase is of uncertain clinical significance, given that alanine transaminase is not known to be associated with the risk of stillbirth or preterm labor in ICP (62) (**table I**). The drug is usually well tolerated, although some patients report nausea and dizziness as adverse drug reaction.

Table I. *Comparative guidelines on intrahepatic cholestasis of pregnancy diagnosis and treatment.*

Guidelines	RCOG	SAMNCP	GWADOH	ACG
Clinical symptoms				
Pruritus	Yes	Yes	Yes	Yes
Ascites	No	No	No	No
Asterixis	No	No	No	No
Jaundice	Yes	Required	Yes	Yes
Steatorrhea	Yes	Yes	Yes	Not specific
Laboratory tests				
Bile acids	Yes	Yes	Yes	Yes
Transaminases	Diagnostic	Yes	Diagnostic	Yes
γGT	Yes	Yes	Optional	Optional
PT	Yes	Yes	Yes	Optional
Bilirubin	Yes	Yes	Yes	Yes
Liver ultrasound			Recommended	Recommended
Laboratory tests for differential diagnosis				
HCV	Yes	Yes	Yes	Not required
EBV	Yes	Yes	Yes	Not required
CMV	Yes	Yes	Yes	Not required
Primary biliary cirrhosis	Yes	Yes	Yes	Not required
Primary sclerosing cholangitis	Yes	Yes	Yes	Not required
Prenatal controls	Not specified	Not specified	Every two weeks	Not specified
Treatment				
UDCA	No dosing provided	Max 750 mg 3-4 times /day	10-15 mg/kg/ day	10-15 mg/kg/ day
S-adenosyl-L-methionine	No	No	No	No
Dexamethasone	Not first line	Not first line	Not first line	May be used for lung maturity
Rifampin		Consider if UDCA fails		
Delivery timing	Depending on laboratory abnormalities	38 wks or earlier If bile acids > 100 µmol/L	37-38 wks or earlier if maternal or fetal impairment	37 wks
Resolution after delivery	In 6 wks	In 6 wks	In a month	Not specified

Royal College of Obstetricians and Gynaecologists (RCOG) "Obstetric Cholestasis (2011)"; South Australia Maternal and Neonatal Community of Practice (SAMNCP) Clinical Guideline "Obstetric Cholestasis (2016)"; Government of Western Australia Department of Health (GWADOH) Clinical Guideline "Cholestasis in Pregnancy (2014)"; American College of Gastroenterology (ACG) "Liver Disease in Pregnancy (2016)".

Another treatment provides the use of Cholestyramine, an anion exchange resin, which binds to bile acids in the intestine, sequestering them from enterohepatic recycling and committing them to fecal excretion. However, according to Kondrackiene J and coll. (63), the use of 8 g daily for 14 days of this drug is less efficient to reduce serum alanine and aspartate aminotransferase activities and endogenous serum bile acid levels compared to UDCA ($P < .01$ and $P < .02$ *vs* ursodeoxycholic acid). Moreover, Cholestyramine is not considered a first-line treatment of ICP for its side effects, such as constipation, diarrhoea, abdominal pain, nausea, and concerns exists regarding the associated decrease in vitamin K levels.

S-adenosyl-L-methionine influences hepatic membrane composition and therefore biliary excretion of bile acids and other steroid hormones, and it is generally used in combination with UDCA. The combined therapy, according to Zhang L. *et al.* (64) significantly improves pruritus, levels of total bile acids, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TB). In their study, singleton pregnancies with ICP that have been randomized into three treatment groups: oral UDCA 4 × 250 mg daily (Group 1, n = 41), intravenous SAMe 1000 mg daily (Group 2, n = 38), and a combination of both drugs (Group 3, n = 41) until delivery. All therapies significantly and equally improved pruritus. The serum levels of total bile acid, alanine amino-

transferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TB) in each group significantly decreased after treatment ($p < 0.05$). Group 1 was more effective than Group 2 in reducing total bile acids concentration ($p < 0.05$), Group 1 and Group 3 showed more effective than Group 2 in reducing AST and TB concentrations ($p < 0.05$), and Group 1 facilitated deliveries at term. No perinatal death or adverse drug reactions were observed.

Dexamethasone has also been proposed as a treatment for ICP given its effect on reducing circulating estriol levels whose levels are increased. Nevertheless, according to Giantz *et al.* (65) the administration of Dexamethasone (12 mg/day) does not improve pruritus or reduce ALT and it is less effective than UDCA at reducing bile acids and bilirubin (66, 67).

Rifampin is a semisynthetic derivative of one of the rifamycins, a group of macrocyclic antibiotics produced by Streptomyces mediterranei. Though not yet studied for use in ICP, there is evidence to suggest that rifampicin is an effective second-line treatment for primary biliary cirrhosis. Further studies are needed to fully establish the extent to which rifampicin may be useful in ICP and to investigate any potential effect on perinatal outcomes. The proposed mechanism of action for rifampicin in cholestasis is enhanced bile acid detoxification and excretion, an effect that is complementary to the upregulation of hepatic bile acid export by ursodeoxycholic acid (68).

Antihistamines such as chlorpheniramine are often used in ICP but there is no evidence of a histamine-mediated pruritus in this pathology thus their use is discouraged (69).

Since itching is generally widespread, topical antipruritic such as menthol is also of limited use. ICP is associated to an increased risk of preterm birth, fetal demise, fetal respiratory distress or meconium staining. For these reasons, it is important to adopt an active strategy to prevent any fetal problem and to program the right time to delivery. The timing of delivery should be approached using risk-stratification based on-specific factors, including total bile acid levels (70, 71).

The Society for Maternal Fetal Medicine (SMFM) recommends that patients with a diagnosis of ICP begin antenatal fetal surveillance at a gestational age when delivery is possible in response to abnormal fetal testing or at the time of diagnosis if it is made later in gestation. Since the optimal frequency of testing is unknown, it may be determined by bile acid levels and comorbidities.

A continuous fetal monitoring in labor is necessary for the higher risk of stillbirth in patients with ICP (72). In Puljic *et al.* (73) retrospective cohort study of over 1.6 million pregnancies, they assess the risk of fetal demise, the risk of infant death and the composite risk of expectant management for every additional week of gestation from 34 to 40 weeks. They found that the balance between the risk of perinatal mortality associated with liver damage and the risk of fetal demise associated with expectant management begin to shift at 36 weeks gestation. A recent metanalyses (74) compares the elevated serum bile acids level above 100 μmol/L to levels below 40 μmol/L. Only women with bile acids above 100 μmol/L have fetal demise rates and an increased risk of stillbirth that are significantly higher than the pooled national rate.

Early term induction is discussed in all guidelines and there are conflicting opinions because there are not published randomized controlled clinical trials sufficiently powered to compare delivery outcomes at different gestational ages, regardless of disease severity, prevention of perinatal morbidity or mortality (**table II**).

Indeed, ACG society recommends delivery at 37 weeks (75). RCOG advises discussing with patients the option of early term delivery *versus* expectant management (76). RCOG notes that induction may be preferential in pregnancies with more severe laboratory abnormalities. SAMNCP advises delivery at 38 weeks for severe disease and to consider earlier induction of labor if bile acid concentrations are > 100 mmol/L (77).

SMFM recommends delivery between 36 and 39 weeks of gestation if total bile acid leves are < 100 mmol/L.

SMSF also recommends delivery at 36 weeks of gestation if total bile acid leves are > 100 mmol/L because the risk for stillbirth is increased substantially around this gestational age, and suggests antenatal administration of corticosteroids for fetal lung maturity for patients delivered before 37 weeks if not previously treated (72). Delivery between 34 and 36 weeks of gestation can be considered if total bile acid leves are > 100 mmol/L, and any of the following: intense and unremitting maternal pruritus unresponsive to drugs, a prior history of stillbirth before 36 weeks of gestation for ICP, a clinical or laboratory evidence of worsening hepatic function in ICP patients with preexisting or acute hepatic disease. GWADOH (Government of western Australia department of health) recommends delivery between 37 and 38 weeks unless

Table II. *Maternal and neonatal outcomes after treatment with UDCA.*

Authors	Study design	Sample size	Therapy	Maternal outcomes	Neonatal outcomes
C M P Rodrigues *et al.* (73)	Longitudinal prospective study	9 patients	UDCA	Symptoms improved bile acids decreased (3-fold)	Apgar: no differences Birth weight: no differences
D Brites *et al.* (74)	Longitudinal prospective study	15 patients	UDCA	Symptoms improved cholic acid decreased (3-fold)	Apgar: no differences Birth weight: no differences
LB Manna *et al.* (75)	Longitudinal prospective study	51 patients	UDCA	Symptoms not valuated cholic acid decreased (4-fold) CDCA decreased (5-fold) UDCA increased (97-fold)	Not evaluated
Joutsiniemi *et al.* (76)	Observational retrospective study	208 patients	UDCA	Total bile acids decreased Pruritus improved Liver function tests improved	Apgar: lower in UDCA users umbilical ph values: no differences Birth weight: not evaluated
Mazzella *et al.* (77)	Longitudinal prospective study	20 patients	UDCA	Pruritus improved Liver function tests improved	Apgar: no differences Birth weight: not evaluated

maternal or fetal compromise dictates earlier induction. Moreover, the majority of early deliveries are induced and there is no evidence that this results in higher rates of emergency cesarean delivery. Indeed, it has been shown in two retrospective cohorts and one prospective study that rates of operative and instrumental delivery are not increased in women with ICP after labor induction (78-80).

CONCLUSIONS

ICP is a relative common disorder of pregnancy, especially of the second and third trimester, occurring in up to 5% of pregnancies. The diagnosis is suspected on clinical symptoms (mainly palm and soles pruritus) and is confirmed by the detection of elevated total bile acids. Maternal risks are often negligible while fetal risks may be considerable, including fetal death, preterm birth, meconium staining, neonatal asphyxia. Its treatment and management remains debated in literature.
Often UDCA is used as first line treatment for its beneficial effects both on pruritus and biochemical abnormalities, but on the reduction on fetal risks has not been definitely proven. Other medications revealed to be inferior compared to UDCA. Management of ICP pregnancy often includes early labor induction between 37 and 39 weeks depending on the severity of total bile acids levels.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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Covid-19 seroprevalence in a group of pregnant women compared to a group of non-pregnant women

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ABSTRACT

Pregnant women are an interesting population to study in the context of the current Coronavirus Disease 2019 (COVID-19); studies are still controversial in concluding if pregnancy is a protective condition or a risk factor for a more severe form of the illness.
We estimated rate of positive serology for SARS-CoV-2 in a population of healthy pregnant women, compared to a population of non-pregnant women of the same age and geographic area. We also made a comparison between the two groups in terms of previous symptoms and lifestyle.
This is a transversal study including pregnant women, above 18 weeks of gestation, aged between 18 and 40 years. The control group consisted of 588 non pregnant women from the same area and the same age group. A total of 344 pregnant women and 588 non pregnant women were recruited.
The rate of positive serology for SARS-CoV-2 was significantly lower in the pregnant group: 9/344 (2.6%) *versus* 75/588 (12.8%) in the non-pregnant group ($p < 0.0001$). The two groups were similar in terms of occupation and in the self-reported habit to leave the house during the lockdown.
Our hypothesis to explain this result is that pregnant women might have adopted a more prudential lifestyle, due to their special condition, which may have led them to behave with more caution, *i.e.*, concerning the responsibility of wearing all the disposable personal protective equipment, and keeping the recommended 6 feet distance from other people.

SOMMARIO

Le donne incinte sono una popolazione interessante da studiare nel contesto dell'attuale pandemia da Coronavirus 2019 (COVID-19); gli studi sono ancora controversi nel concludere se la gravidanza è una condizione protettiva o un fattore di rischio per una forma più grave della malattia.
Abbiamo stimato il tasso di sierologia positiva per SARS-CoV-2 in una popolazione di donne incinte sane, rispetto a una popolazione di donne non gravide della stessa età e area geografica. Abbiamo anche fatto un confronto tra i due gruppi in termini di sintomi precedenti e stile di vita.
Questo è uno studio trasversale su donne in gravidanza, con epoca gestazionale superiore a 18 settimane, di età compresa tra i 18 ei 40 anni. Il gruppo di controllo era composto da 588 donne non gravide della stessa area e della stessa fascia di età. Sono state reclutate 344 donne incinte e 588 donne non gravide.
Il tasso di sierologia positiva per SARS-CoV-2 è stato significativamente inferiore nel gruppo delle gravide: 9/344 (2,6%) contro 75/588 (12,8%) nel gruppo delle non gravide ($p < 0,0001$). I due gruppi erano simili in termini di occupazione e nell'abitudine dichiarata di uscire di casa durante il lockdown.
La nostra ipotesi per spiegare questo risultato è che le donne in gravidanza potrebbero aver adottato uno stile di vita più prudente, a causa della loro condizione particolare, che potrebbe averle portate a comportarsi con maggiore cautela e responsabilità ad esempio nell' indossare tutti i dispositivi di protezione individuale e di mantenere la distanza consigliata di 6 piedi da altre persone.

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Key words
Covid19; SARS-CoV-2; pregnancy; seroprevalence; pandemic.

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INTRODUCTION

The year 2020 has been marked by the spread of the current Coronavirus Disease 2019 (COVID-19) pneumonia pandemic, caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Pregnant women are an interesting population to study in this context. Pregnancy itself can be considered an immunological paradox: the woman must develop an immunological tolerance towards a semi-allogenic fetus, which has exposed pregnant women in history to an increased risk of infection, especially viral (*e.g.*, H1N1 in 2009, SARS epidemics of 2003, Ebola or Zika Virus), with maternal and neonatal unfavourable outcomes (1, 2). Physiological and mechanical changes in pregnancy might theoretically increase susceptibility to infections in general, particularly when the cardiorespiratory system is affected (3).
Concerning COVID-19 and its effect on pregnancy, studies are still speculative and controversial (4). Some theories support the hypothesis that, since COVID-19 is an immune condition marked by reduced lymphocytes and elevated selected proinflammatory cytokines, and similar immune expression has been demonstrated in pregnancy, pregnant women might be on higher risk for a severe form of the illness (5). According to other authors, immunomodulatory mechanisms employed by the pregnant status may mitigate violent immune response, may soften cytokine storm, tightly associated with severely ill COVID-19 patients, and potentially reduce SARS-CoV-2 transmission (6).
Even if it is too early to draw a conclusion, most evidences show that maternal and fetal outcomes do not seem to be unfavourable (7, 8) and, to date, the risk of vertical transmission is possible but very rare (9).
Given the impossibility to accurately estimate the real incidence of the pathology, with all the possible limitations, serological tests for the measurement of immune response could represent an effective tool for the rapid monitoring of the population and estimating the proportion of previous infections in a territory (10, 11).
Some other authors have already used this approach in pregnant women population, reporting data that necessarily need to be widened in order to obtain larger samples for further analysis (12, 13).
The aim of this study is to assess the prevalence of positive serology in a population of pregnant women, compared to a control group of non-pregnant women. Both groups did not perform a baseline molecular oral/nose-pharyngeal swab at the time of enrolment because it is not yet foreseen in the hospital protocol.

MATERIALS AND METHODS

This was a transversal study aiming to estimate the prevalence of immunological response in a population of healthy pregnant women at the time of enrolment. Primary outcome of the research was the rate of positive serology for SARS-CoV-2 in a population of healthy pregnant women, compared to a population of non-pregnant women. Secondary outcome was the comparison between the two groups in terms of previous symptoms and lifestyle.
We included 344 pregnant women attending the Obstetrical Clinic of the "Maggiore della Carità" Hospital (Novara, Italy). Inclusion criteria were: pregnant women, ≥ 18 weeks of gestation, aged between 18 and 40 years, willing to participate in the study.
The control group consisted of 588 non pregnant women from the same area and the same age group, chosen among those women performing the serological assay at the same time and laboratory used for our study population, and giving consent for an anonymous comparison of the results.
Recruitment period was from 27th April 2020 to 1st July 2020.
Women consented to participate in the study after being informed on the nature of the research and data managing and processing; an informed consent was therefore signed.

Data collection consisted of 1) demographics data, 2) serological results and 3) a short survey where they were asked questions on the presence of symptoms, about their lifestyle and about possible contacts with other people in the three months preceding the blood sample collection.
A single serum sample was collected from each participating woman and stored at - 80 °C and subsequently tested for Sars-CoV-2 antibodies in Varelli laboratory (Naples, Italy), blind to the study.
A chemiluminescent microparticle immunoassay (CMIA) (MAGLUMI 2019-NCoV IgG/IgM, Snibe, China) was used to process the samples. This is a highly sensitive (95.6%) and specific (96.0%) assay that has been validated in usage with human serum sample (14-16).
All patients who tested positive to serology were confirmed with RT-PCR.

Analysis

Statistical analysis was performed on frequency distributions of variables, expressed as absolute frequency and percentage. Proportions were compared by Chi-square test or Fisher's exact test, as appropriate. Quantitative variables, reported as medians and interquartile ranges (IQR), were analyzed using the Mann-Whitney U test for unmatched groups. All statistical tests were two sided; a p value < 0.05 was considered as statistically significant. The analyses were performed using Stata 15.1 software (StataCorp LLC, College Station, Texas USA, 2017).

RESULTS

A total of 344 pregnant women and 588 non pregnant women were recruited.
Nine of 344 (2.6%) in the pregnant and 75 of 588 (12.8%) in the non-pregnant group tested positive for IgG ($p < 0.0001$).
In the non-pregnant women group 231/588 (39.3%) reported symptoms in the previous three months; symptoms were reported by 48 of the 75 IgG positive women (64.0%) and by 183 of the 513 IgG negative women (35.7%). In the IgG positive group, 18/75 (24.0%) had high-grade fever and 22/75 (29.3%) had low-grade fever. In the IgG negative group, 76/513 (14.8%) had high-grade fever and 71/513 (13.8%) had low grade fever. Overall fever was reported by 187/231 symptomatic non-pregnant women (80.9%). 94/231 (40.6%) had high-grade fever and 93/231 (40.3%) had low-grade fever.
Cough was reported by 150/231 (64.0%) of symptomatic non-pregnant women. Cough prevalence in the IgG positive group was 27/75 (36.0%), and 123/513 (24.0%) in the IgG negative.
Ageusia was reported by 46/231 (19.9%) of symptomatic non-pregnant women. Ageusia prevalence in the IgG positive group was 27/75 (36.0%), and 19/513 (3.7%) in the IgG negative group.
Anosmia was reported by 53/321 (22.9%) of symptomatic non-pregnant women. Anosmia prevalence in the IgG positive group was 28/75 (37.3%), and 25/513 (4.9%) in the IgG negative group.
Table I summarizes the main characteristics of the non-pregnant group.
In the pregnant group 49/344 (13.9%) women reported symptoms in the previous three months.
Symptoms were reported by 5 of the 9 IgG positive women (55.6%) and by 44 of the 335 IgG negative women (13.1%).
Four of the 9 women with positive serology reported fever (44.4%): 3 (33.3%) with high grade and 1 (11.1%) with low grade. 23 of the 335 women with negative serology (6.9%) reported fever: 10 (3.0%) with high grade and 13 (4.0%) with low grade. Overall fever was reported by 27/344 (7.9%) pregnant women.
Cough was reported by 27/344 (7.9%) pregnant women: 5 of the 9 IgG positive women, and 22 of the 335 negative ones (6.6%). Six women reported ageusia and anosmia, but only one of them was IgG positive.
Table II describes the main characteristics of the pregnant group.
Considering the occupation of the pregnant women there are no evident differences between the two groups, pregnant and non-pregnant. No differences were evident even in the self-reported habit to leave the house during the lockdown.
The only difference we found in both groups was in the higher percentage of positive serology in women who got in contact with a person diagnosed with SARS COV2 disease: 5/9 (55.6%) *versus* 16/335 (4.8%) in the pregnant group and 12/27 (44.4%) *versus* 30/330 (9.1%). This difference was statistically significant within both groups ($p < 0.001$).
The reduced incidence of IgG positivity in the pregnant group was found unrelated to the occupational status, indeed in the housewife or unemployed women subgroup 10/49 (20.4%) of the non-pregnant and 3/96 (3.1%) of the pregnant were IgG positive. In the employed subgroup,

Table I. *Non pregnant women.*

	Total (N = 588)	IgG+ (N = 75)	IgG- (N = 513)	p-value (IgG+ *vs* IgG-)
Age (year), median (IQR)	34 (29-37)	33 (26-38)	34 (29-37)	0.56
Comorbidities, n (%)	27 (4.6)	1 (1.3)	26 (5.1)	0.23
Symptoms				
Symptomatic, n (%)	231 (39.3)	48 (64.0)	183 (35.7)	**< 0.0001**
Asymptomatic, n (%)	357 (60.7)	27 (36.0)	330 (64.3)	
If asymptomatic, recent contact with person affected by SARS COV2	42/357 (11.8)	12/27 (44.4)	30/330 (9.1)	**< 0.0001**
Type of symptoms, n (%)				
Fever	187 (31.8)	40 (53.3)	147 (28.7)	**< 0.0001**
High-grade Fever	94 (16.0)	18 (24.0)	76 (14.8)	**0.043**
Low-grade fever	93 (15.8)	22 (29.3)	71 (13.8)	**0.001**
Cough	150 (25.5)	27 (36.0)	123 (24.0)	**0.026**
Ageusia	46 (7.8)	27 (36.0)	19 (3.7)	**< 0.0001**
Anosmia	53 (9.0)	28 (37.3)	25 (4.9)	**< 0.0001**
Conjunctivitis	26 (4.4)	4 (5.3)	22 (4.3)	0.76
Diarrhea	68 (11.6)	14 (18.7)	54 (10.5)	**0.039**
Others	27 (4.6)	9 (12.0)	18 (3.5)	**0.004**
Occupation, n (%)				
Health worker	74 (12.6)	7 (9.3)	67 (13.1)	0.36
Employed	399 (67.9)	46 (61.3)	353 (68.8)	0.20
Housewife or unemployed	49 (8.3)	10 (13.3)	39 (7.6)	0.09
Student	66 (11.2)	12 (16.0)	54 (10.5)	0.16

Table II. *Pregnant women.*

	Total (N = 344)	IgG+ (N = 9)	IgG- (N = 335)	p-value (IgG+ *vs* IgG-)
Age (year), median (IQR)	32 (29-35)	30 (22-35)	32 (29-35)	0.36
BMI (Kg/m^2), median (IQR)	27 (23-29)	28 (24-29)	27 (23-29)	0.69
Comorbidities, n (%)	46 (13.4)	1 (11.1)	45 (13.4)	1.0
Symptoms				
Symptomatic, n (%)	49 (14.2)	5 (55.6)	44 (13.1)	**0.004**
Asymptomatic, n (%)	295 (85.8)	4 (44.4)	291 (86.9)	
If asymptomatic, recent contact with person affected by SARS COV2	12/295 (4.1)	3/4 (75.0)	9/291 (3.1)	**< 0.0001**
Type of symptoms, n (%)				
Fever	27 (7.9)	4 (44.4)	23 (6.9)	**0.003**
High-grade Fever	13 (3.8)	3 (33.3)	10 (3.0)	**0.003**
Low-grade fever	14 (4.0)	1 (11.1)	13 (4.0)	0.33
Cough	27 (7.9)	5 (55.6)	22 (6.6)	**< 0.0001**
Ageusia	6 (1.7)	1 (11.1)	5 (1.5)	0.15
Anosmia	6 (1.7)	1 (11.1)	5 (1.5)	0.15
Conjunctivitis	0 (0.0)	0 (0.0)	0 (0.0)	-
Diarrhea	7 (2.0)	0 (0.0)	7 (2.1)	1.0
Others	5 (1.5)	0 (0.0)	5 (1.5)	1.0
Occupation, n (%)				
Health worker	31 (9.0)	1 (11.1)	30 (9.0)	0.58
Employed	214 (62.2)	5 (55.6)	209 (62.4)	0.73
Housewife or unemployed	96 (27.9)	3 (33.3)	93 (27.8)	0.71
Student	3 (0.9)	0 (0.0)	3 (0.9)	-
Going out of home, n (%)	244 (70.9)	7 (77.8)	237 (70.8)	1.0
Housewife or unemployed	60/244 (24.6)	2/7 (28.6)	58/237 (24.5)	0.68
Partner health worker, n (%)	10 (2.9)	1 (11.1)	9 (2.7)	0.24
Partner crowded space, n (%)	156 (45.4)	6 (66.7)	150 (44.8)	0.31
Person affected by SARS COV2, n (%)	21 (6.1)	5 (55.6)	16 (4.8)	**< 0.0001**

46/399 (11.5%) of non-pregnant women and 5/214 (2.3%) of pregnant women resulted IgG positive ($p < 0.001$). Between the health workers, the non-pregnant IgG positive were 7/74 (9.4%) while there was 1/31 pregnant woman IgG positive.

Table III shows the characteristics of women with positive serology, comparing 9 pregnant women *versus* 75 no pregnant women. The two groups report a similar amount and variability of symptoms.

Table III. *Women with positive serology (IgG^+).*

	Pregnant (N = 9)	Non pregnant (N = 75)	p-value
Age (year), median (IQR)	30 (22-35)	33 (26-38)	0.20
Comorbilities, n (%)	1 (11.1)	1 (1.3)	0.20
Symptoms			
Symptomatic, n (%)	5 (55.6)	48 (64.0)	0.72
Asymptomatic, n (%)	4 (44.4)	27 (36.0)	
If asymptomatic, recent contact with person affected by SARS COV2	3/4 (75.0)	12/27 (44.4)	0.33
Type of symptoms, n (%)			
Fever	4 (44.4)	40 (53.3)	0.73
High-grade Fever	3 (33.3)	18 (24.0)	0.68
Low-grade fever	1 (11.1)	22 (29.3)	0.43
Cough	5 (55.6)	27 (36.0)	0.29
Ageusia	1 (11.1)	27 (36.0)	0.26
Anosmia	1 (11.1)	28 (37.3)	0.15
Congjunctivitis	0 (0.0)	4 (5.3)	1.0
Diarrhea	0 (0.0)	14 (18.7)	0.35
Others	0 (0.0)	9 (12.0)	0.59
Occupation, n (%)			
Health worker	1 (11.1)	7 (9.3)	1.0
Employed	5 (55.6)	46 (61.3)	0.73
Housewife or unemployed	3 (33.3)	10 (13.3)	0.14
Student	0 (0.0)	12 (16.0)	0.35

DISCUSSION

Large-scale serology testing is critical for estimating how many individuals have been infected during a pandemic status such as the COVID-19 one. Due to widely imposed social distancing requirements, it is mainly difficult to collect serum from the whole population. Pregnant women represent and exception as they continue to have multiple interactions with the medical system for prenatal care and delivery, even during a pandemic, and they might represent a valid sample study of the general population to assess SARS-CoV-2 immunity.

The main and most important result of our study is the difference between the prevalence of positive serology between pregnant and non-pregnant women. This data itself implies two different conclusions: the former one is that the prevalence of positive serology in pregnant women cannot be used to estimate the prevalence of the disease in the general population, as suggested by some authors (17), the latter one is that the pregnant status might be protective towards SARS-CoV-2 infection.

Theoretically, to support this evidence we found that lifestyles did not influence the incidence of IgG positivity in the two groups, as a significative reduction was observed either in the unemployed or the employed subgroup; on the other hand, the media generated awareness in the general population (*i.e.*, for the high mortality of this infection) along with the overall reduced incidence of IgG positivity and symptomatology in the pregnant group might lead us to consider a more prudential lifestyle. Pregnant women may have behaved with more caution, *i.e*, concerning the responsibility of wearing all the disposable personal protective equipment, and keeping the recommended 6 feet distance from other people. This interpretation can be enforced by the observed tendency of pregnant women to be particularly anxious and worried about their health, especially during the pandemic, as demonstrated in previous studies (18).

Data collected in this study support the idea that pregnant women are less infected than the equivalent female population. As previously speculated, this could be either a better immunological response or due to a more prudential lifestyle. Our data do not allow us to understand which of the two hypotheses is true.

Despite our results, we have to consider some limits of our study: low sample size; prevalent use of serological blood, currently not gold standard for diagnosis; and not having performed nasopharynx swab for confirmation.

Due to the lack of conclusive studies on this topic and of the importance of the contagion prevention, more research is necessary to confirm these findings.

CONCLUSIONS

Pregnant women are less infected than the equivalent female population. This could be either a bet-

ter immunological response or due to a more prudential lifestyle. Furthermore, our study does not demonstrate important differences between pregnant and non-pregnant in symptomatology.

ETHICS

This study has been approved by the local Ethical Committee. All women who participated in the study signed an informed consent.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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Effects of laparoscopic salpingectomy *versus* proximal tubal separation on ovarian reserve in management of hydrosalpinx in females undergoing intracytoplasmic sperm injection (ICSI) cycle: a comparative study

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ABSTRACT

Background. Females presented with hydrosalpinx have lower rates of pregnancy by artificial reproductive techniques. There are variable management strategies for hydrosalpinx as: salpingostomy, salpingectomy, proximal tubal ligation and trans-vaginal aspiration; but recent management techniques which proved effective in improving outcome of intracytoplasmic sperm injection (ICSI) are laparoscopic tubal ligation and salpingectomy.
Aim of the present study was to compare the ovarian reserve and ICSI outcomes after performing either laparoscopic tubal separation or laparoscopic salpingectomy in females with hydrosalpinx.
Patients and methods. The study was performed in Department of Gynecology and Obstetrics, School of Medicine, Zagazig University in about 3 years. Patients who fulfilled the inclusion criteria were divided into two groups. Group A included 60 patients that underwent bilateral Laparoscopic proximal tubal separation and group B included 60 patients that underwent bilateral laparoscopic salpingectomy before ICSI. The outcome evaluated parameters of the study were; rates of clinical pregnancy and live births in addition to changes in parameters of ovarian reserve, changes of rate of fertilization, rate of cleavage, rates of implantation and rates of miscarriage.
Results. In the salpingectomy group after surgery we found a significant increase in the serum FSH and serum estradiol levels, a significant reduction in the post-surgery AFC and a significant reduction of levels of serum AMH than the other group ($p < 0.001$). We showed that patients who underwent salpingectomy needed more stimulation days, lower rates of fertilization and higher doses of gonadotropins than patients with bilateral tubal separation ($p < 0.001$).

SOMMARIO

Contesto. Le donne presentate con idrosalpinge mostrano tassi di gravidanza inferiori mediante tecniche riproduttive artificiali. Esistono strategie di gestione variabili per l'idrosalpinge come: salpingostomia, salpingectomia, legatura delle tube prossimali e aspirazione transvaginale; ma recenti tecniche di gestione che si sono dimostrate efficaci nel migliorare l'esito dell'iniezione intracitoplasmatica di spermatozoi (ICSI) sono la legatura delle tube laparoscopica e la salpingectomia.Lo scopo del presente studio era di confrontare la riserva ovarica e gli esiti ICSI dopo aver eseguito la separazione tubarica laparoscopica o la salpingectomia laparoscopica nelle donne con idrosalpinge.
Pazienti e metodi. Lo studio è stato condotto presso il Dipartimento di Ginecologia e Ostetricia, Facoltà di Medicina, Università di Zagazig in circa 3 anni. Le pazienti che soddisfacevano i criteri di inclusione sono state divise in due gruppi. Il gruppo A includeva 60 pazienti sottoposte a separazione tubarica prossimale laparoscopica bilaterale e il gruppo B comprendeva 60 pazienti sottoposte a salpingectomia laparoscopica bilaterale prima di ICSI. I parametri valutati per l'esito dello studio erano: tassi di gravidanza clinica e nati vivi oltre a cambiamenti nei parametri della riserva ovarica, cambiamenti del tasso di fecondazione, tasso di scissione, tassi di impianto e tassi di aborto spontaneo.
Risultati. Nel gruppo salpingectomia dopo l'intervento chirurgico abbiamo riscontrato un aumento significativo dei livelli sierici di FSH e di estradiolo, una significativa riduzione dell'AFC post-operatorio e una significativa riduzione dei livelli sierici di AMH rispetto all'altro gruppo ($p < 0,001$). Abbiamo dimostrato che le pazienti sottoposte a salpingectomia avevano bisogno di più giorni di stimolazione, tassi di fecondazione inferiori e dosi più elevate di gonadotropine rispetto alle pazienti con separazione tubarica bilaterale ($p < 0,001$).

Conclusions. Our study demonstrated the benefits of laparoscopically tubal separation over salpingectomy in patients with hydrosalpinx regarding preventing reduction in ovarian reserve particularly in women underwent ICSI.

Conclusioni. Il nostro studio ha dimostrato i benefici della separazione tubarica per via laparoscopica rispetto alla salpingectomia in pazienti con idrosalpinge per quanto riguarda la prevenzione della riduzione della riserva ovarica, in particolare nelle donne sottoposte a ICSI.

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Key words
Hydrosalpinx; salpingectomy; tubal separation; ovarian reserve.

INTRODUCTION

Infertility due to pathological tubal causes forms about 25-35% of causes of females infertility and it was considered an important indication of artificial reproductive techniques (1). Hydrosalpinx is the most severe manifestation of tubal disease. Females presented with hydrosalpinx have lower rates of pregnancy by *in vitro* fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI) (2). There are variable management strategies for hydrosalpinx as: salpingostomy, salpingectomy, proximal tubal ligation and trans-vaginal aspiration, but the most recent management techniques which proved effective in improving outcome of ICSI are laparoscopic tubal ligation and salpingectomy (3). It was found that the blood supply of the ovary might be interrupted after laparoscopic surgery as the blood vessels supplying the ovary and the oviduct are close to each other that reduced ovarian reserve (4, 5).
The quantitative evaluation of ovarian reserve is very important in patients needing ICSI. Salpingectomy is an easily performed surgical procedure that when performed by an efficient surgeon could reduce injuries to tubal and ovarian blood vessel.
So it will be important to evaluate the benefits and drawbacks of laparoscopic tubal separation or salpingectomy to ovarian reserve before performing ICSI in female patients with hydrosalpinx (6).
Aim of the present study was to compare the ovarian reserve and ICSI outcomes after performing either laparoscopic tubal separation or laparoscopic salpingectomy in females with hydrosalpinx.

PATIENTS AND METHODS

The study was performed in Department of Gynecology and Obstetrics, School of Medicine, Zagazig University in about 3 years from March 2016 to May 2019. An approval was taken from the local ethical committee of School of Medicine, Zagazig University and a written informed consent was acquired from all included participants.

Inclusion criteria

We included female patients with a sure diagnosis of bilateral hydrosalpinx of more than 0.3 cm by ultrasound or by hysterosalpingography (HSG) aged less than 38 years having normal cavity of the uterus and seeking for ICSI.

Exclusion criteria

We excluded female patients having endometriosis, adenomyosis, thin endometrium, uterine synechiae, patients with polycystic ovary syndrome, patients with previous surgery on the ovary and patients with contraindications to laparoscopic surgery.
Several reports found an AMH decline after endometriomas removal and a progressive subsequent recovery of AMH levels after surgery (7, 8), We excluded patients with any ovarian cysts, polycystic ovary syndrome and patients with endometriomas to avoid this findings. Male factor of infertility was excluded.
Patients who fulfilled the inclusion criteria were randomly divided by computer generated randomization into two groups. Group A included 60 patients that underwent bilateral laparoscop-

ic proximal tubal cutting and separation and and group B included 60 patients that underwent bilateral laparoscopic salpingectomy before ICSI. Intracytoplasmic sperm injection (ICSI) is the most recent technique used nowadays.

We assessed all parameters of ovarian reserve to all included patients before surgery as: serum AMH, FSH and antral follicle counts in days 2-5 of the menstrual cycle then repeated in the same phase about eight weeks after performing all surgeries.

The outcome evaluated parameters of the study were: rates of clinical pregnancy and live births in addition to changes in parameters of ovarian reserve, changes of rate of fertilization, rate of cleavage, rates of implantation and rates of miscarriage. We defined pregnancy rate as the presence of a gestational sac containing a fetal pole and cardiac activity during transvaginal ultrasound examination at 6 weeks.

Live birth rate was the percentage of cycles which lead to live birth. Implantation rate was the gestational sacs number determined by ultrasound in comparison with number of transferred embryos. Miscarriage rate was number of losses in pregnancy less than 20 weeks of gestation.

We have performed bilateral salpingectomies and bilateral tubal separation operations laparoscopically for all included patients using bipolar diathermy.

All patients were subjected to standard protocol of pituitary down-regulation on day 21 of the previous cycle using GnRH-a 0.5 mg. confirmation of pituitary desensitization was diagnosed 14 days later by finding serum estradiol levels of < 50 pg/ml, serum LH < 3 IU/l, no follicles > 10 mm in size and thickness of the endometrium was < 5 mm during ultrasound examination.

Later on administration of Gonadotropin 150-300 IU/day was done according to patients' age, BMI and serum AMH. We tracked the follicles serially for assessment of ovarian response to stimulation and doses of gonadotropin were modified accordingly. Trigering all patients with recombinant hCG (250 mcg) if there were a minimal 3 follicles ≥ 18 mm. We performed transvaginal sonar-guided retrieval of the oocyte about 36 hours after trigger. Insemination of retrieved oocytes was done by conventional ICSI. After performing insemination we checked for fertilization about 16-18 hours.

We assessed further cleavage and graded embryos so as to transfer up to 2 embryos with a good quality on Day 3 or 5 under sonar guidance using a soft embryo transfer catheter. We give intramuscular progesterone 100 mg daily for luteal support.

We checked serum β hCG 16 days after transferring embryos and patients with a positive β hCG were confirmed to have clinical pregnancy by ultrasound 4 weeks after embryo transfer.

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20. Quantitative variables were described using their means and standard deviations. Categorical variables were described using their absolute frequencies and were compared using Chi square test and Fisher exact test when appropriate. Kolmogorov-Smirnov (distribution-type) and Levene (homogeneity of variances) tests were used to verify assumptions for use in parametric tests. Mann whitney test (for not-normally distributed data) was used to compare medians of two groups. Independent sample t test (for normally distributed data) was used to compare means of two groups. Percent change was calculated by subtracting postoperative value from preoperative value then divided it by preoperative value*100. Pearson correlation coefficient was used to assess strength and direction of a linear relationship between two variables. Binary logistic regression was used to predict the odds of being a case based on the values of the independent variables (predictors). Linear stepwise regression analysis was used to determine the extent to which there is a linear relationship between a dependent variable and one or more independent variables. The level statistical significance was set at 5% ($P < 0.05$).

RESULTS

During the study period of 3 years a total of 60 patients having bilateral tubal hydrosalpinx were laparoscopically managed by salpingectomy or proximal tubal separation.

Both included groups have no significant differences in demographic or clinical baseline criteria as age, BMI, type or duration of infertility, 2 days serum FSH, serum Estradiol or serum AMH (**table I**).

Post-surgery Serum FSH on day 2 increased in both groups to 6.79 U/L and 6.957 U/L for tubal separation and salpingectomy groups respectively. Post-surgery Serum EH on day 2 increased to 44.4 U/L and 44.5 U/L for tubal separation and salpingectomy groups respectively.

Post-surgery serum AMH decreased significantly in both groups to 3.663 ng/mL and 2.697 ng/mL for tubal separation and salpingectomy groups

Table I. *Comparison between the studied groups regarding baseline data.*

Parameters	Surgical procedures		Test	
	Tubal occlusion	Salpingectomy	χ^2/t	p
	N = 30 (%)	N = 30 (%)		
Age:				
Mean ± SD	27.7 ± 2.961	28 ± 3.434	Fisher	0.731
Range	23-35	23-35		
BMI (kg/m²):				
Mean ± SD	23.9 ± 1.125	24.07 ± 1.112	0.278	0.598
Range	22-26	22-26		
Infertility duration:				
Mean ± SD	4.73 ± 1.337	4.43 ± 1.331	0.287	0.592
Range	2-7	2-7		
Infertility:				
Primary	24 (80)	26 (86.7)	Fisher	0.731
Secondary	6 (20)	4 (13.3)		

χ^2: chi square test; t: independent sample t test.

respectively. In the salpingectomy group after surgery we found: a slight increase in the serum FSH (p value = 0.015), serum EH (p = 0.022) and a significant reduction of levels of serum AMH (p value < 0.001) than in group of patients underwent bilateral tubal separation (**figures 1, 2**).

Days of stimulation within group of tubal separation ranged from 8 to 10 days with mean 9.233 while within salpingectomy group, it ranged from 10 to 12 days with mean 11.033. Total gonadotropin within group of tubal separation ranged from 2800 to 3700 with mean 3187.33 while within salpingectomy group, it ranged from 3500 to 4200 with mean 3793.33. We showed that patients who underwent salpingectomy needed more stimulation days and higher doses of gonadotropins than patients with bilateral tubal separation (p < 0.001) (**table II**).

We found that the rates of fertilization rate in salpingectomy group were lower than tubal separation group (p value = 0.049).

We found no significant differences between both included groups regarding: rates of implantation, rates of clinical pregnancy, live birth or miscarriage rates.

DISCUSSION

Hydrosalpinx was incriminated in reduction of rates of clinical pregnancy and increasing rates of ectopic pregnancy and abortion (9). Moreover it inversely affected IVF, ICSI success rates by unsure mechanisms. There are several suggested mechanism explain how hydrosalpinx inversely affected the fertility; the accumulated fluid of hydrosalpinx might return to cavity of the uterus, negatively affects receptivity of the endometrium, has embryotoxic effects, interfering with implantation or even simply wash out the embryos (10). So treatment of hydrosalpinx either by salpingectomy of by tubal separation before

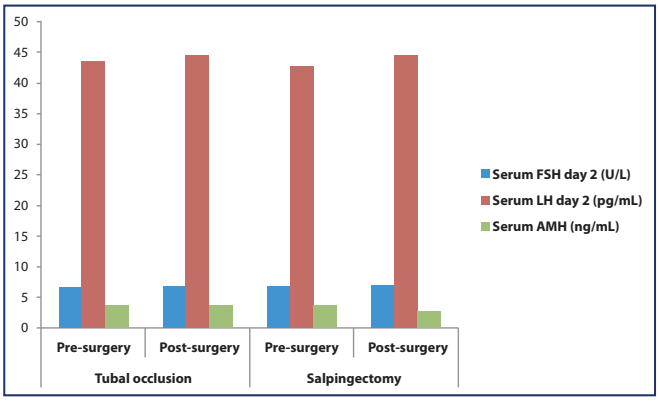

Figure 1. *Multiple bar chart showing hormonal profile among the studied groups before and after surgery.*

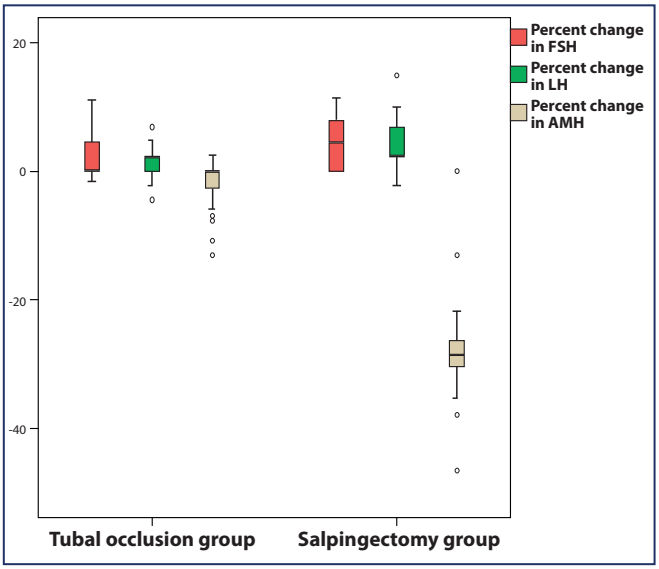

Figure 2. *Boxplot showing percent change in hormonal profile among the studied patients.*

Table II. *Comparison between the studied groups regarding days of stimulation and total dose of gonadotropins.*

Parameters	Surgical procedures		Test	
	Tubal occlusion	Salpingectomy	t	p
	N = 30 (%)	N = 30 (%)		
Days of stimulation:				
Mean ± SD	9.233 ± 0.679	11.033 ± 0.765	- 9.64	< 0.001**
Range	8-10	10-12		
Gonadotropin total dose:				
Mean ± SD	3187.33 ± 369.66	3793.33 ± 206.67	- 7.387	< 0.001**
Range	2800-3700	3500-4200		

t: independent sample t test; **p ≤ 0.001 is statistically highly significant.

starting IVF.ICSI program is needed to increase the rates of success (11). In the current study, we found no significant differences in levels of FSH in the laparoscopic salpingectomy and laparoscopic bilateral tubal separation. We showed that higher AMH levels were found in the tubal separation group than the salpingectomy group similarly Vignarajan *et al*. (12). Salpingectomy as a surgical management strategy for hydrosalpinx was found to inversely affect ovarian vascularity which might decrease ovarian reserve (6). Johnson *et al*. (13) found that salpingectomy might damage ovarian vascular supply while tubal obstruction by ligation has no effect on vascular supply.

Tubal excision may damage the arch of the artery, while tubal ligation at the proximal end and distal salpingostomy may cause less damage to the mesangium (13). A study which assessed salpingectomy effects on the ovarian reserve in patients with tubal disease showed near results to ours that there is marked reduction in levels of ovarian reserve and AMH levels in the salpingectomy group (14). Similarly previous reports showed that proximal tubal occlusion could preserve ovarian reserve more than salpingectomy (15-17). Salpingectomy was found to impair blood supply of the ovary shortly after operation, but the long-term performance was not proved yet (18).

Xu *et al*. (19) metaanalysis showed that patients underwent proximal tubal occlusion has significantly higher AMH levels than salpingectomy but there were no differences between both groups in rates of: clinical pregnancy, implantation or live birth. Their results showed that salpingectomy leads to damage to ovarian reserve than tubal occlusion.

Additionally Vignarajan *et al.* (12) showed that salpingectomy causes more harm to the ovaries than tubal occlusion (**tables III, IV**). Regarding the benefits of salpingectomy over tubal occlusion it was found that salpingectomy leads to removal of any inflammation source, prevents releasing pro-inflammatory cytokines in addition to decreasing incidence of ovarian and tubal malignancies. So salpingectomy is still considered in management of any patients with tubal disease regardless the ovarian reserve (6) (**tables V, VI**).

Although many studies agreed with our results, but other reports who confirmed a regular ovarian function after salpingectomy are found (20, 21), which specify that this problem is not universally proven yet.

CONCLUSIONS

Our study demonstrated the benefits of laparoscopically tubal separation over salpingectomy in patients with hydrosalpinx regarding preventing reduction in ovarian reserve particularly in women underwent ICSI. So proximal tubal separation as suggested as a better surgical management option or treatment of hydrosalpinx in patients undergoing ICSI due to preservation of ovarian reserve. But due to different results of previous studies regarding advantages of tubal separation or salpingectomy, further researches on large number of patients were needed to explore the long term outcomes of surgery on ovarian reserve and ICSI outcome.

Limitations of the study: few number of patients were included aditionally endometriomas were excluded, AMH evaluation was carried out 8 weeks after surgery and no AMH evaluation was carried out later to asses a long term AMH increase as found after surgical treatment of endometrioma which might impair the result of the study, so there is the lack of long term results assessment.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

Table III. *Comparison between the studied groups regarding hormonal profile before and after surgery.*

Parameters	Surgical procedures		Test	
	Tubal occlusion	Salpingectomy	t	p
	N = 30 (%)	N = 30 (%)		
Pre surgery Serum FSH D2 (U/L):				
Mean ± SD	6.643 ± 0.374	6.65 ± 0.365	- 0.07	0.945
Range	6.1-7.2	6.2-7.2		
Post-surgery Serum FSH D2 (U/L):				
Mean ± SD	6.79 ± 0.339	6.957 ± 0.376	- 1.805	0.076
Range	6.2-7.4	6.2-7.9		
P¥	0.001**	< 0.001**		
Pre surgery Serum E D2 (pg/L):				
Mean ± SD	43.5 ± 1.28	42.7 ± 1.44	2.273	0.027*
Range	41-45	40-45		
Post-surgery Serum E D2 (pg/L):				
Mean ± SD	44.4 ± 1.275	44.5 ± 1.28	- 0.303	0.763
Range	42-46	42-46		
P¥	< 0.001**	< 0.001**		
Pre surgery AMH:				
Mean ± SD	3.743 ± 0.353	3.743 ± 0.355	- 0.004	0.997
Range	3.3-4.6	3.3-4.6		
Post-surgery AMH:				
Mean ± SD	3.663 ± 0.329	2.697 ± 0.461	9.35	< 0.001**
Range	3.2-4.6	2.2 – 4		
P¥	0.012*	< 0.001**		

t: independent sample t test; *p < 0.05 is statistically significant; **p ≤ 0.001 is statistically highly significant; ¥paired sample t test.

Table IV. *Comparison between the studied groups regarding percent change in hormonal profile after surgery.*

Percent change	Surgical procedures		Test	
	Tubal occlusion	Salpingectomy	t	p
	N = 30 (%)	N = 30 (%)		
Serum FSH D2:				
Median	0%	4.39%	- 2.443	0.015*
Range	- 1.587, 11.115	0, 11.429%		
Serum LH D2:				
Median	2.27%	2.325%	- 2.284	0.022*
Range	- 44.4, 6.977%	-2.22, 15%		
AMH:				
Median	0%	- 28.57%	- 6.526	< 0.001**
Range	- 13.043, 2.564	0, 46.512%		

Z: Mann Whitney test; *p < 0.05 is statistically significant; **p ≤ 0.001 is statistically highly significant.

Table V. *Comparison between the studied groups regarding outcome.*

Parameter	Surgical procedures		Test	
	Tubal occlusion	Salpingectomy	χ^2/t	p
	N = 30 (%)	N = 30 (%)		
Fertilization rate:				
Mean ± SD	0.932 ± 0.088	0.777 ± 0.059	0.871	0.049
Range				
Clinical pregnancy:				
No	17 (56.7)	19 (63.3)	0.278	0.598
Yes	13 (43.3)	11 (36.7)		
Live birth:				
No	18 (60)	20 (66.7)	0.287	0.592
Yes	12 (40)	10 (33.3)		
Miscarriage:				
No	12 (92.3)	11 (91.7)	Fisher	> 0.999
Yes	1 (7.7)	1 (8.3)		

χ^2: chi square test; t: independent sample t test.

Table VI. *Univariate analysis of parameters associated with ovarian reserve.*

Parameters	Univariate analysis		
	Yes N = 24 (%)	No N = 36 (%)	p
Procedure			
Tubal occlusion	16 (66.7)	14 (38.9)	0.035∞*
Salpingectomy	8 (33.3)	22 (61.1)	
Infertility			
Primary	19 (79.2)	31 (86.1)	0.48∞
Secondary	5 (20.8)	5 (13.9)	
Age	28.38 ± 3.1	27.5 ± 3.23	0.301¥
BMI	24.13 ± 0.85	23.89 ± 1.26	0.425¥
Duration of infertility	4.63 ± 1.35	4.56 ± 1.34	0.845¥
Preop serum FSH D2	6.57 ± 0.38	6.7 ± 0.37	0.17¥
Postop serum FSH D2	6.82 ± 0.36	6.91 ± 0.37	0.33¥
Preop serum LH D2	43.17 ± 1.66	43.06 ± 1.24	0.768¥
Postop serum LH D2	44.5 ± 1.44	44.42 ± 1.16	0.805¥
Preop serum AMH	3.66 ± 0.31	3.8 ± 0.37	0.126¥
Postop serum AMH	3.33 ± 0.66	3.08 ± 0.6	0.146¥
Total gonadotropin used	3344.2 ± 472.96	3587.78 ± 366.86	0.039¥*
Days in stimulation	9.83 ± 1.09	10.33 ± 1.17	0.101¥

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Objective and quantitative evaluation of fetal hiccups by computerized cardiotocography: a prospective observational study

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ABSTRACT

The physiological function of fetal hiccup and its correlation with fetal well-being is a debated topic.
We conducted a prospective observational study in a Tertiary care University Hospital to correlate the fetal hiccups with the antepartum computerized cardiotocography parameters.
Fifty-one nonlaboring women with a term pregnancy were enrolled. We collected data regarding maternal perception of fetal hiccups and the computerized cardiotocographic examination. The pregnant were divided into three groups depending on fetal hiccups perception. There was a statistical difference for the number of fetal movements in an hour between the group of daily perception and the group of no perception.
Changes in fetal movements frequency are essential to recognize pregnancies at increased risk for adverse fetal outcomes. No one studies in the medical literature utilized the computerized cardiotocographic machine to explore fetal hiccups. Then our study showed that a mother with daily fetal hiccups could be considered a low risk considering the significant numbers of fetal movements revealed by computerized cardiotocography. Nevertheless, randomized controlled trials are required to evaluate the fetal hiccups evaluation and its influence on fetal outcomes.

SOMMARIO

La funzione fisiologica del singhiozzo fetale e la sua correlazione con il benessere fetale è un argomento dibattuto.
Abbiamo condotto uno studio prospettico osservazionale in un ospedale universitario di terzo livello per valutare correlazioni tra il singhiozzo fetale ed i parametri della cardiotocografia computerizzata antepartum. Sono state arruolate 51 pazienti gravide a termine non in travaglio attivo. Abbiamo raccolto dati riguardanti la percezione materna del singhiozzo fetale e l'esame cardiotocografico computerizzato. Le gravide sono state divise in tre gruppi a seconda della percezione del singhiozzo fetale. È stata riscontrata una differenza statisticamente significativa per il numero di movimenti fetali attivi in un'ora tra il gruppo con percezione quotidiana del singhiozzo e il gruppo con nessuna percezione.
I cambiamenti nella frequenza dei movimenti fetali sono essenziali per riconoscere le gravidanze ad aumentato rischio di esiti fetali avversi. Nessuno studio nella letteratura medica ha utilizzato la cardiotocografia computerizzata per indagare il singhiozzo fetale. Quindi il nostro studio ha dimostrato che una madre con singhiozzo fetale quotidiano potrebbe essere considerata a basso rischio considerando il numero significativo di movimenti fetali rilevati dalla cardiotocografia computerizzata. Tuttavia, sono necessari studi randomizzati controllati per confermare l'impatto del singhiozzo fetale sugli outcome neonatali.

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Key words
Fetal hiccups; fetal movement; computerised cardiotocography; cardiotocography; fetal heart rate; computerized analysis.

INTRODUCTION

Fetal hiccups are considered quick fetal movements that all mothers can perceive during pregnancy (1). However, current evidence does not fully explain this physiological mechanism, although fetal hiccups are considered healthy and normal fetal functions (2). The prevalence and duration of such phenoms are still unclear. Fetal hiccups can be seen with faltering on the mother's abdomen, and, since it is not a widespread phenomenon, we need much time is usually needed to appreciate it. Additionally, several reports showed no substantial difference in fetal hiccups bouts across different gestational weeks, although the frequency decreases with advanced gestational age, particularly after 28 weeks. Hiccups have been reported during the first trimester of pregnancy (3-5), with increased frequency during the third trimester (6). Other studies have shown a reduced umbilical arterial and venous flow during fetal hiccups (7, 8). However, this finding has not been correlated with adverse fetal outcomes and might be considered a physiological phenomenon (8). During the second trimester, fetal hiccups have not influenced the fetal heart rate (7). In term, hiccups have been related to a modest improvement in fetal heart rate (9), and, as such, it is considered a sign of fetal wellbeing. The best noninvasive indicator of fetal wellbeing in clinical practice remains fetal heart rate (FHR) monitoring through Cardiotocography (CTG) monitoring, which has been recently advanced by the computerized CTG (cCTG) analysis, thus improving fetal surveillance. Normal FHR baseline is indicated as normal in the ranges of 110 to 160 beats per minute (bpm) (10). Computerized CTG is an automatic analysis tool that provides further diagnostic criteria and objective parameters: the baseline fetal heart rate (Basal FHR), the accelerations and decelerations, the long-term FHR variation (LTV), the short-term FHR variation (STV), the episodes of high/low FHR variation, and the number of fetal movements for an hour (FM) (11). We hypothesize the possible analytical correlation between the presence of the fetal hiccups and the maternal perception of the fetal movements can be identified and so applied for future fetal well-being monitoring.

MATERIALS AND METHODS

Subjects and assessment

This prospective observational study was conducted between April 01, 2019, and March 01, 2020, in a single University tertiary care Center. Fifty-one pregnancies were enrolled (**table I**), we included nonlaboring term singleton pregnancies (37 0/7 - 41 6/7 weeks of gestation), who were referred for the fetal antepartum surveillance to the outpatient clinic of the "Obstetrics and Gynecology Unit, AOU Luigi Vanvitelli, University of Campania Luigi Vanvitelli of Naples (Italy)". Gestations complicated by fetal malformations, stillbirths, preterm deliveries, maternal comorbidity (12) or patients with missing data were excluded. For each patient, data on demographics and pregnancy information, including maternal age, maternal height and weight, body-mass index (BMI), gestational age (GA) at delivery, gravity, parity, and smoking, were collected. GA was determined according to the first-trimester ultrasound exam. A cCTG was carried out for each patient using a Sonicaid Team 3 (Huntleigh Healthcare Ltd, Cardiff, United Kingdom) computerized cardiotocography machine. External cCTG was performed at least 20 minutes (maximum 60 minutes), with two transducers fixed on the maternal abdomen: one above the fetal heart level and the other one at the uterine fundus. When evaluating cCTG data, the following measures were assessed: basal FHR (beats per

minute), number of accelerations and decelerations, LTV (minutes), STV (milliseconds), episodes of high/low FHR variation in minutes, and number of FM for an hour (13). Only one tracing per fetus was included. We chose the last tracing before the onset of labor, which occurred within 24 hours. To evaluate the maternal fetal hiccups perception, a trained clinician (M.L.V.) administered an *ad hoc* questionnaire focusing on fetal hiccups perception in the last two weeks to all patients, who took approximately 15-30 minutes to complete it. The questionnaire included if the mother felt the fetal hiccup's presence and, if present, its intensity and frequency, *i.e.*, if the hiccups were perceived daily, occasionally (from two episodes to almost daily perception), or if it was perceived one time only. If one of these questions was not clear for the mother and unable to answer it satisfactorily, it was reported as "does not know". Based on the answers from this questionnaire, we divided our population into three groups: no fetal hiccups perception (G1); occasionally (not daily) fetal hiccups perception (G2); and daily maternal perception (G3). The different parameter of computerized analysis of fetal heart rate (FHR, LTV, STV, episodes of high/low FHR variation in minutes, acceleration and deceleration and FM) were evaluated in each group.

Ethical approval

The confidentiality of all participants was maintained during the whole experimental procedure. Ethical approval was not required since the study was classified as a hospital audit of current clinical practice. The study was registered on www.clinicaltrials.gov database (ID no. NCT04366076) and performed according to the "Strengthening the Reporting of the Observational studies in Epidemiology" (STROBE) guidelines (14).

Sample size

According to available literature, assuming an *a priori* calculation of the minimum sample size to report a significant difference between groups, given 80% power and an alpha level of 0.05, including a 9% opt-out rate, a minimum of 42 women was necessary.

Table I. *Clinical and demographic characteristics of the three groups of women enrolled.*

	No perception (n = 25) (G1)	**Occasionally (n = 14) (G2)**	**Daily (n = 10) (G3)**		
					p-value
Maternal age (years)					
Median, DS	31.1 ± 7.1	34.6 ± 5.6	33.3 ± 7.2	G1 *vs* G2	0.28
Range	18-42	21-43	19-46	G2 *vs* G3	0.87
				G3 *vs* G1	0.67
Body mass index, kg/m^2				G1 *vs* G2	0.22
Median, DS	30.6 ± 6.5	27.5 ± 4.2	26.6 ± 2.9	G2 *vs* G3	0.90
Range	21-50	22-34	23-30	G3 *vs* G1	0.12
Smokers,	4	2	0	G1 *vs* G2	0.97
				G2 *vs* G3	0.56
				G3 *vs* G1	0.39
GA at cCTG,					
weeks, DS	39.1 ± 1.1	39.2 ± 0.6	39.1 ± 1.2	G1 *vs* G2	0.93
range	37.1-40.6	37.1-39.2	37.1-40.4	G2 *vs* G3	0.95
				G3 *vs* G1	0.99
Birth weight, gr					
Median, DS	3,152 ± 859	3,146 ± 567	3,204 ± 455	G1 *vs* G2	0.99
Range	1,900-5,280	2,350-4,070	2,510-3,730	G2 *vs* G3	0.98
				G3 *vs* G1	0.98
Fetuses					
Male	9	4	7	NS	
Female	16	10	5		
Maternal comorbidities					
Gestational Diabetes	4	1	2	NS	
Intrauterine growth restriction	3	3	2		
Pre-eclampsia	2	0	0		
Macrosomia	0	0	0		
Total	9	4	4		

GA: gestational age; cCTG: computerized Cardiotocography.

Statistical analysis

Parametric or non-parametric statistics were used, as appropriate. Namely, the multivariate analysis combined the cCTG parameters to correct for potential confounders during data analysis. The independent variables observed were the following: baseline FHR, number of accelerations and decelerations, episodes of high/low FHR variation in minutes, LTV (min), STV (ms), signal loss (%), and FM. The binary outcome was represented by fetal hiccups (present *vs* absent). The multivariate analysis joined together the cCTG results to correct for potential confounders during data analysis.
All variables are displayed as the means ± standard deviation. Data were compared using a one-way analysis of variance test (ANOVA) followed by the Tukey's honestly significant difference (HSD) test. A P-value (p) < 0.05 was used to indicate a statistically significant difference. Stata 14.1 (Stata corp., College Station, TX, 2013) was used for all data analysis.

RESULTS

Fifty-one pregnancies were initially enrolled (**table I**). Based on the maternal fetal hiccups perception during the last two weeks, the G1 group consisted of 25 women, the G2 of 14 women, and the G3 of 10 women. From the original sample of 51 patients, two were excluded because they were unable to provide any information about the hiccups perception. As shown in **table I**, there could not significative difference between maternal age, BMI, GA, fetal sex, and maternal comorbidity between the three groups. The maximum interval between cCTG and delivery was 24 hours. In order to evaluate whether the fetal weight might have produced a bias, we recorded the fetal weight at the delivery, and no significant difference between the groups was observed. The analysis of cCTG parameters between the three groups showed no significant difference in the percentage of signal loss, baseline FHR, number of accelerations and decelerations, episodes of high/low FHR variation, LTV, and STV at both univariate and multivariate analysis (**table II**). Conversely, we found a statistically significant difference for the number of FM in an hour between the group of daily perception (G3) compared to the group of no perception (G1) (72 *vs* 37 FM, p < 0.05). This statistically significant difference was also confirmed by the multivariate analysis (**table III**).

DISCUSSION

Counting fetal movements is a popular approach used to prevent stillbirths. In addition to fetal movements count, the maternal fetal hiccups perception is usually applied. Nevertheless, limited knowledge about its role in healthy pregnancies is available. Fetal hiccups seem to be shared in primates, but their origin and meaning remain unknown (15). Several theories have been proposed to explain hiccups in fetal life, including respiratory muscles' development, providing the infant for suckling and controlling amniotic fluid in early gestation (15, 16). Some studies reported the qualitative and quantitative aspects of fetal function through continuous real-time ultrasound observations and demonstrated that the fetal hiccups happened episodically with a fluctuating incidence (17-19). A study revealed its increase in maternal perception of fetal hiccups, probably due to a better maternal perception in late gestation (20). Additional researches have focused on the natural aspect of fetal hiccups and its relationship with reduced risk of late stillbirth (21, 22). In this scenario, the present study suggests that the daily perception of the fetal hiccups might be related to an increased number of fetal movements, measured by the cCTG, which seem to be almost twice compared to mothers with no fetal hiccups perception. Given the lack of previous evidence on this topic, this study's finding might significantly support clinicians in screening pregnant women with a real decrease in fetal activity. As such, fetal daily hiccups perception might be considered a marker of normal fetal activity and fetal well-being (23, 24). This study's strength is the prospective design, which allows detailed reporting information on the maternal perception of fetal hiccups concerning the cCTG. Moreover, the assessment of fetal hiccups was performed by an expert physician in order to minimize the maternal questionnaire bias. Another strength was the attempt to reduce the heterogeneity of demographic characteristics of the sample by considering several factors (GA at the cCTG, smoking, age (25), BMI (26), fetus sex, and all maternal comorbidity (27-31). Further, in order to exclude bias related to the fetal size (32), we recorded all the fetal weights at the birth. Finally, this study's peculiarity is the use of cCTG to objectively estimate the real number of fetal movements and all the other cCTG parameters. Several previous publications,

Table II. *Report of maternal fetal hiccups perception during the last two weeks.*

	No perception (n = 25) (G1)	Occasionally (n = 14) (G2)	Daily (n = 10) (G3)		
					p-value
cCTG Duration time, min					
Median, DS	42 ± 17	40 ± 16	40 ± 15	G1 *vs* G2	0.91
Range	20-60	20-60	20-60	G2 *vs* G3	1.00
				G3 *vs* G1	0.92
Signal loss, %					
Median, DS	3.4 ± 4.8	1.6 ± 2.6	5.1 ± 6.7	G1 *vs* G2	0.55
Range	0-20.6	0-8.7	0-18.8	G2 *vs* G3	0.22
				G3 *vs* G1	0.62
FHR, bpm					
Mean, DS	131 ± 7	136 ± 9	129 ± 8	G1 *vs* G2	0.15
Range	121-149	120-150	119-143	G2 *vs* G3	0.09
				G3 *vs* G1	0.77
FM, 1 h					
Mean, DS	37.3 ± 26.2*	42.7 ± 50	72.7 ± 44.6*	G1 *vs* G2	0.90
Range	7-107	4.1-178	21-161	G2 *vs* G3	0.15
				G3 *vs* G1	0.042*
Accelerations, n.					
Mean, DS	9.2 ± 5.9	8.8 ± 6.4	10.5 ± 6	G1 *vs* G2	0.98
Range	0-25	3-24	2-19	G2 *vs* G3	0.79
				G3 *vs* G1	0.84
Decelerations, n.					
Mean, DS	0.1 ± 0.3	0.2 ± 0.5	0 ± 0	G1 *vs* G2	0.68
Range	0-1	0-2	0-0	G2 *vs* G3	0.35
				G3 *vs* G1	0.69
Episode of high variation, n.					
Mean, DS	22.5 ± 13	22.0 ± 14.0	23 ± 14.1	G1 *vs* G2	0.99
Range	0-47	7-57	5-47	G2 *vs* G3	0.98
				G3 *vs* G1	0.99
Episode of low variation, n.					
Mean, DS	8 ± 13.3	9 ± 15.0	5.8 ± 6.8	G1 *vs* G2	0.96
Range	0-52	0-52	0-50	G2 *vs* G3	0.81
				G3 *vs* G1	0.88
LTV, ms					
Mean, DS	50.3 ± 15.9	50.6 ± 17.8	57.9 ± 13.2	G1 *vs* G2	0.99
Range	20-89	19-82	35-76	G2 *vs* G3	0.51
				G3 *vs* G1	0.41
STV, ms					
Mean, DS	9.6 ± 3.5	9.7 ± 3.3	11.8 ± 3.4	G1 *vs* G2	0.99
Range	3.9-21.9	4.9-14.1	7.1-18.6	G2 *vs* G3	0.32
				G3 *vs* G1	0.22

cCTG: computerized Cardiotocography; FHR: Fetal Heart Rate; FM: Fetal Movement; LTV: Long Term Variability; STV: Short Term Variability.

Table III. *Multivariate analysis on fetal movements for an hour.*

			95% CI		
Clinical feature	**B Coefficient**	**SE**	**Lower limit**	**Upper limit**	**p-value**
Age	- 0.137	0.974	- 2.098	1.824	0.889
Body-mass Index	- 2.040	1.217	- 4.492	0.410	0.101
Birth Weight	- 0.009	0.009	- 0.029	0.001	0.305
Gestational age	2.514	8.277	- 14.146	19.176	0.763

indeed, evaluated the importance of fetal movements in women with reduced frequency to prevent stillbirth (33-35), but none of them have been focused on fetal hiccups evaluated with cCTG parameters. This study's main limitations are the relatively small sample size and the lack of fetal outcomes and their correlation with the maternal perception of fetal hiccups.

CONCLUSIONS

Daily maternal perception of fetal hiccups correlated with a double number of fetal movements for an hour than women without perception. Adding support to the evidence that when fetal hiccups are present daily, the number of fetal movements can be adequate. Therefore, these women might not need to contact the maternity care provider. Further randomized and prospective studies are needed to replicate our findings in order to fully include the maternal perception of fetal hiccups in the management of fetal wellbeing.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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The effect of inofolic supplementation on women with polycystic ovarian syndrome (PCOS): a Randomized Clinical Trial study

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ABSTRACT

Objective. Polycystic ovary syndrome is a disorder in women of reproductive age and it is one of the pathological factors that play a role in the failure of laboratory fertilization (IVF). The aim of this study was to determine the effect of inofolic supplementation on women with polycystic ovary syndrome (PCOS).
Material and methods. This clinical trial study was performed on 70 infertile women aged 20 to 40 years with polycystic ovary syndrome referred to the Sanandaj Besat Hospital infertility center in 2019. Patients were randomly divided into intervention and control groups. Patients in the intervention group took Clomiphene and inofolic supplement for 3 months and patients in the control group received only Clomiphene for 3 months. Various parameters such as fasting sugar, LDL, HDL, cholesterol, triglyceride and testosterone level were also measured.
Results. LDL (96.6 ± 19.4 *vs* 105.2 ± 10.1, p = 0.02), cholesterol (158.2 ± 10.4 *vs* 79.8 ± 14.4, p = 0.0001) and triglycerides levels (140.1 ± 30.3 *vs* 160.3 ± 22.0, p = 0.002) was significantly lower in the intervention group than in the control group. The mean HDL level in the intervention group was higher than the control group (47.3 ± 7.5 *vs* 43.2 ± 5.1, p = 0.009). The frequency of follicles (+ 2) in the intervention group (85.7%) was higher than in the control group (37.1%) (p = 0.001). The frequency of clinical pregnancies, pregnancies leading to live births, miscarriages, and preterm births in the two groups did not differ significantly and were almost similar (P > .05).
Conclusions. Inofolic supplementation improved fat profile status, fetal quality and reduced miscarriage and also increased follicles in women with polycystic ovary syndrome.

SOMMARIO

Obiettivo. La sindrome dell'ovaio policistico è un disturbo nelle donne in età riproduttiva ed è uno dei fattori patologici che giocano un ruolo nel fallimento della fecondazione in laboratorio (FIV). Lo scopo di questo studio era di determinare l'effetto dell'integrazione inofolica sulle donne con sindrome dell'ovaio policistico (PCOS).
Materiale e metodi. Questo studio clinico è stato condotto su 70 donne infertili di età compresa tra 20 e 40 anni con sindrome dell'ovaio policistico arrivate al centro per l'infertilità dell'ospedale Sanandaj Besat nel 2019. Le pazienti sono state divise casualmente in gruppi di intervento e di controllo. Le pazienti nel gruppo di intervento hanno assunto Clomifene e integratore inofolico per 3 mesi e le pazienti nel gruppo di controllo hanno ricevuto solo Clomifene per 3 mesi. Sono stati misurati anche vari parametri come glicemia a digiuno, LDL, HDL, colesterolo, trigliceridi e livello di testosterone.
Risultati. LDL (96,6 ± 19,4 *vs* 105,2 ± 10,1, p = 0,02), colesterolo (158,2 ± 10,4 *vs* 79,8 ± 14,4, p = 0,0001) e i livelli di trigliceridi (140,1 ± 30,3 *vs* 160,3 ± 22,0, p = 0,002) erano significativamente più bassi nel gruppo di intervento rispetto al gruppo di controllo. Il livello medio di HDL nel gruppo di intervento era superiore al gruppo di controllo (47,3 ± 7,5 *vs* 43,2 ± 5,1, p = 0,009). La frequenza dei follicoli (+ 2) nel gruppo di intervento (85,7%) era maggiore rispetto al gruppo di controllo (37,1%) (p = 0,001). La frequenza di gravidanze cliniche, gravidanze di nati vivi, aborti spontanei e nascite pretermine nei due gruppi non differivano in modo significativo ed erano quasi simili (P > .05).
Conclusioni. L'integrazione di inofoli ha migliorato lo stato del profilo dei lipidi, la qualità fetale e ha ridotto l'aborto spontaneo ed ha anche aumentato i follicoli nelle donne con sindrome dell'ovaio policistico.

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Key words
Infertility; PCOS; inofolic supplementation; Randomized Clinical Trial; Iran.

INTRODUCTION

Polycystic ovary syndrome is one of the most common causes of infertility in the world, affecting 5-10% of women of reproductive age. This syndrome prevents ovulation and increases the level of androgens in the blood (1). Polycystic ovaries cause irregular menstrual cycles and hyper-androgenism, which can lead to acne, alopecia, hirsutism, insulin resistance, male obesity, dyslipidemia, infertility, and fetal loss in early pregnancy (2). More than 50 percent of women with PCOS suffer from insulin resistance, which can lead to conditions such as metabolic disease, obesity, gestational diabetes, type 2diabetes, and cardiovascular disease (3).
Glucose intolerance affects 30 to 40 percent of PCOS patients, and insulin-sensitizers such as inositol are effective on spontaneous ovulation in patients with PCOS. The inositol phosphoglycan molecule plays a direct role in glucose metabolism. Myo-inositol is a type of inositol isoform in nature and also the human body (ovarian follicular environment) that is obtained from the epimerization of inositol. Epimerization is reduced in type 2 diabetes and PCO patients and is a common cause of endocrine disorders and infertility due to chronic ovulation failure in women of reproductive age (4, 5).
Myo-inositol regulates insulin, FSH, LH and TSH. By reducing insulin resistance, triglycerides, testosterone, and blood pressure, and increasing the sensitivity of insulin receptors, myo-inositol can be effective in inducing ovulation and treatment of patients with PCOS (6). Inofolic supplement contains 2000 mg of myo-inositol and 200 micrograms of folic acid, which is useful in the treatment of insulin-resistant polycystic ovary syndrome and type 2 diabetes (7).
The results of a study by Constantino *et al*. showed that consuming myo-inositol with folic acid for 12 to 16 weeks improved ovulation, metabolic factors, and also hormonal factors in women with PCOS (8). In another study, the use of myo-inositol in patients with PCOS showed an improvement in the number of follicles and a decrease in the number of immature oocytes in the control group (9). It has been suggested that there is a link between reduced levels of myo-inositol and insulin resistance. Several studies have suggested that myo-inositol improves ovarian function in patients with PCOS (8-10).
The present study evaluates the role of myo-inositol in the treatment of infertility in patients with PCOS; also, it examines biochemical parameters and evaluates the effects of myo-inositol on pregnancy rate. The main hypothesis is that treatment with myo-inositol reduces germinal vesicles and oocytes degeneration without compromising total numbers of oocytes.
Regarding that patients with PCOS suffer from infertility due to impaired follicogenesis and oocyte immaturity, the aim of this study was to evaluate the effect of myo-inositol on follicogenesis and oocyte maturation on women with polycystic ovary syndrome (PCOS).

MATERIALS AND METHODS

Patients and gathering their demographic and clinical records

This clinical trial study was performed on 70 infertile women aged 20 to 40 years with polycystic ovary syndrome referred to the Sanandaj Besat Hospital infertility center in 2019. Exclusion criteria were: history of ovarian surgery in the past three months, use of antiepileptic drugs and glucocorticoids, congenital adrenal hyperplasia, hypothyroidism, hyperthyroidism, hyperprolactinemia, androgen-secreting tumors, other causes of infertility and metabolic diseases.
Patients were randomly divided into intervention and control groups. Simple random sampling was used. From a package containing 70 cards, includ-

ing 35 cards labeled "A" for intervention group and 35 cards labeled "B" for control group, patients randomly selected a card and according to the label (A or B) they allocated into intervention or control groups. Sampling was continued in parallel until the number of samples was completed in two groups. Finally total of 70 women with PCOS were enrolled in the study and randomly allocated into intervention group (n = 35) and control group (n = 35). No patient was lost for follow up (**figure 1**).
The data collection tool was a questionnaire that was designed based on the objectives of the study. The questionnaire included two sections: the first

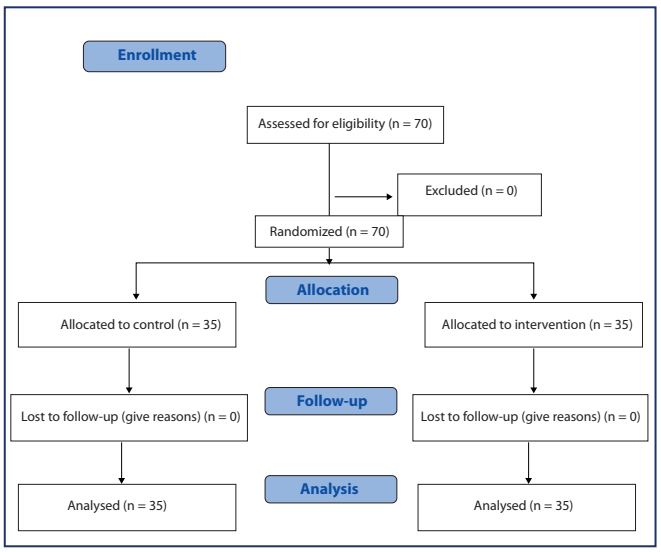

Figure 1. *Flowchart of the study.*

section for demographic information and the second for information on the condition of the follicles, ovaries, pregnancy and its consequences in the patients. Women's demographic information was recorded in the questionnaire and then their body mass index was determined. To determine the fat profile and testosterone levels, 5 cc blood samples was taken. Fasting blood sugar was also taken and recorded.
Patients in the intervention group received Clomiphene and inofolic supplement for 3 months. In the first month of the intervention, 50 mg Clomiphene was administered to the patients for 5 days from the third day of menstruation, inofolic sachets containing 2000 g myo-inositol and 200 µg folic acid was administered daily until the end of the month. In the second month of the intervention, 100 mg Clomiphene was administered to the patients for 5 days from the third day of menstruation, an inofolic sachet containing 2000 g myo-inositol and 200 µg folic acid was administered daily until the end of the month. In the third month of the intervention, 150 mg Clomiphene was administered to the patients for 5 days from the third day of menstruation, an inofolic sachet containing 2000 g myo-inositol and 200 µg folic acid was administered daily until the end of the month.
Patients in the control group received only Clomiphene for 3 months. In the first month 50 mg Clomiphene was administered to the patients for 5 days from the third day of menstruation. In the second month 100 mg Clomiphene was administered to the patients for 5 days from the third day of menstruation. In the third month 150 mg Clomiphene was administered to the patients for 5 days from the third day of menstruation.
After intervention, various parameters such as fasting sugar, LDL, HDL, cholesterol, triglyceride and testosterone were measured. Also, the number of developed follicles, the number of clinical pregnancies, the number of pregnancies leading to live birth and the number of abortions in each group were followed by the researcher and recorded in the questionnaire.
This study was approved by Ethics Committee of Kurdistan University of Medical sciences (IR.MUK.REC.1397.228) and also registered in Iranian Registry of Clinical Trials (IRCT20190825044605N1). Informed consent was taken from all participants of the study.

Statistical analyses

The data were analyzed using SPSS Ver.22 software. The data were summarized using descriptive indicators such as mean, standard deviation, frequency and relative frequency. The normality of quantitatively dependent variables was assessed using Kolmogorov-Smirnov test. To test the relationships between the variables, Kai-Square test, Fisher's exact test and independent t-test were used.

RESULTS

In terms of job, spouse's job, family marriage, medical history, marriage duration, infertility duration, and body mass index, there were no significant statistical differences between the intervention and control groups ($p > .05$), but in terms of average age and age of spouses there was a significant statistical difference between the intervention and control groups ($p < .05$) (**table I**).

Table I. *Comparison of variables in the intervention and control groups.*

Variables		Intervention group N (%)	Control group N (%)	P Value
Women's jobs	Housekeeper	33 (94.3)	27 (77.1)	0.05
	Employed	2 (5.7)	8 (22.9)	
Husband's job	Self-employed	28 (80.0)	23 (65.7)	0.51
	Employed	5 (14.3)	9 (25.7)	
	Army	2 (5.7)	3 (8.6)	
Family marriage	Yes	5 (14.3)	4 (11.4)	0.99
	No	30 (85.7)	31 (88.6)	
Clinical history	Yes	7 (20.0)	4 (11.4)	0.51
	No	28 (80.0)	31 (88.6)	
Women's age		25.9 ± 4.3	31.2 ± 5.6	< 0.001
Husband's age		31.6 ± 4.7	36.2 ± 7.4	0.003
BMI		26.8 ± 3.8	26.9 ± 3.9	0.87
Marriage duration		6.7 ± 3.0	8.0 ± 4.8	0.19
Infertility duration		4.0 ± 3.1	4.6 ± 3.9	0.39

Table II. *Comparison of the frequency of fat profile levels after intervention in the intervention and control groups.*

Variables	Level	Intervention Group N (%)	Control Group N (%)	P Value
LDL	Normal	32 (91.4)	33 (94.3)	0.99
	Abnormal	3 (8.6)	2 (5.7)	
HDL	Normal	6 (17.1)	2 (5.7)	0.15
	Abnormal	29 (82.9)	33 (94.3)	
Cholesterol	Normal	34 (97.1)	31 (88.6)	0.36
	Abnormal	1 (2.9)	4 (11.4)	
Triglycerides	Normal	27 (77.1)	6 (17.1)	< 0.001
	Abnormal	8 (22.9)	29 (82.9)	
FBS	Normal	34 (97.1)	35 (100.0)	0.99
	Abnormal	1 (2.9)	0	
Testosterone	Normal	30 (85.7)	29 (82.9)	0.74
	Abnormal	5 (14.3)	6 (17.1)	

Table III. *Comparison of mean fat profiles, FBS and testosterone level after intervention in the intervention and control groups.*

Variables	Intervention Group	Control Group	P Value
LDL	96.6 ± 19.4	105.2 ± 10.1	0.02
HDL	47.3 ± 7.5	43.2 ± 5.1	0.009
Cholesterol	158.2 ± 10.4	179.8 ± 14.4	< 0.001
Triglycerides	140.1 ± 30.3	160.2 ± 22.0	0.002
FBS	91.0 ± 2.2	89.5 ± .8	< 0.001
Testosterone	4.0 ± 2.5	4.6 ± 2.6	0.36

Table IV. *Comparison of the frequency of follicles in the intervention and control groups.*

Number of follicles	Intervention Group N (%)	Control Group N (%)	P Value
0	1 (2.9)	3 (8.6)	< 0.001
1	4 (11.4)	19 (54.3)	
2	24 (68.6)	9 (25.7)	
More than three	6 (17.1)	4 (11.4)	
Total	35 (100)	35 (100)	

Table V. *Comparison of the frequency of outcomes in the intervention and control groups.*

Outcomes		Intervention Group N (%)	Control Group N (%)	P Value
Clinical pregnancy	Yes	6 (17.1)	5 (14.3)	0.74
	No	29 (82.9)	30 (85.7)	
Pregnancy leads to a live birth	Yes	5 (14.3)	3 (8.6)	0.71
	No	30 (85.7)	32 (91.4)	
Spontaneous abortion	Yes	1 (2.9)	2 (5.7)	0.99
	No	34 (97.1)	33 (94.3)	
Preterm birth	Yes	0	0	-
	No	35 (100)	35 (100)	

The levels of fat profiles did not differ significantly in terms of LDL, HDL and cholesterol after the intervention, but it was significantly different in terms of triglycerides (22.9% in the intervention group and 82.9% in the control group) ($p = 0.0001$). There was no significantly difference between the two groups in terms of fasting blood sugar and testosterone levels ($p > .05$) (**table II**).

When calculating the fat profile average, the findings showed that LDL levels (96.6 ± 19.4 *vs* 105.2 ± 10.1, $p = 0.02$), cholesterol levels (158.2 ± 10.4 *vs* 79.8 ± 14.4, $p = 0.0001$) and triglycerides levels (140.1 ± 30.3 *vs* 160.2 ± 22.0, $p = 0.002$) were significantly lower in the intervention group than in the control group. The mean HDL level in the intervention group was higher than the control group (47.3 ± 7.5 *vs* 43.2 ± 5.1, $p = 0.009$) (**table III**).

The prevalence of 2 follicles and more in women of the intervention group (85.7%) was higher than in the control group (37.1%). This difference was also statistically significant ($p = 0.001$) (**table IV**).

In terms of the frequency of clinical pregnancies, pregnancies leading to live births, spontaneous abortion and preterm births there were no significant difference between intervention and control groups and were almost similar ($p > 0.05$) (**table V**).

DISCUSSION

In our study, the mean LDL, cholesterol, and triglycerides in the intervention (the myo-inositol) group were significantly lower than in the control

group. In the study by Constantino *et al*. triglyceride and cholesterol levels in the myo-inositol group were significantly reduced compared to the control group (8). Monstra *et al*. reported that myo-inositol, alone or in combination with its isomer D-Chiro-Inositol is able to improve the symptoms and outcomes of PCOS patients significantly (11). In a study by Greli *et al*. significant weight loss as well as a significant reduction in LDL was observed in patients treated with myo-inositol (6). In this study, the mean HDL level in the myo-inositol group was higher than the control group. An increase in HDL levels was observed in Greli's study. These data on fat profiles and HDL suggest that treatment with myo-inositol may be helpful in reducing the risk of cardiovascular disease in PCOS women. In a study by Hernandez Marin *et al*. the metabolic profiles of PCOS patients were improved by administrating myo-inositol and alpha-lactalbumin (12). In general, the findings of the mentioned studies were consistent with our study.

Our findings showed that fasting blood sugar and testosterone levels were not significant between the two groups, although testosterone was reduced in the myo-inositol group, but this decrease was not significant. In a study by Constantino *et al*., the results showed that in myo-inositol group the serum level of testosterone was reduced compared with the control group (8). In Regidor's study, the mean testosterone level was reduced from 96.6 to 43.3 ng/dL after administration of myo-inositol (13). The reason may be due to differences in the statistical population of the studies, the prescribed dose of myo-inositol and the duration of the intervention.

In our study the frequency of follicles (+ 2) in the intervention group (85.7%) was higher than in the control group (37.1%). Constantino *et al*. showed that myo-inositol improved ovulation (8). In a clinical trial by Ciotta *et al*., greater numbers of follicles with a diameter greater than 15 mm, higher mean of the transferred embryos, as well as a decrease in the number of immature oocytes were observed in the intervention group compared to the control group (9). Kane and Chiu stated in their studies that high concentrations of myo-inositol in human follicular fluid play a role in follicle maturation and cause the development of oocytes with good quality (14, 15). Goud also showed that myo-inositol had a positive effect on the growth of mature oocytes (16). In a clinical trial aimed to compare the effect of myo-inositol supplementation and D-chiroinositol on the oocytes quality of patients with PCOS the results showed that myo-inositol was able to improve oocytes quality instead of D-chiroinositol (17).

In fact, higher concentrations of myoinositol indicate high quality oocytes. A direct relationship between the concentration of myoinositol and melatonin has been reported by two independent research laboratories (15, 18).

Although in our study, the frequency of clinical pregnancies and pregnancies leading to live births in the intervention group was higher than in the control group, but the differences were not statistically significant. In a study by Abdollahi *et al*., although the frequency of successful pregnancies in the two groups with and without the administration of inofluate was 31% and 40.1%, respectively, but it was not statistically significant (19). A laboratory study has shown that myo-inositol is effective in stimulating the ovaries in women with PCOS (20). In addition, Raffone *et al*. indicated that myo-inositol slightly improved pregnancy rates compared to Metformin (21). These findings confirm the hypothesis that a decrease in insulin levels due to an oral supplement of myo-inositol depends on an increase in IPG which stimulates ovarian, reduces FSH and increases the chance of pregnancy (22). Myo-inositol can affect LH and FSH signaling. These gonadotropins bind to receptors in the ovaries, leading to their effects on steroidogenesis and gametogenesis (23). The effects of gonadotropins on follicle growth, ovulation, and luteinization are associated with differences in FSH and LH receptor concentrations (24). The results of a study by Amjadi *et al*. that evaluated the changes in the genomic profile of comulosa cells in women with PCOS under the influence of inofolic supplementation in assisted reproductive cycles showed that the quality of the oocytes, the quality of the embryos and the fertility rate were improved (25). In areview article, Unfer *et al*. concluded that myo-inositol improves hormonal and metabolic parameters, as well as improves ovarian activity and subsequently increases the risk of fertility in women with PCOS (26).

In our study, the pregnancy rate in intervention group was 17.1% and in a study by Ragidor *et al*., the pregnancy rate was 15.1% (11). The pregnancy rate in a study by Karimzadeh and Javedani was 14.4% (27) and in Legro *et al*. study the pregnancy rate was 12.3% (26) which were consistent with our study.

In our study 97.1% in intervention group and 94.3% in the control group had no spontaneous

abortion, but in a study by Abdollahi *et al*. spontaneous abortion in intervention group was 64.5% and in control group was 77.4% (19). The frequency of spontaneous abortions in our study was lower than Abdollahi *et al*. study.

Notably, in our study, one inofolic powder chassis was prescribed, while in most studies two chassis were used. Also, in our study and other studies, no side effects have been reported for doses of 2 and 4 grams per day, consequently it leads to high patient compliance. We suggest a 4-gram per day dose.

It is better to mention that several therapeutic strategies including fasting have been proposed for the treatment of PCOS, claiming to improve symptoms and signs. Three or more days fasting reduces circulating insulin, glucose levels and IGF-1 (29). Different fasting regimens may have beneficial effects on ovarian function (30). Considering the importance of InsR and compensatory hyperinsulinemia in inducing androgen excess in PCOS women, fasting may improve hyperandrogenism-related symptoms and signs. Although several studies evaluated the correlation between insulin signaling pathways and fasting, there is no adequate data to suggest a clear fasting regime for PCOS patients (31). It seems that more studies should be conducted to prove the above claim.

CONCLUSIONS

The results of our study showed that inofolic supplementation improved fat profile status, fetal quality and reduced miscarriage and also increased follicles in women with polycystic ovary syndrome.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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Future medico-legal implications on Obstetrics and Gynaecology practice in the SARS-CoV-2 pandemic

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ABSTRACT

Since February 2020, the Italian National Healthcare System had to mitigate the possibility of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) transmission to vulnerable patients. Healthcare professionals rapidly reviewed their workflow to maintain a safe and high standard treatment, but weak scientific evidences and organizational limits resulted in the adoption of heterogeneous measures. Adherence to screening protocols and follow-up programs pregnant women and oncological patients has not been always guaranteed: this scenario could evolve in an enormous number of medico-legal actions. This context, showing the weakness of the Italian law No. 24/2017, imposes an urgent reorganization of the legal framework to homogenize the judgements to "protect" healthcare professionals involved in this epochal emergency.

SOMMARIO

Da febbraio 2020, il Sistema Sanitario Italiano ha dovuto arginare il rischio di trasmissione della malattia da Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2) a pazienti vulnerabili. Gli operatori sanitari hanno rapidamente rivisto il loro flusso di lavoro per mantenere uno standard di cure adeguato, ma prove scientifiche deboli e limiti organizzativi hanno portato all'adozione di misure eterogenee. L'adesione ai protocolli di screening e ai programmi di follow-up delle donne gravide e delle pazienti oncologiche non è sempre stata garantita: questo scenario potrebbe evolvere in un numero enorme di azioni medico-legali. Tale contesto, che mostra la debolezza della legge italiana n. 24/2017, impone un'urgente riorganizzazione del quadro giuridico per "tutelare" gli operatori sanitari coinvolti in questa emergenza epocale.

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Key words
COVID-19; gynaecologic liability; Italian law; criminal proceeding; civil proceeding; risk management.

INTRODUCTION

Since February 2020, the coronavirus disease 19 (COVID-19) burdened Italian health institutions because of its high transmissibility. In this emergency, the National Healthcare System had to mitigate the possibility of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission to vulnerable groups, such as pregnant women, which were jeopardized by the virus. In fact, the most recent scientific evidences confirmed the increased risk of preterm birth and caesarean delivery (1, 2) and suspects a vertical transmission for pregnant women affected by COVID-19 (3).

Government-mandated social distancing and lockdown measures were found to be beneficial to limit the spread of the virus. However, they may lead to detrimental effects (in the worst case more important than virus transmission) on pregnant women and patients affected by gynaecological problems due to the partial shutdown of essential care services. For example, antenatal care units have suffered an important resource diversion, which shifted from routinely to essential care of pregnant women and increased the risk of maternal morbidity and mortality, especially for women with lower socioeconomic status (4). Thus, gynaecological and obstetrical medical negligence litigations, related to the consequence of the COVID-19 pandemic, is currently of interest for the Italian public and private health system.

The pandemic evidenced the weakness of complex laws regulating medical liability in Italy, which are structured on an ordinary framework, but inadequate during an emergency. Concisely, healthcare professionals (HPs) worked in an unprecedented setting characterized by poor instrumental and physical resources, shortage and/or contrasting scientific evidences, and overcrowding hospitals, which exposed them to criminal and civil proceedings for medical malpractice (5).

The aim of this article is to underline the background in which HPs had to work and to clarify the current Italian law status based on law No. 24/2017, which regulates HPs liability in both criminal and civil frameworks to propose a possible solution for future potential litigations.

THE EVOLUTION OF SCIENTIFIC EVIDENCES AND RECOMMENDATIONS REGARDING PREGNANT WOMEN AND NEWBORNS IN THE EARLY STAGE OF THE PANDEMIC

The advent of the SARS-CoV-2 pandemic drastically affected the Italian healthcare system, including gynaecology. In the initial phase, between January and May 2020, COVID-19 patients, affected with severe pneumonia, quickly overloaded healthcare facilities, which extremely challenged the in-hospital management of mothers and newborns ensuring an adequate healthcare service and, at the same time, protecting them from transmission. HPs rapidly reviewed their workflow to maintain a secure and high standard treatment, but lacking and weak scientific evidences resulted in the adoption of heterogeneous measures. In fact, the urgency to provide a guidance to manage COVID-19-free patients emerged with pressure during the initial phase of the pandemic. Continuously increasing ambiguous, impracticable and contrasting guidelines and recommendations lead to confusion of HPs whereas the proposed solutions were characterized by low viability from an economical and structural point of view.

On February 27th, the Italian Superior Health Institute (Istituto Superiore di Sanità (ISS)) (6), coordinated by the National Centre for Disease Prevention and Health Promotion (Centro Nazionale di Prevenzione delle Malattie e di Promozione della Salute (CNaPPS)), initiated a task force composed by members of the Italian scientific associations in gynaecology, obstetrics, midwifery, neonatology, paediatrics and anaesthesiology (SIGO, AOGOI, AGUI, FNOPO, SIN, SIMP, SIP, ACP and SIAARTI). The goal of the working group was a weekly online review of clinical practice guidelines for HPs about pregnancy, childbirth and breastfeeding according to current scientific literature on SARS-CoV-2 published on the most popular databases (PubMed, Scopus, Embase and CINAHL).

The weekly communications were essential to: 1) guarantee a continuous learning and guidance for HPs, and 2) share fast scientific evidences and medical experiences about the new virus. This weekly update continued until 7th of May, when the task force considered the knowledge about SARS-CoV-2 more stable, and the working group

decided to inform Italian HPs only in case of relevant and specific developments.
At the end of the first week of their work (February 27th–March 5th), the expert panel of the ISS confirmed the alarming state of scientific uncertainty. In fact, the task force was unable to provide any official recommendation for SARS-CoV-2 positive mothers and/or for those with clinical symptoms of COVID-19 recommending "multidisciplinary case-by-case assessment" (7).
A first document has been published by the Royal College of Obstetricians & Gynaecologists (RCOG) (8, 9) only on March 9th, which summarizes the available evidences on the effects of the SARS-CoV-2 virus on mothers and newborns. The text, for the third time updated on March 18th (10), by the RCOG in collaboration with the Royal College of Midwives, Royal College of Paediatrics and Child Health, Public Health England and Health Protection Scotland, strongly advised "to test for SARS-CoV all women who, at the time of hospital admission for delivery, have symptoms that indicate COVID-19 and to consider all symptomatic women as potentially infected". Subsequently, the 5th version (11) available on March 28th alarmingly signalled the possibility of vertical transmission recommending indications for safe surgical management of suspected or confirmed COVID-19 pregnant women. Moreover, it underlined the prominence that HPs should use effective personal protective equipment, as remarked by the ISS COVID-19 Report No. 2/2020 (12). Up to now, 12 versions (2) of the RCOG's report are available and the last has been published on 14 October 2020.
On March 31st, the Italian Ministry of Health issued the circular 11257 which comprehends key indications related to maternity care during the pandemic (**figure 1**), according to the recommendations contained in the interim guidance on pregnancy, childbirth, and breastfeeding published by the main international agencies (World Health Organization (WHO), Centres for Disease Control and Prevention and the UK Royal Colleges).
Finally, a "Global interim guidance on coronavirus disease 2019 (COVID-19) during pregnancy and puerperium from FIGO and allied partners: Information for healthcare professionals" (13) was published on April 4th by the International Federation of Gynaecology and Obstetrics (FIGO), which focused on the evidences available for the medical treatment of COVID-19 positive women. The manuscript

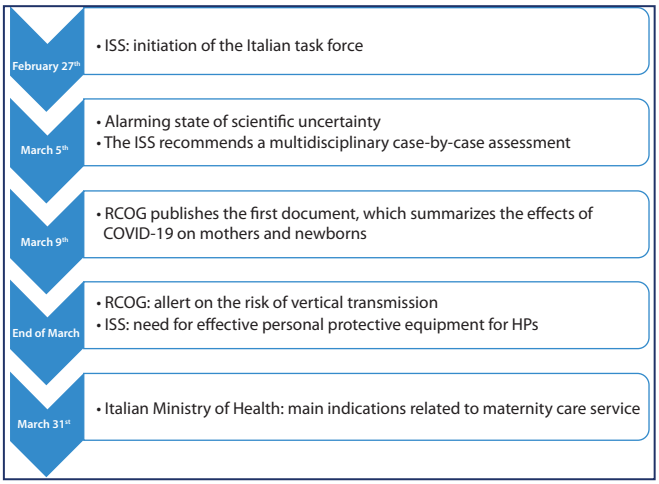

Figure 1. *Prelaminar scientific evidences and recommendations about COVID-19, pregnancy, and newborns in the first month of the outbreak.*

aimed to "response the World Health Organization (WHO) statements and international concerns regarding the coronavirus disease 2019 (COVID-19) outbreak" and focused on four main settings of pregnancy to manage pregnant women adequately: "1) ambulatory antenatal care in the outpatient clinics; 2) management in the setting of the obstetrical triage; 3) intrapartum management; and 4) postpartum management and neonatal care" and to offer "guidance on the medical treatment of pregnant women with COVID-19 infection".
A week later, a narrative revision (14) indicated strategies to reorganize obstetric units during the crisis based on available logistical resources to contrast the emergency and to summarize the most important evidences about the virus during pregnancy.
Additionally, anaesthesiologists offered relevant contributions:

- on April 14th, the Italian Society of Anaesthesia Analgesia Resuscitation and Intensive Care (SIAARTI) (15) proposed the second version of "Indications for the anesthesiological-resuscitation management of patients with suspected or confirmed SARS-CoV-2 (COVID-19) infection in the peripartum".
- On April 22nd, a team of Chinese anaesthesiologists (16) schematized procedures able to reduce the risk of contagion for HPs during caesarean section of infected women.

From March 25th to April 17th, two systematic reviews have been published: the first, (17) disclosed the association between COVID-19 and higher rates

(and pooled proportions) of preterm birth, pre-eclampsia, caesarean section, and perinatal death. The second (18) revealed a favourable outcome for infected women during pregnancy but recognized the relevant adverse risks and the unpredictable fetal consequences of long-standing infections occurring in early gestation. According to the authors, limitations of the reviews regarded the limited number of analysed studies; therefore, results should be interpreted with caution. On April 17th an US narrative review (19) proposed care practices for COVID-19 positive women in pregnancy affirming that "the implications for pregnancy remain largely unknown" and "because no treatment, no vaccine and no herd immunity exist, social distancing is the best mechanism available to protect patients and health care workers from infection". Nevertheless, in the same period different studies (20, 21) suggested no alarming maternal and perinatal outcomes for COVID-19 infected pregnant women.

Another serious global issue has been the availability and effective personal protective equipment in different obstetrical settings. The data of a retrospective multicentre study (22) conducted in the Lombardy region including 42 Italian women with confirmed diagnosis of SARS-CoV-2 infection prior to or within 36 hours after delivery, showed the occurrences COVID-19 symptoms only after delivery. For this reason, the authors stressed the necessity to strengthen safe procedures for HPs during labour assistance. They also affirmed that postpartum infection cannot be excluded, and vaginal delivery may be associated with a lower risk of intrapartum SARS-CoV-2 transmission to neonates. Kabesch *et al*. (23) shared on 20th of April the reorganization of the Regensburg University hospital birth clinic to contain HPs transmission of the virus. The measurements included massive testing of personnel, intensive active monitoring for symptomatic HPs and for their close contacts, increased hygiene measures, and adoption of facemasks and social distancing. Later, (May 1st) the American Journal of Obstetrics and Gynaecology (MFM) (24) published the guidelines edited by the Italian and American colleagues of specialists about clinical and logistics management of pregnant women during the prenatal, labour and delivery phases at the time of the pandemic.

Tips were also offered by:

- the Society of Infectious Disease in Obstetrics and Gynecology (ISIDOG) (25): "Recommendations Concerning COVID-19 and Pregnancy" (April 22nd). The clinical practice recommendations were focused on diagnosis, treatment and management of COVID-19 for pregnant women according to indications provided from the CDC, RCOG and ANZICS.
- The American Journal of Epidemiology: The "General Guidelines in the Management of an Obstetrical Patient on the Labor and Delivery Unit during the COVID-19 Pandemic" (26) (April 28th) offer potential indications for delivery with severe COVID-19 infection.

Following the numerous requests, on 24th April the Italian Scientific Societies (SIGO, AOGOI, AGUI, SIN, FNOPO) (27) presented the document titled "Pregnancy and childbirth in the COVID-19 period: practical advice" to encourage the correct management and needs of mothers, fathers, and children.

As claimed by Shalish *et al*, in a critical review published on May 2nd, the urgent need for guidelines and protocols on diagnosis, management and infection control strategies lead to a "tremendous confusion" due to the sudden development of the disease. For this reason, authors "comprehensively reviewed the current evidence regarding COVID-19 perinatal transmission, respiratory outcomes of neonates born to mothers with COVID-19 and infants with documented SARS-CoV-2 infection, and the evidence for using different respiratory support modalities and aerosol-generating procedures in this specific population".

Finally, on May 31st (28), the ISS published feasible clinical-care practice guidelines based on current available literature (**figure 2**). More than one month later (July 10th), the RCOG (29) proposed the last implemented version (2.4) of the "Guidance for maternal medicine services in the evolving coronavirus (COVID-19) pandemic", which contains pragmatic advices to clinicians on the management of common medical disorders in pregnancy in times of COVID-19.

Nevertheless, the negative impact on pregnancy and birth care is still considered a critical aspect as remarked by a petition (30) signed by 62 members of the European Parliament claiming the weakening of maternity services and requesting funding to avoid the closure of territorial prenatal and birth units. Moreover, the petitioners demanded adequate human, economical and instrumental resources to be employed in maternity services. Additionally, they suggested an identification of necessary protective equipment and policies to ensure the presence of a person to support the woman during labour and delivery, as recommended by the WHO.

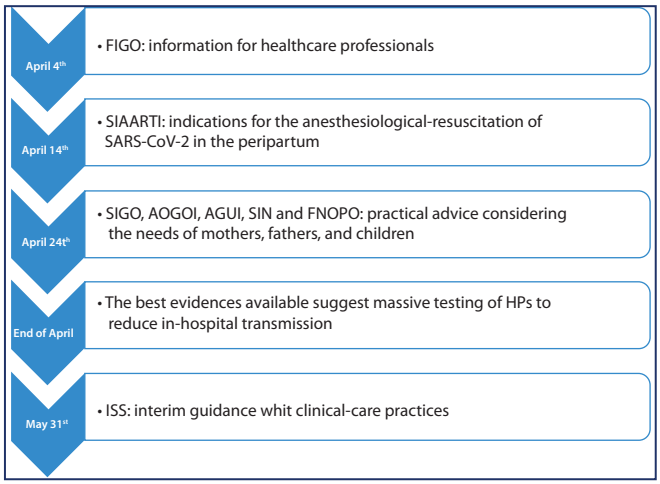

Figure 2. *Scientific evidences and recommendations about COVID-19, pregnancy, and newborns from April to May 2020.*

THE IMPACTS OF THE SARS-COV-2 PANDEMIC ON OTHER ESSENTIAL GYNAECOLOGICAL AND OBSTETRICAL CARE SERVICES

During the COVID-19 pandemic, resources from health system were redirected to respond to the emergency and several essential healthcare services became inaccessible. Particularly, the potential risk of in hospital transmission caused the rejection of COVID-free patients and their adherence to screening protocols and follow-up programs. Furthermore, many patients affected by tumours experienced treatment delays (*e.g.*, surgical, systemic and radiotherapeutic). This contributed to a series of medico-legal problems since the appropriate level of essential gynaecological and obstetrical services has been significantly compromised.

For instance, not all sexual and reproductive health care units, for the treatment of sexually transmitted infections (*e.g.* HIV), were available during the public health emergency. The comment document (31) realized by the Guttmacher Institute estimated that in low- and middle-income countries "10% of women who would normally have a safe abortion instead resorted to an unsafe method" in the event of "countrywide lockdowns forced clinics to close or if abortion was considered a nonessential service". Likewise, the interruptions in regular provision of essential services exposed women and girls, living in fragile socioeconomic context, to manifest medical complications related to gynaecological problems that outweigh the potential risks of in-hospital SARS-CoV-2 transmission (32, 33). In fact, the scientific community emphasized how the deferment dramatically affected vulnerable groups, such as women with oncological pathologies in need of oocyte collection or infertile patients with advanced maternal age and reduced oocyte reserve, underling the necessity to guarantee the access on this essential health services, often erroneously considered luxury (34).

Similarly, the SARS-CoV-2 pandemic constrained a drastically reorganization of cancer surgical recovery plans to reduce the risk of COVID-19 infection. The impact of this rescheduling of oncologic patient care is under investigation by a global expert response study (35) titled CovidSurger-Cancer Gynecological Oncology. Nevertheless, the outcomes related to cancer develop slowly, thus the negative impact of the delay of the medical support will be revealed in the next future.

Furthermore, during the first wave of the pandemic (from March 22nd to May 8th), many of the most important scientific societies of gynaecologic oncology published their recommendations about the diagnosis, treatment and follow-up of gynaecological tumours by providing advices to reorganize surgical services during a health-related crisis aimed to guarantee the high-quality care (36, 37).

Despite the efforts to redistribute facilities' resources, some procedures suffered of radical changes, especially in the hospitals with a poor level of readiness to implement strategic-logistical plans and difficulty to increase medical staff. This has practically materialized in decreased care quality. For instance, in healthcare facilities unable to guarantee access to radiologically guided tissue biopsies to the totality of the patients, physicians were encouraged to fully relay their diagnosis in advanced ovarian cancer to cytology before starting chemotherapy (38).

A global predictive model (39) estimated that during the COVID-19 peak, 12 weeks of disruption caused the cancelation of about 28,505 operations for gynaecological cancer surgery (12-week cancellation rate of 39.3%), 2,175,774 for gynaecological benign surgery (12-week cancellation rate of 81.6%), and 441,611 for obstetrics disorders (12-week cancellation rate of 25.4%). The magnitude of the problem has been recognized by a recent meta-analysis (40), which demonstrated the increased risk of death in some types of tumours caused by the delay in curative cancer treatment leading to unprecedented medical-legal issues.

However, in the context of the COVID-19 pandemic, we should not forget that before, during, or after the surgery the risk of contracting the infection

in hospital has worsened outcomes in COVID-patients compared with COVID-free patients (41, 42). Given this risk, the option to wait for the end of the pandemic or postpone surgery was shared between surgeons and patients, leading to delays related to the choice of correctly informed patients and not to inadequate or weakened health facility response. Furthermore, consequences of oncological treatment delay in the COVID era cannot be considered the same as consequences of a similar delay before the pandemic: the consequences of SARS-CoV-2 infection on patients' health are known.

In obstetrics, scientific evidence of the potential adverse effects of the virus on maternal and perinatal outcomes were published (43). One of the potential explanations of this evidence is the fear of pregnant women to continue their normal follow-up programmes in a period of social distancing imposed by governments.

Similar considerations cannot be neglected when establishing the real cause of unfavourable outcomes during the pandemic. Health responsibility should change its standards following the new scenario, profoundly different from the pre-COVID era, because the pandemic upset the previous balances and paradigms. For example, cancer survival curves, valid in 2019, may not be valid anymore, as some recent studies highlighted in other field (44).

Moreover, hospitals actively reorganize their pathways to ensure high quality and safety for every patient, COVID or not. This means, operatively, that necessarily two different pathways had been constructed for every type of patient: two different obstetrical pathways, one for pregnant women with COVID-19 and one pregnant women without infection; two different oncological pathways, one for oncological women with COVID-19 and one for oncological women without COVID-19 infection. One of the unavoidable consequences of this reorganization is the reduction of healthcare workers: the precedent staff was split and even if new healthcare workers were enrolled the number sometimes resulted inadequate, considering the need of strict segregation among the two equips and the need of ensuring physical distancing in the same spaces (45). Such regulation was mandatory to avoid nosocomial infection among healthcare workers and spread to COVID-free patients. Further, the need to sanitize equipment and environments poses a new limit, forcing the hospital-machinery to another slowdown.

A risk management policy that aims to reduce the burden of litigation due to this different patient management will be successful if able to condense today's difficulties into the medical record, which is the main evidence for the court. The exponential acquisition of constantly updated scientific evidence and adapting experiences are translated daily into organizational choices, sometimes leading to a compromise. The framework that justifies such choices cannot be forgotten. patients who undergo elective surgery should be told that, despite measures to limit the risk of infection, there remains a risk of contracting covid-19 in hospital, whether before, during, or after the operation. The surgeon should explain that, if the risk eventuates, the impact on the patient's health is currently unknown but could at worst lead to complications that require intensi patients who undergo elective surgery should be told that, despite measures to limit the risk of infection, there remains a risk of contracting covid-19 in hospital, whether before, during, or after the operation. The surgeon should explain that, if the risk eventuates, the impact on the patient's health is currently unknown but could at worst lead to complications that require intensi

Another challenge we must face is using our skills to improve our organizations in general, preparing for other challenges that lie ahead.

CRIMINAL ASPECTS

Although the criminal implications of the pandemic are not yet evident and predictable, the dramatic consequences of the outbreak accounting in Italy tens of thousands of victims and more than one million of infected could evolve in an enormous number of undesirable legal actions. Likely, HPs are exposed to be accused of "an event that, even if it happened against the intention, occurred due to negligence, imprudence, unskillfulness or failure to comply with laws, regulations, orders and disciplines" according to article No. 43 of the Italian Penal Code.

The present Italian judicial system for healthcare liability is ruled by article No. 590-*sexies* introduced by articles 6 of the law No. 24/2017, called "Gelli-Bianco", aimed to guarantee the constitutionally protected right to enjoy good health providing safe and high-standard treatments based on the key role of clinical practice guidelines (CPGs) (46). Briefly, law No. 24/2017 ensures healthcare safety by the adoption of effective prevention tools, facilities, structures, technologies and risk management. Moreover, article No. 590-*sexies* clearly states that the punishment is

not practicable as gross fault (*colpa grave*, defined as violation of the basic rules of diligence and a health care below the standards of accepted medical practice) when the medical action is performed according to evidence-based guidelines published by the Italian national health system or, in absence of them, best health care practices (47) adequately adopted on the specific clinical case. This article, inspired by a legislative model called "safe harbour", could have negative impact on the judgment of medical liability during the pandemic, because, especially at the beginning, the guidelines had a consolidated evidence-base that supported their adoption, or many items weren't evidence-based guidelines (48, 49).

At the origin, article No 590-*sexies* produced misunderstanding in proceedings (50, 51) and the United Divisions of the Italian Supreme Court promptly ruled the judgment No. 8770, known as "Sentenza Mariotti") (52) by establishing that: 1) the HPs are liable for slight fault in cases of negligence (*negligenza*), imprudence (*imprudenza*) and unskillfulness (*imperizia*); 2) medical workers could be punished for negligence and imprudence in every case of gross fault, but not if the unskillfulness is committed in compliance with the CPGs and best practices in a complex and extraordinary situation; 3) the specific risk and the special technical difficulties of the clinical case have to be considered in the circumstance of a gross fault for unskillfulness, consisting in a serious deficiency of care and/or a passive behaviour in absence of the essential precautions.

Concisely, the adequate management of a clinical case could be negatively affected by a series of unpredictable situations due to the variability of the clinical picture and the availability of the logistical resources drastically limiting the liability of HPs (49). This legal framework must be recognized in the national emergency caused by the SARS-CoV-2 pandemic.

It is worth emphasizing that Italy was the first Western country to be struck by the devastating new coronavirus. Italian HPs worked in absence of solid scientific evidences without validated, undisputable and convincing evidence-based CPGs because the pathological mechanisms triggered and, consequently, the safety of medications to contrast by the virus, were unknown. Medical workers firstly experienced off-label drug use and experimentation of innovative technical approaches. The COVID-19 outbreak rapidly overwhelmed the health system of the Northern Italy, such as in the Lombardy, one of Europe's wealthiest and most productive areas, causing a shortage of beds and medical supplies and forcing the doctors to make increasingly critical choices. The lack of instrumental and human resources (partially mitigated owing to the extraordinary contribution of young and inexpert HPs), and protective devices worsened the crisis and Italian first-line HPs worked in extreme circumstances.

Even HPs employed in medical divisions not directly involved in the fight against the new coronavirus found themselves in difficulty. For instance, gynaecologists and midwifes faced to treat pregnant women affected by SARS-CoV-2 without exhaustive knowledge about maternal and the fetal effects of the COVID-19 disease, consequence of the disease on the delivery and possibilities of a vertical/intrapartum/post-partum transmission and their relative outcomes on newborns.

It should be stressed that the choice of adequate medical care must be based on CPGs funded on the best evidences of the topic updated at the time of the medical intervention also according to judgment No. 8770 of the Italian United Divisions of the Supreme Court. This statement clarified two capital legal aspects: 1) continuing professional development is mandatory for HPs by acquiring the knowledge, good practice, skills and attitudes useful to offer the state-of-the art treatment; 2) *ex-ante facto* principle has to be the pillar for the robust judgment; thus the judge must evaluate the liability of HPs based on the CPGs and the best practices available at the time of the fact. To date, the situation is still in a very dynamic stage: physicians and scientists daily collect a huge amount of information updating incessantly the state-of-the art about the mechanism of action and transmission of the virus, the development of new techniques of detection and the implementation of novel effective therapeutic strategies. Thus, it has been rather impracticable for medical staff and health care facilities to take the pace day-to-day with the continually improvement of the knowledge about SARS-CoV-2.

Under the above-mentioned situation, it would be reasonable from an ethical and legal point to implement a "criminal shield" for HPs involved in future litigations about their liability in cases of adverse occurrences (5). Undoubtedly, the rapid outbreak of COVID-19 and the consequent overload of the weak Italian health system, coupled with the lack of undisputable CPGs and best practices for the management of the patients affected by a new virus, drastically limited the liability of the HPs calling more attention for a modification of the legal framework by Italian institutions.

CIVIL ASPECTS

Contractual liability

The impact of the SARS-CoV-2 pandemic on the civil litigation related to medical liability could be significate for healthcare institutions (53). According to The Italian Civil Code, article No. 1218 and article No. 1228, public and private healthcare facilities are responsible for negligence or misconduct of their staff (54).
Generally, Italian public and private hospitals are called to respond not only to medical malpractice, but also to personal damage due to organisational shortcomings. In fact, according to the law No. 24/2017, a patient must receive the best standard of care. At the same time, damages related to delays, long waiting list, other pathology, or transmission liability from the HPs to the patients, could involve healthcare facilities in further civil proceedings.
The magnitude of problem is visible considering the hypothesis of hospitals as the main vector of transmission in the first phase of the outbreak. Indictments could be related to negligence or omissions of the employees. Since the early stage of pandemic, healthcare facilities had to ensure: 1) intensive care units for COVID-19 patients consisting in confined areas to avoid in-hospital transmission; 2) well-organized triage to offer suspected COVID-19 patients a satisfactory treatment; 3) adequate provision of personal protective equipment for HPs; 4) information to HPs about risk of transmission; 5) reporting of n positive and suspected new cases to coordination centres. However, potentially infectious persons, pending the result of diagnostic tests, were hospitalized in the same area of confirmed COVID-19 patients favouring transmission. In case of a civil litigation against healthcare facilities about in-hospital transmission in a non-ideal environment, with HPs and the hospitals incapable to satisfy the precautionary measures, the cause and effect link and the fact that the patient would not have been infected in an ideal environment, has to be demonstrated (52).
Considering the high contagiousness of SARS-CoV-2 and the high probability of transmission, in the next future, a significant number of civil proceedings, pressuring the Italian healthcare system (whether private or public) and overloading the law courts, is expected. A possible solution, to mitigate the serious risk of dramatic effects on healthcare costs and workload of the legal system due to civil litigations about HPs liability, is to recognize an *a priori* indemnity for the consequences of COVID-19 directly payed by the state.
Furthermore, the COVID-19 emergency imposed to reschedule the hospitals' agenda and thousands of medical exams have been postponed or, in the worst case, cancelled. Obviously, this decreased the standard of care predictably and exacerbated the pathological conditions of the patients leading to permanent or temporal damages. Likely, it will generate in the next future a tremendous volume of claims that cannot be set in extrajudicial resolution providing a right compensation for injuries.

Extra-contractual liability

Up to now, the discussion was focused on the claims for hospital negligence, but according to article No. 2043 of the Italian Civil Code, the HPs themselves (doctors, midwifes, nurses, technicians, *etc.*) could be attacked. The ultimate result is a considerable enhancement of insurance costs. One must remember that HPs working in the emergency faced an unprecedented condition in absence of convincing and adoptable CPGs based on solid scientific evidence for the management of COVID-19 patients. This is a relevant element since law No. 24/2017 declares that HPs need to act with a scrupulous attention and adequate medical preparation (diligence) complying with CPGs released on the website of the Italian National Institute of Health after a systematic and scrupulous assessment of scientific evidences.
Since the beginning of the outbreak, the efforts of medical associations have been devoted to identify effective care. To date, there is no specific treatment for COVID-19 and care is based on a symptomatic approach, providing supportive therapies with modest results (*e.g.*, oxygen therapy and fluid management). Thus, the eligibility of a claim for active or omissive professional liability cannot exist as ruled by the abovementioned law No. 24/2017. Moreover, article No. 2236 of the Italian Civil Code establishes that "if the performance implies the solution of especially complex technical issues, the contractor is not liable for damages, except in case of willful misconduct or gross negligence".
From a practical point of view, this law supports HPs working in an emergency characterized by a special difficulty to manage patient conditions. Due to the solidity of the reasons that depict the pandemic as a problem of special complexity, plausibly article No. 2236 plays a key role in the ori-

entation of the courts representing a powerful tool for a rational and ethical reduction of the risk of professional liability for HPs (55).
In that regard, article No. 5, subsection 5, of law No. 24/2017, mandates that the quantitative evaluation of the damage, in case of a tort, must be established on the "special difficulty", the complexity of the solution to the problem, not solely from a medical point of view but also based on viability of logistical resources of health facilities.
Overall, the current legal framework limits the liability of HPs in the pandemic, except for wilful misconduct and gross fault (article No. 2236 Civil Code), but its updating is necessary to avoid heterogeneous and arbitrary interpretations. To avoid doubt, legislative action is expected to exclude the liability for medical staff working during the COVID-19 emergency.

CONCLUSIONS

The SARS-CoV-2 pandemic represents a situation of special difficulty for HPs, particularly in its early-stage (56, 57). The rapid transmission of the new coronavirus caused an overload of hospitals where medical staff has treated a relevant number of patients in total or partial absence of undisputable or adoptable CPGs or good health care practices.
The milestone of judgment No. 8770/2018 of United Divisions of the Italian Supreme Court imposed a change of jurisprudential parameters in a special situation adjusting a legal standard. Thus, the case-law is to reject criminal consequences for HPs by considering the unprecedented emergency scenario characterized by the lack of scientific and medical evidences making their performance extremely problematic.
In the above-mentioned exceptional circumstances, the opportunity of applying article No. 2236 of the Civil Code in the criminal process restricts the punishment to wilful misconduct or gross negligence. Moreover, an ethical assessment suggests a necessary revision of the criminal legal framework by legislator to introduce a "shield" for HPs worked in the first line against the SARS-CoV-2 virus.
In case of contractual liability, healthcare facilities are scarcely defensible because of their responsibility on the provision of protective personal devices (in order to reduce in-hospital infections) and logistical deficiencies leading to patient's injuries caused by the COVID-19 disease. Based on the well-established law No. 210/1992, *a priori* indemnity for the resultant law tort directly payed by the Italian State may mitigate the number of civil proceedings. Another main consequence of the overloaded healthcare system has been the delayed medical care of patients affected by different diseases. This compromised the standard of care resulting in permanent or temporal damages opening the door for future civil claims that cannot be simple resolved in extrajudicial settings.
In case of extra-contractual liability, punishment is excluded in a special situation like the SARS-CoV-2 pandemic, according to article No. 2236 of Civil Code and article No. 5, subsection 5, of law No. 24/2017.
Overall, the outbreak imposes urgent reorganization of the legal framework to homogenize the judgments in both civil and criminal processes by excluding the liability of HPs involved in an epochal emergency.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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Caesarean section: a case report of critical attempt to abdominal wall

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ABSTRACT

Introduction. Caesarean section (CS) represents the most widespread and performed procedure in obstetrics. It is an undisputed guaranty in life-threatening conditions, as a primary choice to decrease mortality and morbidity of women and new-borns.
Case report. We describe an exceptional case of a pregnant woman, previously undergone to several surgical procedures because of a unique and complex abdominal and genito-urinary congenital abnormality, with an extended prosthetic abdominoplasty. In 2018 the patient experienced a spontaneous pregnancy, whose course was strictly controlled in our center by a specialized multidisciplinary team. Pregnancy, despite multiple problems, hesitated in an only slightly preterm birth, since the CS was planned at 36 weeks of gestation, in order to avoid possible complications both for the mother and the baby over the last weeks. The CS was mandatory because of the complex congenital abnormality of the patient. It was performed by a lateral paramedian abdominal incision, in order to avoid any transection of the abdominal mesh. Both the post-operative period and the follow-up were uneventful. Newborn was in good condition and completely breastfeed.
Conclusions. The individualized technique used for CS demonstrate the importance to adapt the surgical technique even in a procedure whose approach is standardized all over the world.

SOMMARIO

Introduzione. Il taglio cesareo (CS) rappresenta la procedura più diffusa ed eseguita in ostetricia. È una garanzia indiscussa in condizioni di pericolo di vita, come scelta primaria per diminuire la mortalità e la morbilità delle donne e dei neonati.
Caso clinico. Descriviamo un caso eccezionale di donna incinta, precedentemente sottoposta a diversi interventi chirurgici a causa di un'anomalia congenita addominale e genito-urinaria unica e complessa, con addominoplastica protesica estesa. Nel 2018 la paziente ha avuto una gravidanza spontanea, il cui decorso è stato rigorosamente controllato nel nostro centro da un team multidisciplinare specializzato. La gravidanza, nonostante i molteplici problemi, ha esitato in un parto solo leggermente pretermine, poiché il CS è stato programmato a 36 settimane di gestazione, al fine di evitare possibili complicazioni sia per la madre sia per il bambino nelle ultime settimane. Il CS era obbligatorio a causa della complessa anomalia congenita della paziente. È stata eseguita con un'incisione addominale paramediana laterale, al fine di evitare qualsiasi recisione della maglia addominale. Sia il periodo post-operatorio sia il follow-up sono stati tranquilli. Il neonato era in buone condizioni e completamente allattato.
Conclusioni. La tecnica individualizzata utilizzata per CS dimostra l'importanza di adattare la tecnica chirurgica anche in una procedura il cui approccio è standardizzato in tutto il mondo.

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Key words
Caesarean section; paramedian incision; genito-urinary malformation; transitional care; multidisciplinary team.

INTRODUCTION

Caesarean section (CS) is one of the most commonly performed abdominal procedures in women. Although it was introduced in clinical practice as a life-saving procedure both for mothers and babies, nowadays there is an alarming globally increase in CS rates, related to the augmentation of unnecessary procedures (1).
CS is associated with short and long-term maternal morbidity, including consequences for future pregnancies, such as an increased risk of spontaneous preterm birth, uterine rupture, and abnormal placentation, that may result in maternal bleeding and hysterectomy (2). Moreover, newborn may suffer respiratory problems, with long-term increase in autoimmune and obesity related problems (3).
On the other hand, CS represents a fundamental option in selected cases, in order to reduce risk events for both the mother and the newborn (1).
Nowadays there is a widespread use of standardized CS surgical technique (4, 5). However, the obstetric surgical skills have to be adapted to challenging conditions. Therefore, the expertise in surgical options could act as a major ally when the optimal choice is necessarily directed towards alternative techniques. In our case, CS was mandatory in order to preserve the mother and the newborn wellbeing. It was carefully planned by a multidisciplinary team because of the complexity and unicity of the case and her hostile abdomen. Moreover, the site of the CS was judiciously chosen by the same team, thanks to the preoperative scans of the patient.

CASE REPORT

The patient was born with a gastroschisis and a complex malformation of the urogenital tract (double bladder, vaginal and urethral septation, unique vestibular ostium, pubic diastase, abdominal wall defect). At birth she underwent surgery to treat the gastroschisis. At the age of 7, a complex surgery was accomplished: bilateral posterior iliac osteotomy, vaginoplasty, unification of the two bladders, urethroplasty with anterior bladder flap according to Tanagho technique, clitoridoplasty, umbilicoplasty, and anterior wall plastic were realized. At the age of 9, a new procedure was performed because of a complete urinary incontinence: closure of bladder neck, end-to-side transurethral-ureterostomy of the right ureter on the left, pro-urethra appendix (orthotopic Mitrofanoff), distal vascular right ureteral stump (orthotopic), and positioning of abdominal monofilament polypropylene mesh (Marlex mesh; Bard Davol Inc, Warwick, RI) to strength the abdominal wall defect were realized.
However, urinary continence was achieved thanks to clean intermittent catheterization and oxybutynin per oral. Nevertheless, the patient suffered recurrent lower urinary tract infections (UTIs) and bladder stones. Over the following years, the patient underwent several lithotripsies through cystoscopy.
At 28 years of age, a surgical procedure was performed to treat bladder stones: through laparotomy and cystotomy, bladder stones were removed. However, in order to access the hostile abdomen, it was necessary to remove the mesh and several adhesions. A bowel resection was necessary due to a double intestinal perforation, and an end-to-end jejunal-ileal anastomosis was performed. On the left side, persistent complex adhesions between the sigmoid intestine and abdominal wall were left in place, in order to avoid further bowel resections. A new abdominal mesh was positioned (non-cross-linked porcine dermal scaffold, XenMatrix™; Bard Davol Inc, Warwick, RI). A 15 by 10 cm mesh was placed. It was located in the central and lower abdominal quadrants, in order to fill the gap given by the abdominal wall defect of the patient. Finally, an abdominoplasty under severe tension was accomplished. A vacuum-assisted closure (VAC) therapy was required during the healing of the wound.
At the age of 30 the patient had a spontaneous pregnancy and was referred to the high-risk pregnancy clinic of our Obstetrics and Gynaecology Department. Pregnancy was at high risk of UTIs (as the patient has suffered several UTIs), preterm birth, and intra-uterine growth restriction (IUGR), because of the complex malformation of the urogenital tract of the mother as well as the strong and copious endo-abdominal adhesions, the merely absence of the muscles of the abdominal wall, and the presence of an abdominal mesh. The pregnancy implanted normally in the right uterine wall, with a normal first trimester course. The Down screening at 12 weeks of gestation showed nuchal translucency NT 2.6 mm, with a risk of 1/4,662 and a cervical biometry at the lower limits (26 mm). During the second trimester, the abdominal ultrasound vision got obscured by the mesh. Therefore, the scan was carried out transvaginally and through a 3D reconstruction of the section planes. No major fetal abnormalities were identified; the

cervical length remained stable (cervical biometry 25mm at 20 weeks of gestation). Moreover, a normal placental implantation in the right-side wall, and a regular uterine dopplers were detected.
At 22 weeks of gestation, the patient suffered a febrile UTI with a suspected sepsis. She was then admitted as inpatient in order to receive an intravenous antibiotic treatment.
Throughout the third trimester, an apparent normality of fetal anatomy was confirmed by ultrasound (US)-scan, but IUGR was detected (abdominal circumference < 5th centile, with a stable uterine dopplers detected). Biometry and Doppler follow-up were therefore set.
The patient experienced a further hospitalization at 32 weeks of gestation, because of threatened preterm labor with increasing inflammation markers. A single tocolytic course and betamethasone were performed for lung maturation therapy with antibiotics.
Given the increasing IUGR, the need for an elective planned CS, and in order to avoid possible complications both for the mother and the baby over the last weeks, the timing of birth was set by a specialized multidisciplinary team (*i.e.*, Head of Obstetrics and Gynecology Unit, Head of Neonatal Intensive Care Unit, Head of Anesthesiology Unit, and Head of Pediatric Surgery Unit) after the completing of 36 weeks of gestation. The multidisciplinary team excluded a vaginal delivery because of the issues related to the complex urogenital malformations of the patient (*i.e.*, a very fragile and irregular urethra, with problematic urinary continence and a mandatory clean intermittent catheterization, the absence a real abdominal wall, and the presence of the abdominal mesh). Therefore, the team did prefer not to stress or further compromise the fragile urogenital and abdominal anatomy of the patient. The timing of birth set after the completing of 36 weeks of gestation was accurately set by the same team. For instance, Neonatologists required as much lung maturation as possible. On the other hand, the team carefully evaluated both the increasing IUGR and the possible risks either for the mother and the baby given by the increasing weight and height of the fetus during the last weeks of gestation.
The surgical approach was individualized on the basis of multiple goals: 1) to avoid abdominal access extension into the left abdomen, where extensive adhesions were located, 2) to avoid mesh transection, 3) to obtain a safe and rapid access to the pregnancy site, in order to prevent difficult fetal extraction. The best abdominal access appeared to be the right lateral paramedian access. The CS was performed under spinal anesthesia. Through accurate preoperative US-scan, the right lateral margin of the mesh and the presumed anterior edge of the placental implant were identified and marked on the operative field for surgical incision (**figure 1**). A CS with right paramedian access (**figure 2**) was performed. It took close to 20 minutes to achieve the uterus for the hysterotomy. A high uterine incision (**figure 3**) avoiding mesh transection and placental insertion (**figure 4**). A breech extraction of the cephalic fetus was easily performed (**figure 5**). Oxytocin with ergometrine maleate were used for the third stage, with normal blood loss. Uterus was sutured in two layers. The length of all surgical procedure was close to 120 minutes. The male newborn weighted 2,280 grams, Apgar score was 6 and 9 at the 1st and the 5th minute, amniotic fluid was meconium stained. The newborn needed to be aspirated and ventilated and he was hospitalized

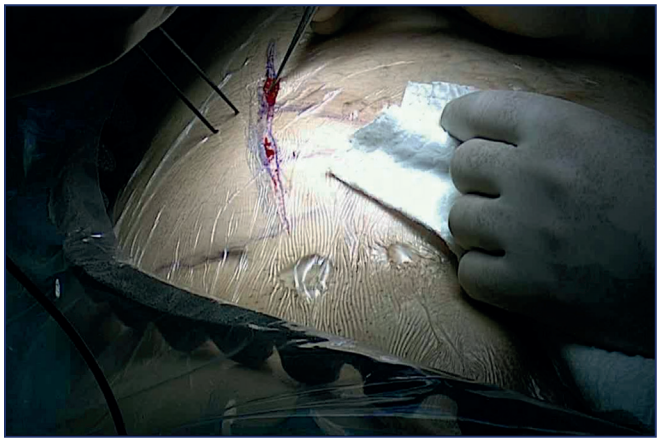

Figure 1. *Right lateral paramedian incision. Following a preoperative US-scan, this incision was established in order to avoid mesh transection.*

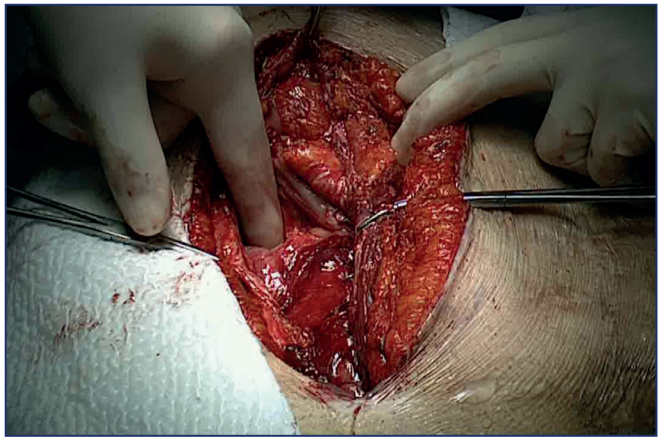

Figure 2. *Right lateral paramedian access to the uterus, with a longitudinal incision near the lateral border of the rectus sheath.*

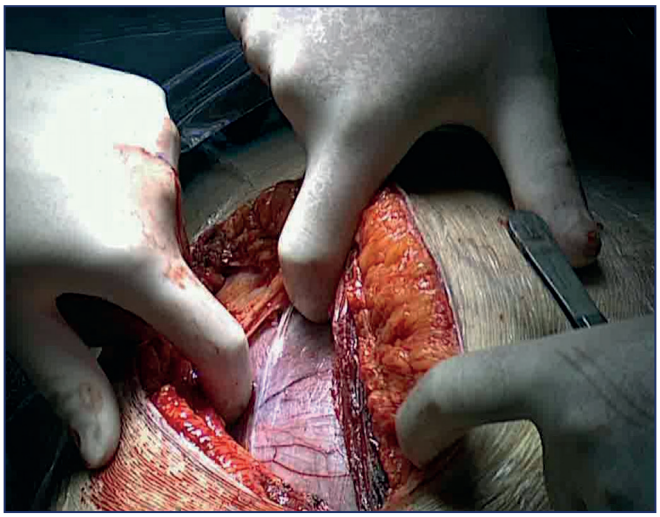

Figure 3. *Site of uterine incision. A high uterine incision was performed, as per preoperative US-scan.*

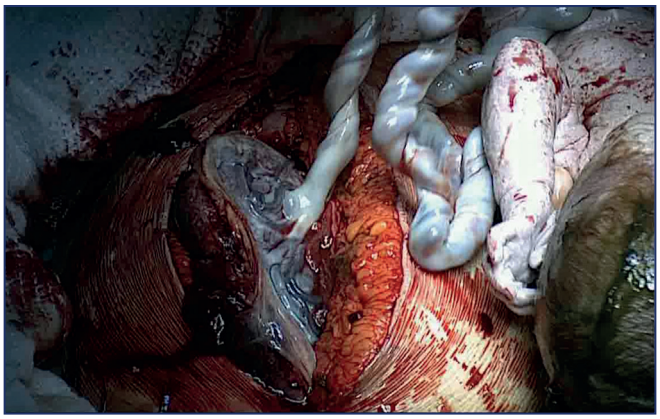

Figure 4. *Placental insertion. Thanks to the high uterine incision, placental insertion was avoided.*

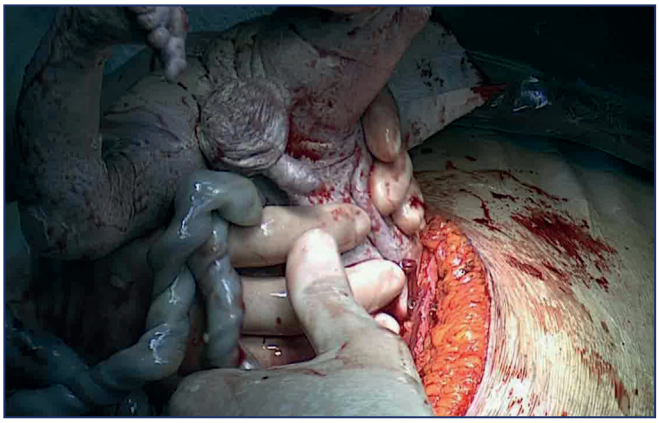

Figure 5. *Breech extraction of the cephalic fetus.*

for 3 days. Post-operative period was uneventful. Antibiotic and thromboembolic prophylaxis were performed for 7 days. Oxybutynin was stopped during breast-feeding. At 2-years follow-up, the patient and the child were in good health conditions.

DISCUSSION

During last decades, advances in reconstructive surgery have improved both the health and the quality of life in women with complex genito-urinary tract abnormalities, thus reaching reproductive age and considering pregnancy. However, literature suggests impaired fertility and higher risk with pregnancy (6). The case report we have presented was affected by multiple malformations, not classified as a discernable syndrome, but reported as a unique case. However, the patient underwent to similar techniques as for those affected by bladder exstrophy complex (7). Therefore, she developed the same complications during pregnancy, as reported in bladder and cloacal exstrophy patients (8).

Even though a CS could have represented a risk for a damage of the bladder and a vaginal delivery might be considered as optimal, there was a significant risk for maternal surgical injury and neonatal sequelae due to the hostile abdomen, with related risk of delayed access to the fetus in an emergent situation during the labor. Hence, vaginal delivery could have been considered when the pregnancy is uncomplicated and a senior obstetrician and urologist should be available for emergency delivery (9). Surgical complications, such as injuries to the urinary reservoir, transection of the ureter, fistula formation, and postpartum hemorrhages, should not be underestimate (10).

Abdominal entry by paramedian incision could be chosen rather than classical or transverse incisions; this technique has been proposed for women with impaired abdominal access (*e.g.*, women with previous correction for exstrophy-epispadias complex) or severely kyphotic parturient (whose supine cannot be achieved) (11). The favorable aspects of this technique are based on the relatively avascular linea alba. Two variants are known: the "medial" paramedian incision, in which the rectus sheath and rectus muscles are transected close to the linea alba, and the lateral paramedian technique, which consists of a longitudinal incision near the lateral border of the rectus sheath. In this approach, the rectus muscle is separated and then medially retracted. This lateral retraction prevents dissection of the deep epigastric vessels. Finally, the posterior rectus sheath (above the arcuate line) and the peritoneum are opened in the same plane as the anterior rectus sheath (**figure 2**) (12). This technique is more complex than the midline incision, resulting in an increased opening time and potential blood loss. The possibilities for extend-

ing the incision superiorly are limited by the costal margin (13). A vertical or classical uterine incision at the upper uterus was completed in order to avoid any injury to the lower urinary tract. In the present case, we do choose the lateral paramedian technique trying to prevent any mesh transection.
There are several reviews with regards to CS from the pre-operative to the post-operative care, in order to suggest the best standard (14, 15). Unfortunately, to the best of our knowledge, there are no reviews for standard care in those patients with hostile abdomen, except of small series reporting single center experience (8, 10, 11).
The lateral paramedian incision matches some of the advantages of the midline incision, such as wider exposure, richly vascularized wound bed, decreased risk of blood vessel dissection, and preservation of rectus muscle. Furthermore, the most remarkable characteristic of the paramedian incision is the significant reduction of incisional hernia incidence (approximately 0-1%). An explanation of this low rate is the so-called "shutter mechanism" the rectus muscle would provide in this approach: the muscle, located medially to the wound, would enable abdominal muscles contraction to bring the wound edges together (13).

CONCLUSIONS

CS represents a crucial point of care in women when complex comorbities would advise against a vaginal delivery. Expertise in surgical options could act as a major ally when the optimal choice is necessarily directed towards alternative techniques. Furthermore, a multidisciplinary involvement is essential to provide the best management for these complex cases. A lateral paramedian abdominal incision could represent an appropriate option of care in those patients where classical CS procedure would be contraindicated.

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ETHICS

A written informed consent from parents has been achieved.

CONTRIBUTORS

Each author gave a substantial contribution for the preparation of the manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.
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