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EDITORIAL

Infertility and ovarian failure in celiac disease

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Abstract

Unexplained infertility in females may be a devastating event for the reproductive-aged female. However, infertility may be due to ovarian failure associated with celiac disease, an immune-mediated disorder that may have few or no symptoms and can be successfully treated. In some prospective serologically-based studies, over 4% of infertile females may prove to have celiac disease. Serological screening for celiac disease is relatively inexpensive and involves testing for antibodies to tissue transglutaminase. If positive, a small intestinal biopsy should be done to confirm the diagnosis. The initial treatment for this disorder is a gluten-free diet. To date, a number of reports have indicated that this treatment for celiac disease may result in successful pregnancy, in spite of prolonged periods of infertility. Celiac disease, when untreated, may also lead to several adverse events following pregnancy including increased risk of recurrent abortions, low birthweight and impaired fetal growth. Recent molecular and pathological studies from different laboratories suggest that altered placental function may be due to binding to cells in the trophoblast by tissue transglutaminase antibodies impairing embryo implantation and leading to failure of early pregnancy or retarded intrauterine growth.

Key words: Celiac disease; Infertility; Ovarian failure; Autoimmune disease; Polyglandular syndrome

Core tip: Females with unexplained infertility should be screened for celiac disease. This involves use of a simple and inexpensive serological quantitative method for detection of tissue transglutaminase antibodies, a marker for celiac disease. If positive, biopsy evaluation should be done to determine if pathological features of untreated celiac disease are present in the small intestinal mucosa. A gluten-free diet may lead to effective management of celiac disease and may promote a favorable pregnancy outcome.

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INTRODUCTION

Infertility is a significant, even devastating clinical issue for some reproductive-aged women. Although infertility

may result from a number of factors, premature ovarian failure may be responsible. Ovarian failure normally occurs with the process of aging, premature failure of ovarian function may be defined as the failure of estrogen production by the human ovaries, usually before the age of 35 to 40 years. There may also be other significant long-term health consequences associated with premature ovarian failure including osteoporosis, heart disease, autoimmune disorders and increased risk of mortality[1]. There are many causes of premature ovarian failure in adults that need to be considered, including celiac disease.

FACTORS ASSOCIATED WITH FEMALE INFERTILITY

A number of different factors may lead to investigation of altered female fertility and, traditionally, these may be considered anatomically to include local factors in the cervix (*e.g.*, altered cervical mucus) and uterus (*e.g.*, congenital uterine abnormalities), fallopian tubes (*e.g.*, adhesions) as well as ovarian failure. Ovarian failure *per se* may have several causes, including polycystic ovary syndrome, hyperprolactinemia or hypothalamic amenorrhea, often associated with pituitary disorders, including tumors, or some medications. Ovulatory dysfunction may also occur in association with endocrine disorders, particularly those associated with an altered autoimmune process, including hypothyroidism or adrenal insufficiency (if together, Schmidt's syndrome, and when coupled with diabetes, Carpenter's syndrome). Either of these, but particularly autoimmune thyroiditis, may be frequently observed with celiac disease. Alternatively, an autoimmune polyglandular syndrome affecting several endocrine tissues can lead to ovarian failure. Polyendocrinopathy-candiasis-ectodermal dystrophy syndrome (APECED) is considered a rare autosomal recessive disease caused by mutations in the autoimmune regulator (*AIRE*) gene. Typical clinical findings include candidiasis, Addison's disease, hypoparathyroidism, diabetes, alopecia, vitiligo, ectodermal dystrophy, autoimmune thyroiditis, pernicious anemia, chronic active hepatitis, celiac disease and premature ovarian failure[2].

CELIAC DISEASE

Celiac disease is an immunologically-mediated gluten-sensitive small intestinal mucosal disorder primarily detected in genetically-susceptible persons[3,4]. Gluten peptides found in grains appear to be a triggering environmental factor following dietary exposure. Mechanisms involved in the pathogenesis of celiac disease are beyond the scope of this article, but have been recently detailed[4]. Chronic diarrhea, malabsorption of major and minor nutrients, and weight loss may occur. Development of serum antibodies to tissue transglutaminase also occurs in celiac disease and quantitation of these antibodies is a useful method for serological screening of populations or case finding in clinical practice. Endoscopic biopsies of the proximal small intestinal mucosa in untreated celiac disease show typical inflammatory changes along with moderate to severe alterations in mucosal architecture. Usually, these clinical, serological and pathological changes in untreated celiac disease respond to a gluten-free diet. Recognition of celiac disease is important because of the potential for later development of other co-morbidities, including osteoporosis and malignancy. Extra-intestinal or autoimmune processes may also occur, including dermatological changes, such as dermatitis herpetiformis, and endocrine disorders, including hypothyroidism associated with autoimmune thyroiditis. Often, these extra-intestinal features may be the presenting symptoms to an underlying small intestinal mucosal disorder that may be clinically silent.

CLINICALLY SILENT CELIAC DISEASE

Celiac disease has now become more often appreciated to have few or limited intestinal symptoms. Minimal diarrhea without weight loss may be evident. Often, females with celiac disease and reproductive disorders have no overt symptoms, or perhaps, only decreased energy with iron deficiency or, if more significant, iron deficiency-associated anemia[5]. As a result, impaired female fertility or changes that include delayed onset of menses, amenorrhea and early menopause may conceivably be the initial clinical presentation that eventually leads to recognition of underlying celiac disease. Owing to the increased ability to serologically screen for celiac disease, up to 1% to 2% of individuals in the general population in some countries have been detected with this disorder, especially in women of child-bearing age[4]. Screening biopsies may also be done and these studies have demonstrated that young adult females, in particular, are the most common adult age group first diagnosed with celiac disease[6]. Conceivably, premature ovarian failure leading to infertility could be reflect underlying immune-mediated mechanisms occurring in celiac disease, or alternatively, negative nutritional consequences of impaired absorption in celiac disease *per se*.

CELIAC DISEASE AND POLYCYSTIC OVARY SYNDROME

An important consideration in the broad differential diagnosis of premature ovarian failure is the polycystic ovary syndrome, initially described by Stein and Leventhal in 1935. Since then, this syndrome has been extensively investigated, and appears to be very heterogeneous with clinical features that include menstrual changes, chronic anovulation and excessive androgen levels, often with hirsutism. Anatomically, large cystic ovaries are often detected, but in up to

Table 1 Celiac screening studies in females with infertility

Number[1]	Country	Celiac disease[2]	Ref.
150 (98)	Finland	2.7% (4.1%)	[12]
99	Italy	3.03%	[14]
192	Israel	2.65%	[15]
47	Finland	2.1%	[16]
200[3]	Italy	2.5%	[18]
51	United States	5.9%	[20]
29	Brazil	10%	[21]

[1]Number with infertility (number in parentheses, unexplained infertility subgroup); [2]Celiac disease suspected with serological screening, usually with tissue transglutaminase or endomysial antibodies, and confirmation with small intestinal biopsy; [3]Infertility group for assisted reproduction technology.

a third of women with the same clinical features, the ovaries appear to be normal. Gonadotropin secretion appears to be abnormal with elevated levels of luteinizing hormone, or LH, combined with normal or low levels of follicle stimulating hormone, or FSH. In some, a mild elevation of serum testosterone is evident. The cause of the polycystic ovary syndrome still requires clarification. An earlier study suggested that the polycystic ovary syndrome may also occur in celiac disease[7]. Unfortunately, the data needed to fully examine this relationship (even with development of easy-to-perform serological screening measures) are currently not available. Some patients labeled with the polycystic ovary syndrome, especially with normal-appearing ovaries, could also conceivably have occult celiac disease, potentially amenable to a gluten-free diet.

ALTERED FEMALE FERTILITY IN CELIAC DISEASE

First cases noted a possible relationship between celiac disease and female infertility[8,9]. In one, subsequent treatment with a gluten-free diet resulted in pregnancy, confirming observations in 2 other reports[10,11]. Later, more extensive population-based studies were done to explore this potential relationship (Table 1). A detailed serological evaluation of 150 women with infertility due to all causes from Tampere, Finland demonstrated an apparent overall rate of celiac disease (*i.e.*, 2.7%)[12], (although subsequent serological screening studies from Finland in otherwise healthy subjects have revealed a relatively high rate of celiac disease in Finland compared to other countries[13]). For women with unexplained fertility, however, this study also reported rates of detection of celiac disease of over 4%[14]. Similar results were later reported in 99 couples from Northern Sardinia[15], estimated to be a rate of approximately 3%. Using more modern serological assays, a study from Israel employed assays for both tissue transglutaminase and endomysial antibodies in 192 Arab females with unexplained infertility. Among these, positive serological tests were noted in 2.65%[15]. In all, small intestinal biopsies were positive for changes of celiac disease, if serological studies were positive. Like most serological screening studies of populations, however, biopsies in serologically negative patients were not defined. Other studies have provided different results. Interestingly, a different center in Finland[16] could not confirm earlier study results from the same country[12]. A Czech investigation reported increased serum antibody positivity for celiac disease in women with infertility, but biopsies were not reported[17]. In a study from Italy that evaluated a group of infertile women specifically referred for assisted reproduction, a significant association with serology could not be defined[18] while a Swedish population-based cohort study of biopsy-defined celiac disease suggested that fertility was not decreased until the final 2 years preceding diagnosis[19]. Data from the United States demonstrated a prevalence of celiac disease in 5.9% of patients with unexplained infertility[20], while a Brazilian study evaluated 170 infertile women screened for tissue transglutaminase antibodies followed by small bowel biopsies in serologically-positive patients[21]. In this aforementioned Brazilian study, the prevalence of celiac disease was 10.3% in women with unexplained fertility. This contrasted with a prospective primary care study of over 2 million women from the United Kingdom where no overall impairment in fertility was recorded in celiac disease compared to non-celiac disease women[22]. However, in the same study[22], infertility rates were over 40% higher in celiac disease patients between ages 25 to 29 years compared to a similar age-matched population without celiac disease. Finally, a recent report[23] describing a meta-analysis in 105 relevant studies reported that "all-cause" infertility was 3.5 times higher in women with celiac disease compared to controls while "unexplained infertility" in women with celiac disease was 6 times higher than controls. Thus, recent population-based studies of women with unexplained infertility suggest a significant occurrence of occult celiac disease. Moreover, some of these celiac patients were reported to have a successful pregnancy following treatment with a gluten-free diet.

OTHER FERTILITY-RELATED CHANGES IN CELIAC DISEASE

Delayed onset of menses, amenorrhea, early menopause, repeated abortions and diminished pregnancy rates in celiac disease could indicate a possible impairment in fertility. In 74 patients from the United Kingdom[10], the reproductive period appeared to be more prolonged for celiacs on a gluten-free diet compared to celiacs not on a gluten-free diet. Otherwise, maternal health did not appear to be significantly altered. Nonetheless, a lower incidence of spontaneous abortions was noted in celiacs treated with a gluten-free diet. These findings were supported by a subsequent study from Italy[24]. A delay in the onset of menarche

(13.5 years *vs* 12.1 years) was also noted compared to age- and "sexual behavior-matched" controls. Amenorrhea and repeated abortions were more frequent in the celiac group, but menopause onset was not affected. A Polish study[25] suggested that the age of menarche in celiacs could be regulated by a gluten-free diet, while an Italian study[26] suggested that menarche was mainly impacted by the female's maternal history of menses onset. A United Kingdom study showed that celiacs may have limited fertility with a higher incidence of stillbirths and perinatal death[27]. However, the rate of miscarriage appeared to be improved after diagnosis of celiac disease and treatment with a gluten-free diet[27]. Finally, in a report from Brazil[28], gluten-free diet therapy appeared to be instrumental in promoting a positive nutritional state, relevant to reproductive health in patients with celiac disease.

Some earlier studies have evaluated pregnancy outcomes in celiac disease[29,30]. If untreated, celiac disease is associated with an increased risk of recurrent miscarriage and premature deliveries, along with reduced fetal growth and term birthweight[29]. In an Italian study[30] of 94 untreated and 31 treated celiac patients, relative risks of either abortion or delivery of a low birthweight infant were increased while breast feeding duration was reduced. The investigators noted improvement with a gluten-free diet. Similar observations have been recorded by others[31]. Lower birthweight and retarded intrauterine growth have been recorded from European centers[32-35]. For example, in a further Italian study[33], celiac disease was more common than most diseases normally screened for during pregnancy in their health care facility. In another European evaluation[34], undiagnosed maternal celiac disease appeared to be a far greater risk factor than diagnosed celiac disease. However, a later report was not able to confirm an unfavorable pregnancy result[36]. Later studies also suggested that celiac disease in the father was not a risk factor for an adverse outcome of the pregnancy[37,38].

PLACENTAL STUDIES

The precise mechanism for adverse pregnancy outcomes are not clear but have been evaluated to a limited extent. Placentas of some mothers affected with celiac disease, in particular, may be abnormal. Using immunohistochemical methods and *in situ* hybridization, tissue transglutaminase expression and apoptosis were increased in trophoblast cells[39]. This suggested a possible injury mechanism in both fetal and placental portions of the placenta[39]. Maternal celiac disease autoantibodies may bind directly to the syncytiotrophoblast and inhibit placental tissue transglutaminase activity leading to an impaired placenta[40]. Other studies in female celiacs have raised the issue of early pregnancy loss due to altered coagulation affecting placental or fetal microvascular function[41]. Finally, binding of antibodies to tissue transglutaminase by the trophoblast might represent a crucial mechanism causing impaired embryo implantation and pregnancy outcome in pregnant celiacs[42]. Additional studies are needed to explore these intriguing early findings in celiac disease.

CONCLUSION

A preponderance of evidence from multiple studies indicates that infertility or ovarian failure in females may be increased in immune-mediated disorders, including celiac disease. In celiac disease, an immune-mediated small intestinal mucosal disorder is triggered by gluten-containing peptides found in food grains. The primary treatment of celiac disease involves administration of a strict gluten-free diet. In females with untreated celiac disease and infertility, successful pregnancy may occur solely after treatment of the celiac disease with a gluten-free diet. Widespread use of serological screening in different populations suggests that celiac disease may occur in 4% to 10% of females with unexplained infertility. Serological screening using tissue transglutaminase antibodies represents a very inexpensive method for evaluation, and if positive, should be followed by small intestinal biopsies to define the presence of celiac disease and lead to treatment.

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Preeclampsia - What is to blame? The placenta, maternal cardiovascular system or both?

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Abstract

Preeclampsia (PE) is a pregnancy-specific syndrome, complicating 2%-8% of pregnancies. PE is a major cause of maternal mortality throughout the world with 60000 maternal deaths attributed to hypertensive disorders of pregnancy. PE also results in fetal morbidity due to prematurity and fetal growth restriction. The precise aetiology of PE remains an enigma with multiple theories including a combination of environmental, immunological and genetic factors. The conventional and leading hypotheses for the initial insult in PE is inadequate trophoblast invasion which is thought to result in incomplete remodelling of uterine spiral arteries leading to placental ischaemia, hypoxia and thus oxidative stress. The significant heterogeneity observed in pre-eclampsia cannot be solely explained by the placental model alone. Herein we critically evaluate the clinical (risk factors, placental blood flow and biomarkers) and pathological (genetic, molecular, histological) correlates for PE. Furthermore, we discuss the role played by the (dysfunctional) maternal cardiovascular system in the aetiology of PE. We review the evidence that demonstrates a role for both the placenta and the cardiovascular system in early- and late-onset PE and highlight some of the key differences between these two distinct disease entities.

Key words: Preeclampsia; Placenta; Maternal cardiac function; Cardiovascular; Aetiology

Core tip: The conventional paradigm is that preeclampsia (PE) is solely due to placental dysfunction. However not all cases of placental dysfunction result in the syndrome of PE. Equally, placental dysfunction - as evidenced by impaired uterine artery Doppler indices, low birth weight and abnormal histology - is not always present in PE. Therefore, the heterogeneity observed in PE cannot be solely explained by placental dysfunction. There is now strong evidence supporting the role of the maternal cardiovascular system in PE. In this mini-review, we evaluate the evidence supporting a dual

aetiology of PE, involving both the placenta and the maternal cardiovascular system.

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Preeclampsia (PE) is a pregnancy-specific syndrome, complicating 2%-8% of pregnancies[1]. PE is a major cause of maternal mortality throughout the world. Over half a million women die during or after childbirth every year, and it is estimated that 60000 of these maternal deaths worldwide are attributable to hypertensive disorders of pregnancy[1,2]. In the United Kingdom, PE/eclampsia was the fourth leading cause of direct maternal deaths between 2009-2011[3]. In the triennium 2009-2011, 10 deaths in the United Kingdom were attributable to PE, giving a mortality rate of 0.42 per 100000[3], considerably lower than the mortality figures worldwide. The management of hypertension in conjunction with the use of national and local guidelines are credited with the reduction in morbidity and mortality observed in the developed world.

PE can also result in morbidity to the fetus, including fetal growth restriction, placental abruption and stillbirth. In addition, there is a considerable financial burden as a result of increased antenatal surveillance, investigations and hospital admissions along with an increased risk of operative intervention and peripartum monitoring. Following delivery, prematurity and the associated care costs, both in the short and long term, further contribute to the financial burdens associated with PE. At present, the only recognised cure for PE is delivery of the placenta. As a result, PE is the leading cause for iatrogenic preterm birth[4], which is associated with greater neonatal morbidity than delivery at term.

The importance and magnitude of PE on maternal health and neonatal morbidity is well accepted. Despite this being such an important disease, our knowledge of its aetiology is still incomplete. Forming a better understanding will aid us in making an accurate diagnosis, perform better screening and improve our ability to triage disease severity, offer targeted preventative and therapeutic measures and formulate appropriate short- and long-term postpartum management plans.

PE is a heterogenous disease exhibiting a diverse range of both fetal and maternal disease presentations. At present, we aim to identify and diagnose PE based on organ dysfunction both in the mother and in the fetus. The timing of onset of PE is as diverse as its organ involvement and can occur from the late second trimester, through to term and can even present in the postnatal period.

Although often classified as mild, moderate and severe, a newer and perhaps more relevant method of clinical classification would be based upon timing of onset. Early onset PE is thus defined as PE occurring prior to and requiring delivery before 34 wk gestation and late onset PE developing and requiring delivery after this gestational age. There are distinct differences between these two types of PE, in terms of their pathophysiological basis, effects on mother and fetus as well as their long term implications. Late PE accounts for approximately 75% of cases, whilst the remaining 25% are early onset PE[5,6].

Ten percent to fifteen percent of pregnant women will develop some form of hypertensive disease that requires further assessment and follow up. Currently, United Kingdom guidelines on screening for identification of those at high risk of developing PE is performed in the first trimester and is based on maternal demographics and obstetric/medical risk factors[7]. The NICE model recommends commencing preventative measures (*e.g.*, low dose aspirin) and formulation of an appropriate antenatal management plan in high risk women. However the NICE model categorises more than 60% of women as high risk, but predicts less than 30% of those destined to develop PE[8].

PATHOPHYSIOLOGY - THE ROLE OF THE PLACENTA

The precise pathophysiological basis of PE remains an enigma with multiple, plausible aetiological theories involving a combination of environmental, immunological and genetic factors.

The conventional, leading hypotheses, which has stood the test of time, for the initial pathophysiological insult in PE is inadequate trophoblast invasion and thus incomplete remodelling of uterine spiral arteries This leads to placental ischaemia, hypoxia and thus oxidative stress[9,10]. The placental hypoxaemia sets off a biochemical cascade of angiogenic/antiangionic factors which leads to subsequent endothelial cell dysfunction with its observed maternal signs and symptoms of PE. Whilst placental hypoxaemia and the subsequent oxidative stress is a significant contributing factor, we acknowledge that a definitive link between PE and placental hypoxia is not confirmed and further research is required.

However not every pregnancy characterised by abnormal placentation results in PE - fetal growth restriction is one such example in where there is suboptimal placentation with detrimental fetal effects but minimal maternal effects. Others will go on to develop the syndrome of PE which is characterised by hypertension and proteinuria and multiple organ involvement.

One possible reason for the development of PE is that there is a concurrent maladaption of the maternal cardiovascular system along with abnormal placentation, and this predisposes an individual to developing PE. This would also explain why not all women with poor placentation with its sequalae (such as fetal growth restriction) develop PE; their cardiovascular system adapts appropriately to the ongoing pregnancy and associated haemodynamic changes.

IS PE SOLELY DUE TO PLACENTAL DYSFUNCTION?

The development of PE does not necessarily require a uterus or indeed a fetus, as the condition has been reported in abdominal[11] and molar pregnancies[12] respectively. However, a placenta is essential for the disease to occur, and is therefore central in the pathogenesis. Furthermore, it is well documented that the cure for PE is delivery of the placenta, further supporting the crucial role played by the placenta in the development of PE.

The paradigm is that the "placenta causes PE". Whilst we firmly believe that the placenta is a pre-requisite and therefore crucial to the development of PE, herein we critically review the evidence that the placenta is indeed the only organ of causality in PE.

CLINICAL EVIDENCE FOR THE AETIOLOGY OF PE

The majority of the evidence for the placental origin hypothesis of PE is based on the clinical features of the disorder. Broadly speaking, they constitute clinical risk factors, placental blood flow evaluation, fetal growth restriction and placental biomarkers.

Risk factors for PE

Both PE and fetal growth restriction (FGR) are thought to share clinical risk factors such as increased maternal age, ethnic origin, increased body mass index (BMI), diabetes and other co-morbidities. These associations are conventionally advocated by national guidelines[7,13] as a method to screen routine populations for their risk of developing PE. However, analysis of data from 40000 pregnancies collected for the World Health Organization Antenatal care trial[14] showed that surprisingly, PE and fetal growth restriction had different risk factor profiles. Mothers with PE compared with those with fetal growth restriction were more likely to have a history of diabetes, renal or cardiac disease, chronic hypertension, previous PE, increased BMI and extremes of maternal age. Conversely, fetal growth restriction was associated with higher risk of low birth weight in previous pregnancies, but not with previous PE. The same analysis demonstrated that PE and gestational hypertension shared many risk factors - conventionally associated with cardiovascular disease in the non-pregnant population. These data infer that the aetiology of PE is not necessarily as a result of placental dysfunction.

Placental blood flow

The pathological hallmark of placental insufficiency is incomplete spiral artery remodelling, and this is seen in both fetal growth restriction as well as in some, but not all cases of PE. Uterine artery Doppler waveform studies have been shown to aid in the prediction of pregnancies that will be complicated by fetal growth restriction or PE[8,15-17]. Increased uterine artery resistance indices are related to incomplete trophoblast invasion of maternal spiral arteries, which results in a high-resistance placental circulation and therefore an underperfused fetoplacental unit. This placental hypoperfusion is a feature seen in both PE and fetal growth restriction.

The association with increased uterine artery Doppler resistance indices and a higher propensity to develop PE and FGR is widely reported and used in current clinical practice in the management of high risk pregnancies. Velauthar *et al*[17] carried out a meta-analysis of over 55000 patients looking at the predictive value of performing uterine artery Doppler measurements in the first trimester. The authors reported a sensitivity and specificity of 47.8% and 92.1% respectively for the detection of early onset PE. The sensitivities and specificities for detection of late onset PE were 21.5% and 90.3%; for PE at any gestation these were 26.4% and 93.4%. Although first trimester uterine artery Doppler screening is a highly specific means of screening for early PE, it is less so for late PE.

Verlohren *et al*[18] have corroborated this finding in their published data of second trimester uterine artery Doppler screening. The investigators looked at outcomes in over 27000 cases that had uterine artery measurements in the second trimester. They reported that the prevalence of resistance indices above the 90th centile were present in 63.6% of early PE, 15.5% of late PE and 8.8% in the control group. This significant finding shows that increased uterine artery Doppler indices, are a poor predictor of late onset PE.

It is well recognised that increased uterine artery dopplers are an index for inadequate placentation, and thereby a surrogate marker for increased risk of PE and FGR. Data from these large-scale, high quality studies indicate that inadequate placentation is a key feature of early onset PE and FGR. The reduced prevalence of increased uterine artery indices observed in late-onset PE lends further support to the argument that the heterogeneity observed in PE, is due to early onset PE being related to a dysfunctional placenta, whilst late onset PE may not be associated with placental insufficiency.

Fetal growth

The fetal effects of a dysfunctional placenta include fetal growth restriction, and this is seen in many cases of early-onset PE, but less so in cases of late PE. The majority of neonates in late PE are of normal size[18]. This observation is consistent with the notion that whilst early PE due to placental insufficiency is also closely correlated to FGR, there is an additional aetiological factor to placental insufficiency in late onset PE.

Verlohren *et al*[18] reported on the distribution of small-for-gestational-age (SGA) neonates in their large cohort. They found the incidence of SGA to be 66.2% in the early PE group, 16.7% in the late PE group and 10.8% in the control group.

The distribution of large-for-gestational age neonates did not observe a similar pattern; the prevalence being greatest in the late PE group as compared to all other groups. Interestingly, both SGA and large-for-gestational-age (LGA) births were more prevalent in the late PE group, demonstrating a bimodal skewed birth weight distribution in late PE. Whilst the association between PE and LGA babies has been observed previously, the bimodal distribution is certainly a novel finding.

The increased incidence of SGA births in early-onset PE, associated with increased uterine artery impedances, is an expected finding given the underlying placental insufficiency, unlike the reported increased prevalence of both SGA and LGA in late-onset PE. The authors postulate that this finding implies that whilst the SGA form of late-onset PE is due to placental insufficiency, the LGA form (with its associated normal uterine artery impedance measurements) is secondary to the inability of the maternal heart to meet the demands of the (oversized) placenta. The subsequent placental hypoperfusion as a result of the inadequate pump action, leads to placental hypoxia and the biochemical cascade that leads to endothelial cell dysfunction observed in PE.

Placental biomarkers

The oxidative stress in the placenta leads to an imbalance in angiogenic and antiangiogenic factors. An imbalance in these factors can adversely affect vascular homeostasis, and therefore contribute to the array of symptoms displayed in PE. The angiogenic factors that are believed to play a role include vascular endothelial growth factor (VEGF) and placental growth factor (PlGF)[10,19,20], the latter being used as a potential diagnostic biomarker for PE[19,20]. PlGF is produced by trophoblasts and interacts with cell surface receptors such as Flt-1. The soluble form of this, soluble fms-like tyrosine kinase-1 (sFlt-1) is an antiangiogenic factor which has also emerged as a key factor and potential biomarker in PE. sFlt-1 has been shown to block PlGF and therefore play a causative role in the development of PE[10,21]. Maynard *et al*[10] demonstrated a rise in arterial pressures and proteinuria with exogenous administration of sFlt-1. Furthermore, uteroplacental ischaemia has been shown to increase sFlt-1 levels in experimental models, as well as a decrease in VEGF/PlGF[22,23].

Another biological factor that has been studied and may have a role in PE is Heme Oxygenase-1 (HO-1). HO-1 and its metabolites carbon monoxide (CO) and bilirubin exert protective effects against oxidative stimuli, and *in vitro* studies have reported that HO-1 downregulates sFlt-1[24]. HO-1 has been shown to inhibit sFlt-1 release[24,25] and it is possible that a loss of HO-1 plays a part in the pathophysiology of PE. There are no published studies looking at the differences in the expression of HO-1 in early-onset *vs* late-onset PE.

Poon *et al*[26] conducted a first trimester screening study using uterine artery Doppler indices, serum pregnancy associated plasma protein A (PAPP-A) and placental growth factor measurements in a large cohort of over 57000 cases. They found that in the pre-term SGA group, serum PAPP-A and PlGF were reduced, indicating an underlying pathology of impaired placentation. The authors reported that for a false-positive rate of 5%, the detection of early-onset PE (< 34 wk gestation) using PlGF was 59.3%, however in all cases of PE below 42 wk gestation, the detection rate was significantly lower at 29.1% - this figure included all of the early-onset along with late-onset cases of PE. The evidence suggests that placental biomarkers have a good performance for screening for early-onset PE, but a poor performance when screening for late-onset PE.

The biomarkers discussed above have been used in clinical trials as a potential screening tool for the detection of PE. Elevated sFlt-1/PlGF ratios have been observed in early-onset PE[19,20]. Chappell *et al*[19] published data from a multicentre trial using a PlGF assay (Triage, Alere, California, United States). PlGF concentrations below the 5th centile had a sensitivity of 96% and a negative predictive value of 98% for PE requiring delivery within the next fourteen days, in gestational ages below 35 wk. Beyond 35 wk, the test was not as good at excluding PE. This further strengthens the argument for a role of placental insufficiency in early cases of PE, with a weaker association between PlGF and late-onset PE.

PATHOLOGICAL EVIDENCE FOR THE AETIOLOGY OF PE

Many scientific evaluations of the pathology associated with PE have been used to justify the placental origins hypothesis of PE. These may be genetic, molecular or histological in nature.

Genetic

There is an unquestionable link between the development of PE and subsequent cardiovascular disease. Despite the incomplete understanding of the aetiology

of PE, familial clustering has been observed[27,28] and reported in PE and therefore supports a genetic link. Many susceptibility genes for PE have been reported in the literature - the function of the majority of these loci remain unknown. The very few PE genetic loci with known associations have previously been implicated in adult cardiovascular disease. Despite further work in this field being required, it does seem entirely plausible that due to the similarities between PE and cardiovascular disease with clinical risk factors and postpartum cardiovascular legacy, there is indeed a shared genetic link between PE and cardiovascular disease.

Molecular

Recently published data on the molecular biology, also provide insightful evidence. Yung *et al*[29] investigated whether there was molecular evidence of a difference in placental stress response between cases of early and late PE. Investigating multiple stress-signalling pathways, the investigators demonstrated that activation of stress-signalling pathways was negatively correlated with gestational age, with a clear inflection for placentae beyond 34 wk gestation. Activation of these pathways was significantly higher in early PE than in late PE or controls. The authors reported no difference between placentas in late PE and normotensive controls. It appears that this group of investigators have provided the first molecular evidence of placental stress response in early PE with a lack of such a response in cases of late PE. This supports a placental cause for early PE, but further suggests that placental dysfunction may not necessarily be implicated in late onset PE.

Histological

Histopathological studies of placentae have also reported a difference between early and late PE[30-32]. Whilst in the majority of cases of PE the placentae are histologically normal, it is also found that histopathological lesions are mainly seen in preterm PE[22]. Pathak *et al*[33] carried out a blinded analysis and reported that placental hypoperfusion in the form of massive perivillous fibrin deposition was much more frequent in those with PET (OR 20.2) and SGA (8.9). However we acknowledge that this was in a small sample of pathological cases. Ogge *et al*[32] demonstrated the prevalence of placental hypoperfusion lesions were greatest in cases of early-onset PE (58%), as compared to 33% in late onset PE and 16% in term controls.

An interesting, recent study investigated placental perfusion using magnetic resonance imaging in early and late PE[34]. The study reports that women with early PE did indeed show smaller placental perfusion fractions when compared to controls. Interestingly, women with late onset PE had a larger placental perfusion fraction when compared to matched controls for gestational age. The increased placental perfusion fraction seen in late PE further supports the argument that late-onset PE is associated with larger placentae and babies, and therefore is unlikely to be due to placental insufficiency.

PATHOPHYSIOLOGY - THE ROLE OF THE CARDIOVASCULAR SYSTEM

The maternal cardiovascular system undergoes a series of changes in pregnancy, all of which have a role in ensuring adequate uterine perfusion, oxygen delivery and provision of nutrients to meet the demands of the growing fetus. These adaptations enable the pregnancy and the growth of the fetus to continue unimpaired. They include a rise in cardiac output, heart rate and plasma volume and a concomitant fall in total vascular resistance (TVR).

Despite a significant volume of literature regarding placental dysfunction in PE, data regarding the cardiac changes associated with PE are more scant, and also more controversial. The conventional belief was that early-onset PE is associated with reduced cardiac output and increased total vascular resistance, with maternal cardiac function succumbing early in the disease process. With regard to late-onset PE, the original data implied that this was a condition of raised cardiac output and reduced total vascular resistance, however this model has not been reported consistently[35,36]. The discrepancies reported in the cardiovascular profiles in women who present with PE can be attributed to certain confounding factors - antihypertensive medication, co-morbidities, different gestational ages and stages of labour.

Another significant reason for the discrepancies reported in the literature with regards to haemodynamics in PE is the use of cardiac output instead of the corrected index for body surface area, cardiac index. Cardiac index (CI) represents the cardiac output per square meter of body surface area. We believe that using this corrected index is superior to using cardiac output, which does not factor in height or weight of the subject. As human beings come in different shapes and sizes, with varied metabolic demands, we feel that comparison of cardiac output without correcting for body surface area is both inadequate and inaccurate.

Prior to the onset of symptoms of early-onset PE, there is a shift towards a reduced cardiac index in conjunction with a raised total vascular resistance, increased mean arterial pressure, contracted intravascular volume and reduced venous reserve capacity[37-42]. These findings suggest that the high resistance/low volume haemodynamic profile observed in women destined to develop early-onset PE is observed in the latent phase of the disease at mid-gestation[39,40].

In a study by Melchiorre *et al*[39] women who subsequently developed late-onset PE presented with raised total vascular resistance, but importantly, there was no difference in cardiac index between late-onset

PE and control groups. Whilst these findings have not been reported consistently in the literature, it is worth noting that in studies that did not corroborate the findings observed by Melchiorre *et al*[41], corrected cardiac indices were not employed.

In preeclamptics medicated with anti-hypertensives, the CI normalises and is comparable to CI in normotensive controls. The observed reduction in arterial compliance, has also been shown to be corrected following antihypertensive treatment in patients with PE[42].

Although some of the initial work into cardiac changes in PE showed conflicting results, more recent work has demonstrated a more consistent collection of findings. This is in part due to improved and newer techniques such as colour tissue Doppler and 2-dimensional speckle tracking derived strain and strain rate imaging, which are able to detect early and subtle changes in myocardial function in an objective manner, when used in conjunction with validated diagnostic algorithms.

Prior to the onset of clinical symptoms in women who develop early onset PE, there is an abnormal remodelling of the left ventricle which consists of concentric remodelling and hypertrophy[39,41]. Moreover in this group of women, there is evidence of mild diastolic dysfunction as well as impaired myocardial relaxation[39,41]. This impaired diastolic function is thought to be associated with the increased cardiac afterload (increased TVR) and abnormal left ventricular remodelling[39,41]. The abnormal pattern of remodelling observed in PE is similar to that observed in non-pregnant individuals with essential hypertension and is consistent with an impairment that is afterload-induced[39,41,43].

Studies assessing early myocardial changes, have reported that mild-moderate isolated left ventricular diastolic dysfunction is seen in approximately half of women with early onset PE[39,41,43] with one in five women having biventricular systolic dysfunction with associated left ventricular hypertrophy[41,43]. Melchiorre *et al*[39,41] have also demonstrated impairment in myocardial contractility in early onset PE using colour tissue Doppler and 2-D speckle tracking. Left atrial remodelling in PE has also been reported. These findings suggest that the pregnant heart in PE is working at its maximum potential capacity, and any additional stress could result in a deterioration of cardiovascular function.

Other authors have suggested raised intra-abdominal pressure (IAP) as a major aetiological factor to the development of PE[44,45]. They hypothesize that women with raised IAP will have compromised venous return to the heart, which will result in decreased blood flow in the vascular beds of the placenta, uterus, kidneys and liver. The result of this impaired blood flow and venous congestion would include placental ischaemia, oedema of the lower extremities, glomerulopathy associated with hypertension and proteinuria and hepatic dysfunction[44,46]. This hypothesis is in concordance with the cardiovascular origin of PE proposed in this review. The myocardial dysfunction of PE noted in many studies will also compound the compromised venous return to the heart. These two theories are therefore not mutually exclusive.

WHAT IS THE CARDIAC LEGACY OF HAVING PE?

Although we have classically believed that treatment of PE is by delivery of the placenta in order to avoid impending maternal harm, the subtle, subclinical cardiac changes associated with a diagnosis of PE are not cured by birth of the infant or delivery of the placenta. Melchiorre *et al*[47] showed that at 1 year postpartum 56% of women with early onset PE had asymptomatic left ventricular dysfunction compared to 14% of women with late onset PE and 8% of healthy controls. The same authors also reported a 40% incidence of essential hypertension at 2 years post-delivery.

Indeed a diagnosis of PE is considered a risk factor for long term cardiovascular disease[48,49]. Following a diagnosis of PE, in particular early onset PE, it therefore seems prudent that these patients are adequately risk assessed in the years following pregnancy, in order to address and mitigate future cardiovascular risks.

CONCLUSION

Herein we argue that in order for PE to manifest in an expectant mother, there is an element of both a dysfunctional placenta as well as an abnormal cardiac response. Figure 1 depicts the common and specific key components of impaired placentation and cardiovascular dysfunction. We cannot ignore the role of the maternal cardiovascular system in the development of PE, and thus PE must be recognised and managed, both in the acute and in the long term phases, as a cardiovascular-placental syndrome.

PE is a heterogenous disorder. There is an abundance of evidence supporting the role of the placenta in PE. The evidence indicates that placental dysfunction is prevalent in early-onset PE, however this is not as clear in cases of late-onset PE, as evidenced by uterine artery Doppler indices, birthweight distribution, placental biomarkers, histological and pathological studies. Table 1 summarises the main differences in placental and cardiovascular parameters observed in early-onset vs late-onset pre-eclampsia

The majority of PE is late-onset, and has classically been described as "maternal" or "heterogenous" PE. By and large, maternal PE remains unexplained. Several theories involving an exaggerated maternal systemic inflammatory response and increased systemic levels of oxidative stress have been published in the past[50].

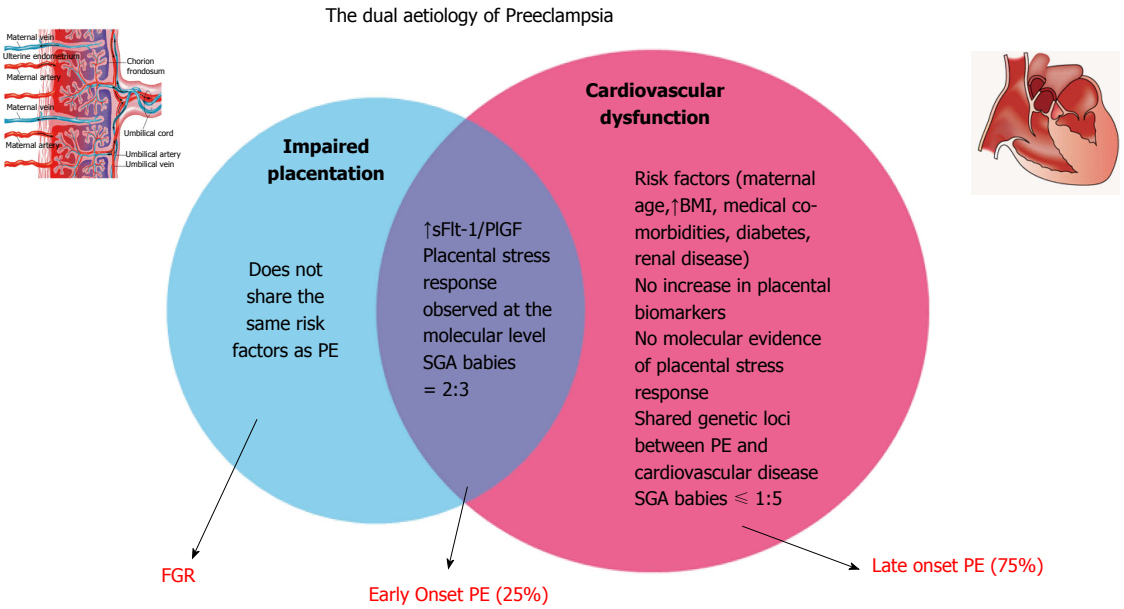

Figure 1 Illustration of the key components, both specific and common to impaired placentation and cardiovascular dysfunction and their presence in fetal growth restriction, early-onset and late-onset preeclampsia. FGR: Fetal growth restriction; PE: Preeclampsia; PlGF: Placental growth factor; sFlt-1: Soluble fms-like tyrosine kinase-1; SGA: Small-for-gestational-age; BMI: Body mass index.

Table 1 A summary of the key differences observed in placental and cardiovascular parameters in early-onset *vs* late-onset preeclampsia

	Early onset PE	Late onset PE
Fetal growth restriction	↑↑↑	↑
Hormones - PlGF	↓	↔
Hormones - sFlt-1	↑	↔
Hormones - PAPP-A	↓	↔
Uterine artery Doppler resistance	↑↑↑	↑/↔
Molecular stress response	↑	↔
Histological evidence of hypoperfusion	↑↑	↑/↔
Haemodynamics - Cardiac index	↓	↔
Haemodynamics - TVR index	↑↑	↑
Left Ventricular geometry – concentric remodelling	↑↑	↑↑
Left Ventricular geometry – concentric hypertrophy	↑	↓
Chamber diastolic function	↓↓	↓
Chamber systolic function	↓	↔

PE: Preeclampsia; PlGF: Placental growth factor; sFlt-1: Soluble fms-like tyrosine kinase-1; PAPP-A: Pregnancy associated plasma protein A; TVR: Total vascular resistance.

Whilst this is supported by the observed increase in PE in women with certain pro-inflammatory systemic conditions (autoimmune disease, renal disease, *etc.*)[51], another, and we believe stronger, argument can be made for the role of the maternal cardiovascular system in the development of PE.

PE is a complex disorder which is no doubt closely related to placental insufficiency. However we strongly believe that the placenta is not the only culprit; the inability of the maternal heart to adapt to placental dysfunction also forms a significant, however as yet incompletely understood, part of the enigma.

We propose that whilst intrinsic placental dysfunction and the mal-adaptation of the maternal cardiovascular system leads to early-onset PE, late PE is associated with an acquired placental dysfunction as a result of the maternal heart not being able to meet the demands of the placenta. Both the intrinsic and acquired placental dysfunction results in placental hypoxia which sets off a cascade of events result in the multisystem disorder of PE. In view of the evidence, we propose a paradigm shift of our current understanding of pre-eclampsia, as a disease entity not solely due to the placenta, but as a cardiovascular-placental syndrome. In answer to our original question which forms the title of this review, the answer is both - as an element of dysfunction in both the cardiovascular system and the placenta are required in order for PE to develop.

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MINIREVIEWS

Universal screening for hemoglobinopathies in today's multi-ethnic societies: How and when

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Abstract

Increasing multi-ethnicity in countries endemic or non-endemic for hemoglobinopathies has brought fundamental changes to the screening strategies for these traits. While in the past pre-screening on microcytosis was a reasonable method to economize upon follow up analysis, selecting low mean corpuscular volume means today missing all those normocytic carriers of common traits associated with severe conditions. Therefore, blood count should not be considered as a pre-selection tool but as additional information to be used for the interpretation of the provisional results, obtained by routine high throughput separation and measurement of the hemoglobin (Hb) fractions. Moreover, the moment of screening should be well planned depending on the social and cultural situation. Screening for genetic diseases in a modern multi-ethnic society should be offered to couples seeking progeny when both partners are more likely to be equally concerned with the good health of their children. In several societies screening before marriage and changing partner choice is culturally accepted. However, new generations are bound to disagree with these more or less imposed conditions and may decide not to renounce the choice of their partner asking for other preventive methods. In addition, a carrier state during pre-marital screening may in some cultures stigmatize the carrier, mostly the female with adverse social consequences. Therefore, screening for hemoglobinopathies early in pregnancy is the most sensible alternative in modern countries. Adding hemoglobinopathies to the routine rhesus screening using a simple separation of the Hb fractions on dedicated devices (high performance liquid chromatography or capillary electrophoresis) will virtually identify all female carriers of all common traits responsible for the severe conditions mainly sickle cell disease and thalassemia major in time for partner analysis, counseling and primary prevention.

Key words: Sickle cell disease; Thalassemia; Diagnosis; Prevention

Core tip: All women in most modern countries are offered screening for rhesus antagonism and infectious diseases early in pregnancy. Hemoglobinopathy (HBP) screening done together with rhesus screening using an inexpensive routine high performance liquid chromatography or capillary electrophoresis analysis could identify all women carriers of the frequent traits associated with the severe forms of HBP. Subsequent partner analysis could identify all couples at risk to be confirmed by molecular analysis in time for prenatal diagnosis when requested. This procedure will allow the prevention task to be offered at the most logical moment and by the most specialized hands of the gynecologists and obstetricians in collaboration with the local labs and the genetic counselors.

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INTRODUCTION

While up to a recent past were the Mediterranean countries those concerned with endemic hemoglobinopathy (HBP), with the thalassemia syndromes being the almost exclusive traits to be identified, today both Southern and Northern Europe are becoming a melting pot of different cultures from Africa, Middle and Far East, with different HBP traits to be identified[1]. In fact the same ethnic changes are observed all over the world and without prevention strategies many immigration countries will be confronted with a dramatic increases of severe HBP leading to human suffering and to increased costs of public health for the management of these incurable and expensive diseases[2,3]. To prevent all this, a broader spectrum screening for relevant HBP would have to be done in the endemic countries of Southern Europe and new national screening and managing strategies would have to be introduced in all other non-endemic countries experiencing massive immigration[4].

Over the past decades, regular beta thalassemia trait screening was based upon pre-selection of healthy but slightly anemic and microcytic carriers by measuring their low mean corpuscular volume (MCV) eventually persisting after iron therapy. This pre-selective screening method was considered practical and economically justifiable due to the fact that a complete blood count (CBC) was less expensive than screening by hemoglobin (Hb) electrophoresis and manual measurement of the HbA2 fraction[5]. This strategy might still be practical for large population screening campaigns for thalassemia in emerging countries. However, for screening and regular diagnostics in (non-endemic) developed countries this pre-selective strategy is inefficient and not cheaper at all.

Table 1 Cross table indicating the risk for the progeny of carriers of the common β globin gene defects

β traits	β minor	HbS	HbE	HbC	HbD	HbY
β minor	β major					
HbS	SCD	SCD				
HbE	β major	SCD	β minor			
HbC	β minor?	SCD	β minor	β minor		
HbD	β minor	SCD	β minor	Normal	Normal	
HbX	?	?	?	?	?	?

Hb: Hemoglobin; SCD: Sickle cell disease; HbD: HbD^{Punjab}.

In fact, even if well applied, this procedure implies at least two visits of the anemic carrier to the general practitioner (GP), two CBC's and at least one iron prescription. Moreover, healthy but slightly anemic thalassemia carriers are often kept on iron therapy for a long time before the hemoglobinopathy option is considered and investigated. In this scenario, delayed diagnosis of beta thalassemia carrier status in couples at risk might easily happen, resulting in the birth of a first severely affected child.

To avoid this unfortunate way of (mis) managing diagnostics and prevention, carrier screening campaigns at school, before obtaining the marriage certificate and before or early in pregnancy have been organized for several decades in most endemic Mediterranean countries, but often following the same strategy, being CBC as the first step followed by Hb separation and HbA2 measurement in case of low MCV[6-13].

Today these methods are inappropriate. Dedicated high performance liquid chromatography (HPLC) and/or capillary electrophoresis (CE) devices are available in all modern laboratories where separation and estimation of all normal and abnormal Hb fractions is obtained automatically (Figure 1). These automatic methods are much faster and cheaper to public health than the procedure mentioned above, involving GP visits, inadequate iron therapies and repetitions of non-conclusive laboratory analyses. Moreover, carriers of common traits like HbS and HbC or less common traits like HbD^{Punjab} or HbO^{Arab}, all associated with sickle cell disease (SCD), are not anemic or microcytic unless an independently inherited alpha thalassemia trait is also present. As a matter of fact, those few SCD carriers diagnosed today are identified just by chance, because of their border line MCV due to a coexisting alpha + thalassemia trait (-α/αα) or because of diabetes control with HbA1c later in life. Moreover, carriers of HbE, at risk for both severe beta thalassemia and SCD in their progeny, are often just border line microcytic, especially if vitamin B12 deficiency is also present. Therefore, pre-selecting by MCV in a multi-ethnic society, means missing the majority of the carriers of relevant traits associated with severe conditions in the progeny (Table 1). Screening should also not be based upon ethnic origin selection but should be offered at

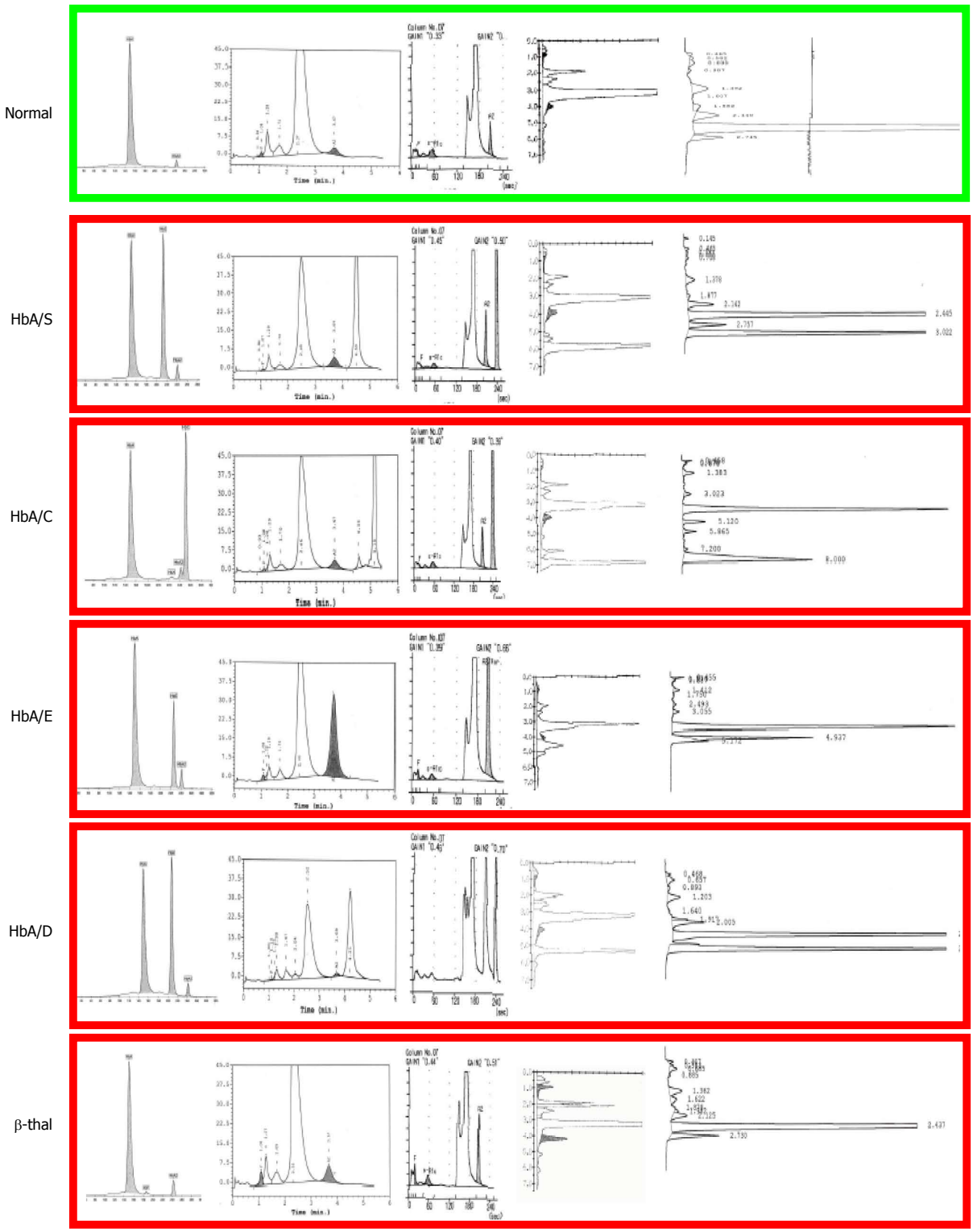

Figure 1 Examples of putative diagnostic results identifying carriers of the common β globin gene defects associated with sickle cell disease and β-thalassemia major on different high performance liquid chromatography or capillary electrophoresis devices. From left to right separations on Sebia CE and Bio-Rad, Menarini, Tosoh and Ultra2 HPLC devices. HPLC: High performance liquid chromatography; CE: Capillary electrophoresis; Hb: Hemoglobin.

the national level and at the most logical moment. Ethnic selection has been matter of discussion since HBP in some non-endemic European countries has been considered as a rare "import" disease, too infrequent in the general population to justify universal screening. Indeed HBP are not found very frequently in the so called "low risk" North-European populations but low risk is not "no risk" and carriers of beta thalassemia, alpha thalassemia and HbD^{Punjab} are regularly diagnosed in North-Europeans and not only because of distant (forgotten) "ethnic ancestors" but also due to "local" mutations. In fact, selecting for

ethnic origin means discriminating both the included and the excluded ones, and adds to the risk when having found a carrier in the high risk population, the low risk partners in mixed couples are not checked, just supposing that for them the chance of being a carrier is "very low".

Because of all these considerations newborn screening (NBS) is universal in most countries that offer this option. However, NBS is not providing primary prevention and not even retrospective primary prevention to couples at risk if they are not properly counseled[4,5,14].

For primary prevention, in the new multi-ethnic societies, screening should be offered to couples at risk before the first affected child is born and how and when universal screening should be organized starting with separation and measurement of the Hb fractions on HPLC or CE and using CBC as auxiliary data is further explained in this review.

The HBP's in a nutshell

To understand the HPLC/CE and CBC results one should keep in mind that thalassemias and abnormal hemoglobins are both HBP.

Thalassemias are caused by mutations on the globin genes that impair the expression of the genes reducing the amount of globin which is needed to form normal hemoglobin. This explains the microcytic hypochromic state of the red cells, the typical erythromorphology and the qualitatively normal HPLC/CE separations.

Abnormal hemoglobins (like the common HbS, HbC, HbE and HbD) are caused by mutations on the (beta) globin genes that change the structure of the globins and herewith of the more or less normally expressed abnormal hemoglobins. This explains the normocytic normochromic state of the red cells and the abnormal HPLC/CE separation. An exception among the common variants is HbE which is an abnormal hemoglobin with lower expression (approximately 25%) with a mild thalassemic phenotype in the carrier.

The genes involved in Hb expression during pre and postnatal life are the embryonic epsilon and zeta (ε and ζ), the fetal gamma (γ), the full expression beta (β) and alpha genes (α) and the low expression delta (δ) genes. All Hb molecules expressed during embryonic, fetal and postnatal life are tetramers. From the fetal life on, these tetramers are formed by 2 alpha and 2 non-alpha like globin chains. In normal conditions, the expression of postnatal hemoglobin HbA (α2/β2) and HbA2 (α2/δ2) is coded by two β-globin genes (one of paternal and one of maternal origin) located on chromosome 11, four α-genes (two of maternal and two of paternal origin) located on chromosome 16 and two δ genes, adjacent to the β-genes. The four γ genes needed for fetal HbF (α2/γ2) expression during fetal life, are silenced during the first year of life. Therefore the expected expression of the Hb fractions in normal adults will be approximately 96%-97% HbA 2.5%-3% HbA2 and 0.5%-1% HbF. All changes found in these parameters can be associated with a relevant hemoglobinopathy.

METHODS: SCREENING HOW

The samples

Materials and methods for "basic" HBP carrier screening are very simple and available in virtually all laboratories[15]. Screening should always include separation and estimation of the Hb fractions on automated HPLC or CE and the separation results should be compared for compatibility with the CBC parameters. Samples should be collected in EDTA and be processed directly for the best CBC and Hb separation results. However, samples kept refrigerated and processed a few days later are still valid for separation and measurement of the fractions and for DNA extraction if required. Dry samples, as eventually used for newborn screening, are suitable for obtaining a provisional result from the separation on HPLC or CE but are obviously not suitable for CBC and DNA extraction from dry samples requires special methods.

Separation of the Hb fractions on dedicated devices

Separation and measurement of the Hb fraction has been done automatically for more than two decades using dedicated devices. The most popular HPLC and capillary electrophoresis devices (CE) evaluated by Van Delft *et al*[16] are available in virtually all laboratories.

These devices identify rapidly and at very low cost all carriers of high HbA2 beta thalassemia and of the common and relevant HbS, HbC, HbE and HbD which will represent most of the positive results to be expected in a multi-ethnic population[16].

HbA2 levels above 4% are diagnostic for beta thalassemia trait with HbF levels that may or may not be slightly elevated[15]. Some cases of beta thalassemia trait may however present with HbA2 levels lower than 4%. In these cases, if the CBC remains microcytic, alpha and beta thalassemia should be further investigated at the molecular level, especially when risk is suspected in a putative carrier couple. In some cases, interfering delta globin gene defects may cause low HbA2 (δ2/α2) levels and especially delta thalassemia may interfere with the diagnosis of high HbA2 beta thalassemia[17].

Carriers of the common Hb variants (HbS, HbC, HbE and HbD) are identified at 100% sensitivity and over 95% specificity (Figure 1)[16]. The high specificity is partially due to the precise positions in which these fractions migrate but mainly due to the fact that these variants are the most common. To exclude rare variants moving in the same position as HbS the fraction can be confirmed with 100% specificity using the sickle test[18]. For all presumed couples at risk confirmation should be performed at the molecular level. All rare variants not corresponding to the common one should also be investigated at the molecular level to exclude genetic risk.

Table 2 Genotype/phenotype correlation, indicative MCV/MCH values, HPLC/CE separations and presence of HbH or Hb Bart's and genetic risk in alpha thalassemia

Genotype	α-thalassemia Condition name	Phenotype	MCV MCH	HPLC CE	HbH (adult) Hb Bart's (newborn)	Genetic risk for the progeny of carriers
αα/αα	Normal	Normal	Normal	Normal	Absent Absent	None
-α/αα	α^+ heterozygous	Eventually anemic	borderline	Normal	Absent 0%-5%	HbH
-α/-α	α^+ homozygous	Mildly anemic	Low	Normal HbA2↓	Absent 5%-10%	HbH
--/αα[1]	α° heterozygous	Mildly anemic	Low	Normal HbA2↓	Absent 5%-10%	HbH or Hb Bart's HF
--/-α[2]	HbH disease	Intermediate hemolytic anemia	Lower	HbH Hb Bart's HbA2↓↓	0%-10% 10%-30%	HbH or Hb Bart's HF
--/--	Hb Bart's Hydrops Fetalis	Severe intrauterine hemolytic anemia	Severe morphology	No HbA or HbF in newborn	Hb Portland Hb Bart's	Lethal to newborn. Life threatening for mother

[1]Rare positive cells after inclusion bodies staining; [2]Many positive cells after inclusion bodies staining (Figure 2). MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; Hb: Hemoglobin; HPLC: High performance liquid chromatography; CE: Capillary electrophoresis.

Table 3 Indicative parameters usually associated with carrier states

Carrier of	Hb	MCV	MCH	RBC	HbA2	HbF	Approximately HbS (%)	Approximately HbC (%)	Approximately HbE (%)	Approximately HbD (%)	Smear
Alpha thal	L[2]	L[2]	L[2]	N	N[1]	N					TC, APC
Beta thal	L	L	L	N or E	E[4]	N or E					TC, APC
HbS	N[1]	N[1]	N[1]	N	N[3]	N	45-25[6]				Normal[5]
HbC	N[1]	N[1]	N[1]	N	N	N		45-25[6]			TC
HbE	L-N	L-N	L-N	N	N	N or E			± 25		TC, APC
HbD	N[1]	N[1]	N[1]	N	N	N				± 45	Normal

[1]Could be lower in case of alpha thal; [2]Low depending on the form of the defect (see Table 2); [3]Could be higher due to HPLC artifact; [4]Some rare beta thal carriers have normal HbA2; [5]Positive on sickle test; [6]%HbS or C reduced in the presence of alpha thal. L: Low; N: Normal; E: Elevated; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; RBC: Red blood cell; TC: Target cells; APC: Anisopoikilocytosis; Hb: Hemoglobin.

Results of HPLC/CE screening and prevention

Screening with HPLC/CE methods will practically identify all carriers of "beta gene defects" that are important for offering primary prevention of severe hemoglobinopathies in a multi-ethnic society. Adult carriers of mild alpha gene defects are not identified on HPLC/CE but can be easily identified during newborn screening by the presence of Hb Bart's (Table 2).

Due to the lethal outcome of homozygous alpha zero defects and the usually mild to intermediate phenotype of HbH disease, carriers of alpha gene defects represent a relatively less severe problem to public health. However, and also because of maternal risk, screening for these traits, common in Southeast Asian and Chinese populations, should be taken into serious consideration (read further).

Correlation between HPLC/CE and CBC parameters

The CBC parameters should be compatible with the HPLC or CE results. As mentioned above, carriers of thalassemia are characterized by a mild microcytic (MCV↓) hypochromic (MCH↓) anemia (Hb↓) not improving on iron therapy. Conversely, carriers of the common Hb variants, separable on HPLC or CE are not microcytic, unless also carriers of coexisting alpha thalassemia. In addition, a low MCV value tends to increase with time and in case of borderline values measured often in mild alpha thalassemia, one can better rely on the MCH value which remains more constant. The best discriminating CBC parameter to differentiate between iron deficiency and thalassemia will be the red blood cell (RBC) count, which is often elevated in the compensated beta thalassemia carrier with sufficient folic acid intake. Indicative parameters are summarized in Table 3.

The microcytic anemic condition is less evident in carriers of mild alpha thalassemia. This depends from the presence of 4 alpha genes and from the number of alpha genes left active. CBC can then be nearly normal in the mildest alpha thalassemia forms and strongly abnormal in the severe HbH disease form (Table 2).

Erythromorphology

While often disregarded in modern laboratories because time consuming and because it requires trained observers, the smear gives away the diagnosis of thalassemia to the expert eye who will identify the typical erythromorphology of the target cells and anisopoikylocytosis (last column in Table 3), the last being the visual equivalent of the differential

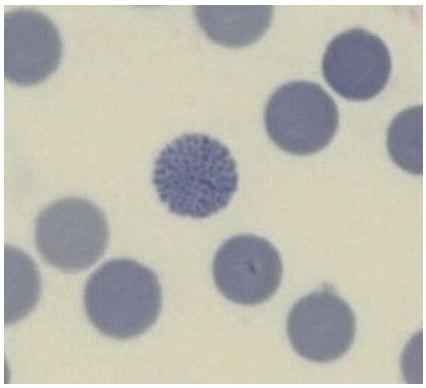

Figure 2 Typical hemoglobin H inclusion bodies found sporadically in carriers of alpha zero thalassemia (--/αα) and abundantly in patients with hemoglobin H disease (--/-α).

parameter "red cell distribution width" (RDW). Moreover, smears stained with supravital brilliant cresyl blue (reticulocytes staining), will identify the rare HbH inclusion body cells, specific to the alpha zero thalassemia carrier (at risk for hydrops fetalis in the progeny) (Figure 2).

Significance of HbA2 and HbF measurement

As mentioned above, an adult β-thalassemia carrier will usually show 4% or more HbA2. Then, if matching with the expected microcytic hypochromic CBC parameters, the provisional diagnosis will be "carrier of β-thalassemia". However, some rare β-thalassemia mutations show HbA2 values in the "grey zone" (3.5%-3.9%) or even normal levels. In these cases excluding a β-thalassemia trait because of a value just below the normal cut of, trusting on the presumed precision of the measurement is very imprudent, especially when CBC indicates persistent microcytosis in absence of iron depletion. In these cases further investigation is needed as one may have a borderline HbA2 β-thalassemia, an α-thalassemia or a large β deletion thalassemia defect[19]. Some HbF (up to approximately 7%-8%) will eventually be present in the beta thalassemia carrier. Higher HbF levels are usually associated with delta/beta thalassemia deletion defects that will present with normal HbA2 levels and masked microcytosis due to the larger size of the HbF cells.

Dedicated devices may overestimate or underestimate the HbA2 levels in the presence of Hb variants. However, measurement of HbA2 in the presence of HbA and any β variant is obsolete. When both HbA and the β variants are expressed (in non-transfused patients) it means that both β genes are expressed and that there is no need to measure HbA2 to establish the presence of a non-expressed β thalassemia gene.

HbA2 and alpha thalassemia

Adult α-thal carriers will show no specific characteristics on HPLC or CE except for a marginal reduction in HbA2 expression. The only exception is HbH disease, which will usually present with significantly low HbA2 and a rapid migrating or eluting but unstable and quickly disappearing HbH fraction.

When suspecting α-thal because of the CBC parameters in the absence of iron depletion and low HbA2, then molecular analysis is needed to detect the common deletions or point mutations. On the other hand, α-thal can easily be detected at birth by the presence of Hb Bart's. The presence of Hb Bart's observed during newborn screening should be reported to avoid a more laborious diagnosis later in life.

In the presence of a single non active α gene Hb Bart's value between 0% to 5% is usually measured. The rates rises from 5% to 10% when two α genes are compromised (either in *cis* --/αα or *trans* -α/-α), while in HbH disease (-α/--), Hb Bart's level can go from 10% to 30%. In case of the lethal hydrops fetalis syndrome (--/--) no HbF is present in the fetus or in the severely compromised newborn but traces of HbH (β4), Hb Bart's (γ4), (ε4), Hb Gower-I (ζ2ε2) and the embryonic Hb Portland (ζ2γ2) are detected (Table 2).

The folic acid issue

Together with iron are vitamin B12 and folic acid essential for erythropoiesis. Folic acid is prescribed before and during pregnancy also to prevent neural tube defects like spina bifida. Folic acid is a vitamin with limited body storage that can be rapidly exhausted during increased cell division, a condition not only present in pregnancy but also chronic in thalassemia carriers. As mentioned above, the RBC count tends to be elevated in well compensated beta thalassemia carriers. Compensation is folic acid dependent and anemia may become more pronounced in beta thalassemia carriers in case of low folic acid intake. If folic acid is sufficiently present, RBC counts tend to be higher and by this compensatory mechanism Hb levels tend to be higher as well, while the packed red cells (PCV) value remains within normal levels. Measurement of serum folic acid gives no clue since it is the limited reserve of this vitamin which determines maintenance of the compensating mechanism. Therefore a folic acid prescription for anemic thalassemia carriers with poor dietary intake can be beneficial.

SCREENING WHEN

Newborn screening

Although SCD and thalassemia major do not need special treatment during the first 5-6 mo of life, several immigration countries have included HBP in the ongoing newborn screening (NBS) originally intended for treatment of metabolic diseases directly after birth[20-24]. Although NBS comes one child too late, one could expect some retrospective and prospective primary prevention for couples who had an affected or a carrier child. Unfortunately, after 7 years of NBS in the Netherlands no changes in the incidence of these severe diseases and in the requests for prenatal

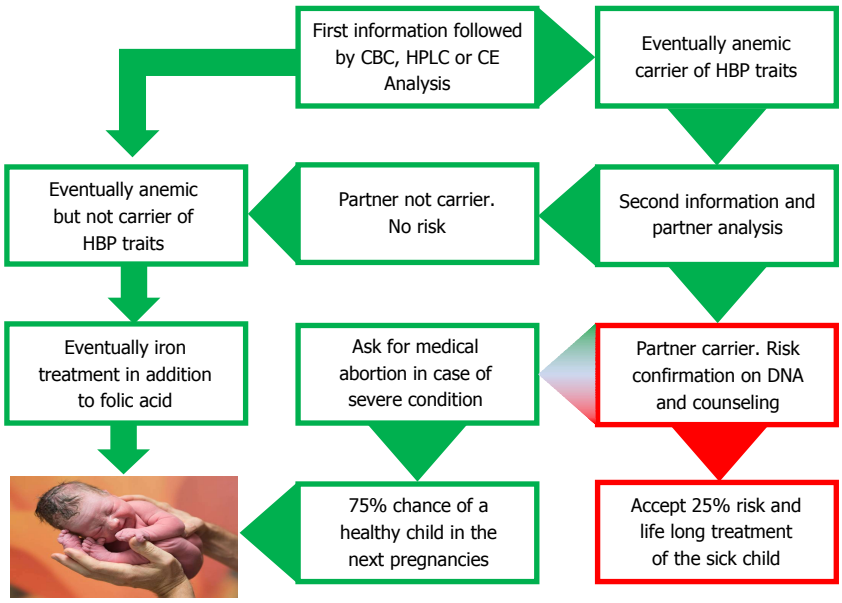

Figure 3 Genetic risk for hemoglobinopathies and practical prevention flow chart after carrier screening early in pregnancy. HPLC: High performance liquid chromatography; CE: Capillary electrophoresis; HBP: Hemoglobinopathy; CBC: Complete blood count.

diagnoses have been registered[14].

More efficient screening

To introduce a more efficient screening for primary prevention in multi-ethnic societies, one needs to identify healthy couples at risk before the birth of the first affected child. Moreover, one needs to choose the most logical moment, to provide the most consistent structure and to apply the most economically and socially convenient strategy[24]. If we look at the experience from decades of prevention in endemic countries[7-13], we see mandatory screening options before marriage which are not likely to become recommended in modern multi-ethnic societies unless prescribed by specific cultures. Screening when planning a pregnancy would be ideal but no structures are available to deal with different cultures at this stage except perhaps the scarcely experienced and reluctant GP's. In fact, pre-pregnancy counseling is not available in most of the non-endemic immigration areas where little awareness is present in the daily healthcare structures and in the public. Putting up such a structure would take lot of efforts, lot of time and money. Regarding PGD, most countries offer this only in case of fertility problems and/or embryo selection when a suitable stem cells donor is needed for an affected child.

Screening early in pregnancy has been proven to be most effective and requested by well-informed couples in the endemic countries of Southern Europe and it is bound to be the most sensible options in most immigration countries (Figure 3). Screening early in pregnancy comes at a time when both parents are concerned with the good health of their progeny. Both parents are generally involved and the female partner has lesser chances to be stigmatized. Pregnancy care structures are usually well organized in most immigration countries where dedicated midwifes or obstetricians can provide information and offer screening and prevention[25]. The most practical option would then be to anticipate the first pregnancy visit and to offer hemoglobinopathy screening together with the rhesus and infectious diseases screening which is routinely offered to all pregnant women in most developed countries.

Screening results: Information vs counseling

An important task for the obstetrician/gynecologist and for the laboratory analyzing the blood is to provide a thorough explanation. This can be done verbally and by using a standard letter when offering screening. Once the (pregnant) female carrier is diagnosed, short term partner analysis should be advised and to avoid anxiety it should be explained that being a carrier is not a disease but a recessive hereditary trait that only if present in both parents may affect their children with a chance of 1 in 4 causing a severe condition. Couples at risk should be made aware of the fact that the risk of 1 on 4 goes for every pregnancy and that having had one affected child does not mean that the next 3 should be healthy. Carriers should be reassured by explaining that each human is a carrier of several recessive traits and that knowing which one is an advantage that allows prevention if necessary.

Once the partner of a carrier of a relevant trait is found positive, then the putative couples at risk should be confirmed at the molecular level and counseled by a genetic counselor well informed about the severity of the diseases and the prognosis to be expected in the progeny. If the parents opt for prevention, prenatal diagnosis should be provided. Counseling should not be directive, neither toward treatment nor prevention,

but based upon thorough and realistic information and should not be provided by doctors that may propose very expensive treatment without any hope of a definitive cure[3]. Parents should be counseled about the difference between treatment and cure. They should be made aware of the fact that the disease can be treated but cannot be cured and that, in spite of the best treatment, the severity of the disease will only progress until premature death. Bone marrow (stem cells) transplant (BMT) the only available "cure" today, may also fail causing severe graft versus host diseases and even when "successful" may leave the patient in a chronic post BMT condition requiring life-long treatment and monitoring.

Some bottlenecks

Information on genetic risk can be interpreted in different ways in different cultures. In some cultures, when the pregnant female is diagnosed as a carrier and the male partner is asked to be checked, he might refuse to comply. In some culture the male might blame the female for getting a sick child. There have been cases of pregnant HbS carriers who had a child affected with SCD because the father did not show up, leaving the mother alone to take care of the sick child. It would therefore be ethically correct to offer prenatal diagnosis to female carriers also in absence of the father and in particular when the supposed father belongs to an ethnic group of high prevalence.

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MINIREVIEWS

Emergency contraception: What is new?

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Abstract

Unintended pregnancy rates remain high throughout the World and increase the risk of poor maternal and infant outcomes. Most of unintended pregnancies occur in women who were not using contraception or who became pregnant despite the reported use of contraception. Women who have had recent unprotected intercourse including those who have had another form of contraception fail are potential candidates for this intervention. Currently used emergency contraceptive methods are pills that contain combined estrogen-progesterone, only progestin, antiprogestins and copper intrauterine devices. The most common form of this type of contraception is oral progestin-only pills (levonorgestrel). The most effective method is copper intrauterine devices followed by anti-progestins and oral progestin-only pills. The major pathogenesis of oral emergency contraceptives is the prevention or delay of ovulation. Although conception is possible on only a few days of the cycle, emergency contraception is offered when indicated without regard to the timing of the menstrual cycle because of uncertainty in the timing of the ovulation. Levonorgestrel and E/P regimes are most effective as soon as possible after unprotected sexual intercourse. A linear relationship has been shown between effectiveness and the time of dose. The effectiveness continues for 120 h, but it is recommended to be used within 72 h after intercourse. Intrauterine devices may prevent pregnancy when 5 d after ovulation.

Key words: Emergency contraception; Levonorgestrel; Mifepristone; Ovulation; Ulipristal acetate

Core tip: Emergency contraception methods have varying ranges of effectiveness depending on the method and timing of administration. The major pathogenesis of oral emergency contraceptives is the prevention or delay of ovulation or prevention of fertilisation. Combined and progestin-based emergency contraceptives should be used as soon as possible to enhance the efficacy. Emergency contraception offers a final chance to prevent pregnancy.

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INTRODUCTION

Emergency contraception is defined as contraceptive methods used after unprotected sexual intercourse or sexual assault and in cases with contraceptive failure. Currently used emergency contraceptive methods are pills that contain combined estrogen-progesterone, only progestin, antiprogestins and copper intrauterine devices.

The most common form of this type of contraception is progestin-only pills (levonorgestrel). The most effective method is copper intrauterine devices followed by anti-progestins and oral progestin-only pills. The major pathogenesis of oral emergency contraceptives are presumed to be a delay or prevention of ovulation. Levonorgestrel in particular is ineffective as emergency contraception after ovulation. The major role of copper-induced intrauterine devices in emergency contraception is the prevention of fertilization[1]. There is much evidence suggesting that implantation of the fertilized ovule cannot be prevented by emergency contraception.

The possibility of pregnancy after unprotected sexual intercourse changes between 12%-30% in a population of young couples in their mid-twenties depending on the day of the menstrual cycle[2]. While the possibility of pregnancy is higher on the day of ovulation, emergency contraception can be used at any time of the menstrual cycle. It is independent of the day of ovulation. The most widely used EC methods are summarised in Table 1.

Indications for possible emergency contraception fallow as[3]: When no contraceptive was used during sexual intercourse within the previous 120 h; When there is a contraceptive failure or incorrect use of a contraceptive within the previous 120 h, including: (1) condom breakage, slippage, or incorrect use; (2) three or more 30 to 35 mcg ethinyl estradiol pills have been missed (or two or more 20 to 25 mcg pills); (3) progestin-only pill (minipill) taken more than three hours late; (4) more than two weeks late for injection of depot-medroxyprogesterone acetate; (5) dislodgment, breakage, tearing, or early removal of a diaphragm or cervical cap; (6) dislodgment, delay in placing, or early removal of a contraceptive hormonal skin patch or vaginal ring; (7) failed coitus interruptus (*e.g.*, ejaculation in vagina or on external genitalia); (8) failure of a spermicide tablet or film to melt before sexual intercourse; (9) miscalculation of the periodic abstinence method or failure to abstain on fertile day of cycle; and (10) expulsion of intrauterine contraception.

Table 1 Summary of emergency contrception methods

Contraceptive methods	Mechanism of action	Effective time	Most common side effects
Contraceptive Pills (Yuzpe Regimen)	Inhibition or delay of ovulation	72 h recommended (Up to 120 h)	Same as COC
Progestin-only ECP	Inhibition or delay of ovulation	72 h recommended (Up to 120 h)	Nausea and vomiting Irregular bleeding
Ullipristal	Inhibition or delay of ovulation	Up to 120 h	Nausea and vomiting Abdominal pain
Copper induced IUD	Prevention of İmplantation	Up to 5 d (recommended)	Genital infection Bleeding with unknown etiology Abnormalities of the uterus Wilson disease

ECP: Emergency contraceptive pills; IUD: Intrauterine devices; COC: Combined oral contraceptives.

COMBINED EMERGENCY CONTRACEPTIVE PILLS (YUZPE REGIMEN)

Many clinical studies have shown that ovulation can be prevented or delayed with emergency contraceptive pills that contain estrogen (E) and progesterone (P)[4,5]. After the first studies showing prevention of pregnancy with high dose of estrogen in 1974, Yuzpe *et al*[6] defined a low dose regime containing 200 mcg ethinyl estradiol and 1 mg levonorgestrel.

Some studies suggested that emergency contraceptive pills (ECP) prevent the implantation of the fertilized ovule by changing the endometrial receptivity in addition to the biochemical and histological changes in the endometrium after use of this regime. However, these suggestions were not confirmed in recent studies. Other possible mechanisms of ECP include changes to the function of the corpus luteum, thickening of cervical mucus, changes in tubal transport and prevention of fertilization[7-9].

Preven was approved for emergency contraception by Food and Drug Administration (FDA) in 1998 and was withdrawn in 2004. However, combined oral contraceptives (COC) that are not packed for emergency contraception can be used as 100 mcg ethinyl estradiol and 0.50 mg levonorgestrel with the same dose repeated in 12 h[10]. Levonorgestrel and E/P regimes are most effective after as soon as possible after unprotected sexual intercourse. There is a linear relationship between efficacy and the dose time. The efficacy continues for 120 h, but use within 72 h is recommended. Patients must be warned about this reduction in efficacy - especially after 96 h. In a meta-analysis of 3800 women, the success of the Yuzpe regime in the prevention of unwanted pregnancies was found to be 56%-89%[11]. However,

the Yuzpe regimen is not recommended because side effects are more common, and the efficacy of ECP is approximately half of the use of only levonorgestrel[12].

PROGESTIN-ONLY ECP

Early studies showed that levonorgestrel disrupts ovulation and the luteal functions. No effect on the endometrium was reported in these studies despite a study suggesting that the use of levonorgestrel glycodelin just before the luteinizing hormone (LH) surge changes the luteal phase secretory pattern. Levonorgestrel has no effect on the endometrial implantation of the embryo and endometrial receptivity markers[13-18]. The original prescription is the use of 0.75 mg oral levonorgestrel in the first 72 h with a repeated identical dose after 12 h. The same efficacy was reported with a 1.5 mg single dose in different studies. In addition to the studies supporting the same side effects for the two methods, most studies reported more headaches and breast sensitivity in the single dose regime[19,20]. In another study, 2 doses of 0.75 mg levonorgestrel repeated at 24 h had the same efficacy. Levonorgestrel is more effective than the Yuzpe regime in the prevention of pregnancy (RR = 0.51, 95%CI: 0.31-0.83) with fewer side effects (nausea RR = 0.43, 95%CI: 0.39-0.48; vomiting RR = 0.24, 95%CI: 0.18-0.31; headache RR = 0.83, 95%CI: 0.69-1.00; breast tenderness RR = 0.84, 95%CI: 0.69-1.01)[21].

ANTIPROGESTINS

Ullipristal and mifepristone are similar antiprogestins with similar chemical structures. The major mechanism is *via* prevention or delay of ovulation in addition to the effects on endometrium causing disruption of implantation[22].

MIFEPRISTONE

Mifepristone is a first-generation progesterone receptor modulator and is approved for use in many countries for first trimester abortion. In randomized studies, a dose of 5-600 mg mifepristone (RU-486) has the same or better efficacy in the prevention of pregnancy than oral emergency contraceptives (up to 99%-100%)[23-26].

The optimal dose is not clearly defined but may be 25-50 mg with no serious side effects. A delay in menstrual bleeding after the use of antiprogestins decreases doubts of pregnancy and anxiety[27,28]. The use of mifepristone in pill form is prevented for widespread use as emergency contraception. Mifepristone is only approved in Armenia, China, Russia and Vietnam as an emergency contraceptive.

ULLIPRISTAL

A selective progesterone receptor modulator with antiprogestin effects that can cause 5 d in delay of ovulation. There are studies showing that it can cause endometrial changes, however it remains controversial if it inhibits implantation. Ullipristal is used as a single dose of 30 mg. It is the most effective ECP in Europe and United States with an efficacy of 62%-85%[29-31]. Ullipristal is effective in the late follicular phase in which a rise in the level of LH has begun but no peak was achieved. In a meta-analysis comparing the efficiencies of levonorgestrel and ullipristal, the latter was shown to be higher at 0-24, 0-72 and 0-120 h after intercourse with no differences in side effect profiles[31].

COPPER-INDUCED INTRAUTERINE DEVICES

Implantation occurs 6-12 d following ovulation. Intrauterine devices may prevent pregnancy when applied 5 d after ovulation. Copper induced intrauterine devices (IUD) are the most effective emergency contraceptives with a major advantage for continuous contraceptive effect. In a multi-centre study with 1013 patients, its efficiency was 96.9% when used 120 h after intercourse[32]. The contraindications of applying an IUD at the same day of intercourse are acute cervicitis and other known medical contraindications. It should be applied 5 d after unprotected sexual intercourse, however there is limited data suggesting that the efficiency continues to 7 d[33].

OTHER INTRAUTERINE CONTRACEPTIVE METHODS

No data was found in the literature about the use of LNG-IUDs in emergency contraception. It was not recommended for this indication[34,35]. The LNG-IUDs can only be applied after the exclusion of pregnancy and only if the patient is 7 d or more into the menstruel cycle. If not, an additional contraceptive method or ECP for 7 d is recommended[35].

The frameless copper IUDs produced for adolescents and nullipar women (GyneFix® 330) have the same efficiency as ordinary copper IUDs in emergency contraception[36].

THE FACTORS CHANGING THE EFFICIENCY OF EMERGENCY CONTRACEPTIVES

Timing of treatment

Combined and progestin-based emergency contraceptives should be used as soon as possible (0-72 h) to enhance the efficacy[37]. Both drugs can be started up to 120 h after sex, but patients must be informed about the reduced activity after 96 h[38]. Ullipristal is the only approved oral agent for emergency contraception

from 72-120 h. Copper IUDs can be used for up to 5 d because of the post-fertilization effects. A systematic review reported the efficiency of IUDs up to 7 d, but there is limited data supporting this claim[39].

Body mass index

The efficiency of levonorgestrel ECP is decreased in obese women with an increase in unwanted pregnancies; however, no such effect was seen for ullipristal[39]. Because of these results, one commercial form of levonorgestrel added a warning of reduced efficiency in patients over 75 kg. After revision of the study, the European Medicines Agency described no reduction, and the warning was removed[40].

SAFETY

There is no physical examination or laboratory testing required before the use of oral progestin-based emergency contraceptives beyond the unnecessary pregnancy test unless there is no doubt of pregnancy due to symptoms or last menstruation date. No teratogenic effects on the foetus or side effects have been reported, however pregnancy must be excluded in cases using ullipristal or IUD.

Emergency contraception did not show any causal connection to death or serious complications. According to profit and loss rate data from the United States Medical Eligibility Criteria for Contraceptive Use (US MEC), no risk factors were reported to prevent the use of combined ECP and progestin-only pills[34,35]. ECP can be used in patients with lactation or a history of ectopic pregnancy, cardiovascular disease, migraine, and liver disease. ECPs can be used in patients contraindicated for combined oral contraceptives because of the short use time and lower total hormone dose[35]. Ullipristal acetate, progestin-only ECP, or copper-induced IUDs are preferred in women with changes in coagulation factors or a history of venous thromboembolism (VTE) or pulmonary embolism (PE). Repeated ECP use is safer than pregnancy even if there is no sufficient data for repeated ECP use. A warning to not repeat the dose in one menstrual cycle is included in the prospectus of ullipristal asetate[41].

CONTRAINDICATIONS

The contraindications for hormonal contraceptives cannot be adapted to women using emergency contraception. This is especially true in cases with cardiovascular diseases, thrombotic diseases, migraine, and liver disease. The advantages of use overpower the potential risks[42,43]. These guides do not include ullipristal. The contraindications for use of ullipristal are suspected pregnancy, uncontrolled asthma and liver diseases. The contraindications for IUD are uterine distortion, active pelvic infection, allergy to copper, and suspected pregnancy.

SIDE EFFECTS

Nausea-vomiting, abdominal pain, breast tenderness, headache, dizziness and weakness that regressed spontaneously in 24 h are the major side effects. Nausea and vomiting are seen in nearly half and 20% of the patients using combined ECP, respectively. Nausea and vomiting are less common in levonorgestrel than in combined ECP[12].

Some authors suggest a repeated dose in case of vomiting. This should be given 2 h later. In combined ECPs containing E/P, a repeated oral levonorgestrel and ullipristal dose is given at 1 h and 3 h after vomiting, respectively. Meclisine can be used to prevent nausea but it has no effect on dizziness. Vaginal administration is described, but the efficiency remains unclear. The application of the IUD is preferred instead.

ECPs containing levonorgestrel may shorten menstrual cycle when used in the early days of the cycle. ECPs have no effect on the duration of the cycle, but the duration of bleeding may extend to the next cycle. The duration of the menstrual cycle extends when used in periovulatory and postovulatory phases. Inter menstrual bleeding is seen in 15% of patients.

EFFECTS ON PREGNANCY

In a study of 332 pregnant women who used levonorgestrel in during the conception phase, there was no rise in birth defects[44]. ECPs do not increase the risk of extrauterine pregnancy in future pregnancies[45,46].

USE IN LACTATION

There is no restriction on the use of levonorgestrel or combined ECPs in lactating women[44]. In a study researching the pharmacokinetics of the use of 1.5 mg levonorgestrel for emergency contraception in lactating women, it is recommended to break from breastfeeding for 8 h during the excretion of levonorgestrel to breast milk. Feeding should restart in 24 h[47].

In a study comparing efficiencies of progestin-only oral contraceptives and progestin-only ECPs in lactating women, no difference was detected in maternal and foetal effects[48]. Seven days hiatus from breastfeeding is advised after a single dose of ullipristal. The milk must be collected and discarded during this time to continue to stimulate lactation[49].

DRUG INTERACTIONS

Drugs that induce liver enzymes may reduce the efficiency of levonorgestrel and ullipristal. Therefore, IUDs may be recommended for emergency contraception in patients using drugs inducing liver enzymes such as anti-epileptics and antiviral agents in the last 28 d[50]. In addition, ullipristal should not be

used with drugs increasing gastric pH.

PROCEEDING OR STARTING HORMONAL CONTRACEPTION

Because of the unknown duration of emergency contraceptive effects, it is unclear how to proceed with continuous contraception at the same menstrual cycle. The patient must be informed about using a safe contraceptive method[51]. According to pharmacokinetic studies and expert opinions, barrier methods and hormonal contraceptives may be started after application of emergency contraception. In the first seven days of hormonal contraceptives, an additional failsafe method must be used.

The use of long-term contraceptives is not recommended prior to exclusion of pregnancy. The efficiency of hormonal contraceptives may decrease in patients using ullipristal depending on the progesterone antagonistic effect. The FDA does not recommend the use of hormonal contraceptives within 5 d of ullipristal[52].

EXPERIMENTAL METHODS

Prostaglandin inhibitors

Cyclooxygenase enzyme (COX-1 and COX-2) inhibitors have female reproductive functions of oocyte maturation and ovulation. Many studies targeting this effect in emergency contraception are still underway. Studies have shown 15 mg of meloxicam with 1.5 mg levonorgestrel is more effective on follicular rupture in 5 d in patients with a follicular size over 15 mm than levonorgestrel only[53,54]. In addition, a 400 mg dose of celecoxib may delay or prevent luteal changes[55].

CONCLUSION

Emergency contraception is the last chance to prevent pregnancy after unprotected sexual intercourse or contraceptive failure. The efficiency increases by starting the medication as soon as possible. It is important to remember the rate of unsuccessful contraception and to exclude pregnancy especially in obese or drug-contraindicated patients. Of course, patients must be informed and encouraged to use regular and effective contraception.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Outcomes of surrogate pregnancies in California and hospital economics of surrogate maternity and newborn care

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Abstract

AIM: To describe maternity and newborn charges for an economic analysis of surrogate pregnancies on the health care resource utilization.

METHODS: A retrospective chart review of all women identified as being surrogates and the infants born from these pregnancies was performed between January 1, 2012 and December 31, 2013. Selected maternity diagnoses, mode of delivery, duration of hospitalization, and hospital charges were collected together with infants' birth weights, gestational age, length of hospital stay, and hospital charges. Charges associated with the *in vitro* fertilization cycles, artificial insemination, or embryo(s) transfer into the surrogate were not considered in the maternity charges. A ratio contrasting the maternity hospital charges for the surrogate carrier was compared as a ratio to the mean charges for 2540 infants delivered in 2013 after natural

conception and adjusted to the baseline hospital charges for both maternity and newborn care.

RESULTS: Analysis of sixty-nine infants delivered from both gestational and traditional surrogate women found an increased in multiple births, NICU admission, and length of stay with hospital charges several multiples beyond that of a term infant conceived naturally and provided care in our nursery. Among singletons and twins (per infant) hospital charges were increased 26 times ($P < 0.001$) and in triplets charges were increased 173 times ($P < 0.0001$) when compared to a term infant provided care in a normal nursery at our center.

CONCLUSION: Maternity costs for surrogates exceed those of women who conceive naturally, and these costs are especially magnified in women with triplets and multiple births.

Key words: Surrogacy pregnancy; Assisted reproductive technologies; Prematurity; Multiple gestations

Core tip: Surrogate pregnancies result in higher maternity and newborn costs with increased rates of multiple births and creates a moral hazard for hospitals. This increase occurs despite of the fact that surrogate mothers are prescreened for health and reproductive ability. Reduction in multiple embryo transfer would reduce the adverse economic impact of surrogate pregnancy, maternity and newborn costs.

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INTRODUCTION

In the United States approximately 7.4% of married couples are affected by infertility[1]. The causes of infertility are multiple and range from advanced maternal age, uterine malformation, hysterectomy, fallopian tube blockage, previous tubal ligation, lack of oocyte reserve in women, male factor infertility associated with oligospermia, pervious vasectomy with failed reconstruction, and other causes. In addition to fertility, in our evolving society where non-traditional family models are increasingly accepted, more and more single adults, or adults in same-sex relationships or marriage also desire to become parents and rear a family. In many such situations prospective parents may enter into an agreement to obtain oocytes or sperm, or use the surrogate's own egg and serve as a traditional surrogate for a pregnancy[2]. In other situations, a couple that has genetically related embryos created through *in vitro* fertilization (IVF) requires another women, a gestational carrier, in whom an embryo(s) and fetus(es) may develop. After birth, through a contractual relationship arranged prior to pregnancy, the gestational carrier relinquishes the infant(s) to the intended parents[2].

In many countries and in some United States states, traditional and gestational surrogacy is illegal. In the United States and its territories, a patchwork of laws regarding surrogacy exists[3]. Some United States states, limit the use of surrogacy, or permit surrogate pregnancies or use of gestational carriers only among married couples or the use of gametes from relatives, and in most states surrogacy contracts and their enforcement are determined by case law. Nevertheless, surrogacy is gaining greater societal acceptance in the United States. For instance, in California, one of the most liberal United States states in this respect, the law permits both traditional and gestational surrogacy in exchange for payment, and designates independent legal counsel for the surrogate and the intended parents, and the creation of a contract with judicial review and approval under the Uniform Parentage Act as amended in 2012[4]. However, the recruitment of women as traditional or as gestational surrogate carriers is unregulated in California. Further informed consent with thorough discussion of the risks associated with oocyte retrieval for some embryo transfers used in gestational surrogacy is unregulated in all states except California, and significant gaps have been identified in adherence to state statutes[5]. Despite the growing popularity of surrogacy, the medical complications associated with surrogacy and the related costs have not been precisely quantified to date. While anecdotal evidence suggests that these complications and costs are much higher than in normal pregnancies no peer reviewed data are available for documentation. This is a critical question to explore since such complications have not only financial and social costs, but may raise ethical issues for prospective parents, physicians, and hospitals. These issues need to be quantified and clarified, so that proper information and counseling/guidance can be provided to the potential parents and to women wishing to be surrogates.

In 2012, the Society for Assisted Reproductive Technology reported that among 379 of their member clinics, 165172 cycles or procedures involving *in vitro* fertilization were performed, and that infants conceived using *in vitro* fertilization procedures constituted 1.5% of all births in the United States[6]. However, the number of infants being born using either traditional or gestational surrogacy is not known. For 2009, the Centers for Disease Control and Prevention (CDC) released information regarding 145244 assisted reproductive procedures performed

in the United States. California ranked the highest with 18405 procedures performed, with 7545 infants born from the use of these technologies. Only 52.7% of the infants born were singletons - in contrast to 96.8% of naturally conceived infants[7], and these data did not distinguish between surrogate and other IVF births.

IVF pregnancies are considered high-risk pregnancies due to the increased risk of prematurity, pregnancy related complications, and increased incidence of multiple gestations. These factors may directly relate to the increased medical charges associated with these pregnancies[8]. There are multiple costs specific to surrogacy, many of which are beyond the purview of this report, which focuses on the hospital costs associated with surrogate births. For example, the costs of acquisition of surrogate or gestational carrier women (often through the use of agencies who advertise for eligible women), attorneys who specialize in preparing contracts between prospective parents and the surrogate, and other costs such as specialized social services, psychological counseling for the intended parents and often for the surrogate herself.

We hypothesized that hospital charges for maternity and newborn care would be significantly greater for women serving as surrogates than those delivering after natural conception and that the hospital charges for the infants would also be significantly greater than for infants delivered after natural conception and at term among naturally conceived infants. As a major medical center in Southern California we believe that baseline data from our center may be useful in informing those contemplating surrogacy pregnancies.

MATERIALS AND METHODS

The Institutional Review Board of Loma Linda University evaluated this study and determined that it was exempt from informed consent. Selected maternity diagnosis, mode of delivery, duration of hospitalization, and hospital charges were collected from women who were identified by their obstetrical provider as being a surrogate (traditional or gestational carrier). Infants born of these pregnancies had their birth weights, gestational age, length of hospital stay, and hospital charges tabulated, as well as their stay in either the normal nursery or neonatal intensive care unit between January 1, 2012 and December 31, 2013 tabulated from medical chart review. All hospital charges data were independently tabulated by the Office of Finance based on the surrogate's or infant's medical record number, as well as, the source of payment such as private payment, third party insurer, or charged to a national health insurance scheme for international surrogacy arrangements.

Charges associated with the IVF cycles, artificial insemination, or embryo(s) transfer into the surrogate were not considered in the maternity charges. A ratio contrasting the maternity hospital charges for the surrogate carrier was compared as a ratio to the mean charges for 2540 infants delivered in 2013 after natural conception. 2013 was chosen as the baseline hospital charges for both maternity and newborn care, as the electronic medical system and financial accounting system change occurred in late December 2012. Between 2012 and 2013 there was a 9% increase in hospital charges. Therefore hospital charges for both maternity care for 2012 were adjusted by this increase in hospital charges. Charges for infant care in "normal nursery" or in the Neonatal Intensive Care unit were similarly tabulated and charges for 2012 adjusted to charges in 2013 because of the increase in hospital charges.

Table 1 Characteristics of Surrogate Women prior to surrogate pregnancy: mean, range and SD

Surrogates	Age (yr)	Gravidity	Parity
n = 45	27	2.7	2.3
Range	20-43	1-8	1-7
SD	4.6	3.6	3.3

RESULTS

According to the CDC, in 2011 and 2012 there were 1766 cycles in gestational carriers in the State of California that resulted in the birth of 1067 infants of whom 36% (in 2011) and 39% (in 2012) were born prematurely. Approximately 15% were multiple births (CDC)[9]. Data from traditional surrogacy pregnancies or outcomes are not collected by either the CDC or by the California Department of Health Services.

At our center, 45 women served as surrogates (24 gestational and 21 traditional) from January 1, 2012 until December 31, 2013. These women averaged 27 (range 20-43) years of age with a mean of 2.7 prior pregnancies prior to being a surrogate during the 24 months of our study (range 0-8 previous pregnancies). These women had an average of 2.3 living children (range 1-7) prior to the surrogate pregnancy. These data (and standard deviations) are summarized in Table 1.

According to maternity documents, prenatal care began in the 4.5 wk of embryo transfer or artificial insemination. Among women delivering at our center with embryo transfers (genetically related or not) 55.5% were with multiple embryos. Sperm from the intended father[7], donor semen[3], or mixed sperm from one male couple were impregnated into the 21 traditional surrogates. The cesarean section rate was 52% for surrogate gestations contrasted to 33% among women who conceived naturally. This increased operative mode of delivery may account for the increased average length of hospitalization among women who were surrogates. Table 2 documents the births as singleton or plural births, surrogate length of stay (LOS) for maternity care pre and post birth, and hospital charges as a ratio to women who delivered after natural conception. In the only triplet gestation

Table 2 Maternal characteristics for surrogate pregnancies related to singleton, twin or triplet delivery

Surrogates	Maternity LOS (d)	Ratio	Hospital charges (± SD)	Ratio
Singleton births (n = 20)	4.2 (1.2)	1.3	$31115	1.2
Twin births (22)	3.5 (0.8)	1.1	$29692 ± 11892	1.1
Triplet births	15	4.7	$102673	3.8

Hospital Length of Maternity Stay (LOS) and charges compare surrogate carrier charges related to LOS and maternity charges for naturally conceived term infants requiring normal nursery care (mean ± SD).

there was a significantly longer length of stay and her maternity charges were considerably higher than compared to either singleton, or twin gestations.

Sixty-nine live-born infants resulted from surrogate gestations. Four infants died soon after birth due to extreme prematurity (although the legalized parents refused resuscitation for 24 wk twins). There was one fetal death in a twin pair, and the surviving infant was classified as a singleton, and among a triplet gestation there was fetal reduction of one fetus, and the infants born were classified as twin. Among the 69 infants born, 78% were born prior to 37 completed wk and 17.4% were born less than 30 wk. The mortality rate was 5.7% among infant born using assisted reproduction technologies in contrast to 0.7% of naturally conceived infants and having their initial admission to the normal nursery. Table 3 documents the infant characteristics by birth weight, gestational age, length of hospitalization, and the ratio of charges compared to naturally conceived infants. Compared to naturally conceived singleton or twin infants admitted to the normal nursery with a mean length of stay of 2.1 d, infants delivered of surrogates had a substantially greater length of stay. This longer length of stay was undoubtedly associated with the greater number of infants admitted to the NICU after delivery to a surrogate. Hospital charges were increased 26 times for both singleton and twin deliveries (tabulated per infant) to surrogates, and 173 times for each triplet infant (the sole triplet set that were born alive).

DISCUSSION

Data regarding outcomes of surrogacy pregnancies in California using a gestational carrier and from our center (both gestational and traditional surrogates) reveal a higher rate of prematurity and lower birth weight than among pregnancies resulting from natural conception. The higher cesarean rate may be explained by the higher multiple gestation pregnancies among surrogates and is consistent with the report on the increasing cesarean section rate among twins[10].

Charges for hospital services for these women and the infants delivered provide new information regarding the consumption of medical services by these pregnancies. A discussion of healthcare economics is relevant to the data presented by our experience at a single center. While many healthcare economic discussions center on dwindling reimbursement, the issue is quite different with provision for services to surrogates. Commercial insurance coverage was available for all but one of the women serving as surrogates, and of the 69 infants all but 8 also had commercial insurance with the other women or infants classified as "self pay" resulting in a net profit for our center for maternity care. Newborns were similarly covered except that national health plans in France and Spain would not cover the costs of neonatal intensive care. Combining a well-insured population with a profitable service line such as neonatal intensive care at our center produces a favorable financial outcome for our center. However, in an environment where state-sponsored insurance payments are declining and more people are migrating towards lower-paying insurance exchanges, medical centers are inclined to protect their major sources of margin. This raises the concern of the "moral hazard" of surrogacy. As illustrated surrogate women and the infants delivered have greater rates of cesarean section, premature birth, and low birth weight infants at significantly higher rates than the population of infants born after natural conception. The same is true for IVF/Assisted Insemination pregnancies[8]. Kissin *et al*[11] recently calculated the increased medical costs attributed to Assisted Reproductive Technologies by state. California led with this economic burden for 2013 estimated at $158800418.

A "moral hazard" occurs when the system that helps create the higher risk pregnancy also stands to profit from the additional care that the women and babies are likely to require. The interests of the 3 decision-making parties - intended parents, healthcare system and insurance system - are not aligned. Although gestational surrogacy represents a fraction of all IVF related births, these increased costs and potential profitability are not aligned with value-based health care. The overwhelming desire of prospective parents is to have a normal infant ideally delivered at term. In most cases, these couples, or even single adults will have attempted multiple other means of having a child before settling on the significantly more complicated method of hiring a surrogate. Most families will be paying cash for the surrogate pregnancy ($20-30000 for a surrogate, if an egg donor is required another $5-10000, the fertility clinic and reproductive endocrinologist $15000 per cycle, the surrogacy agency $10-20000) and attorneys fees of about $10000[12]. However, the cost for prenatal care, maternity charges, and expenses associated with neonatal intensive care may exhaust some intended parents resources. While many intended parents may be able to afford the $50000 or so to begin a pregnancy with the assistance of a surrogate, we have encountered many who have been unprepared for the charges associated with the care of

Table 3 Infant characteristics after birth from surrogate pregnancy

Infant(s)	Birth weight	GA	LOS	Hospital charges	Ratio
Singleton (n = 19)	3798.3 ± 832.9	35.9 ± 2.9	11 ± 3	$154874 ± 326415	26.2
Twins (n = 44)	2151.5 ± 750.5	33.8 ± 4.3	12.7 ± 4	$154885 ± 339442	26.2
Triplets (n = 3)	1337.2 ± 91.8	30.0 ± 0	75.0 ± 0	$1025927 ± 99097	173.8

Hospital charges are expressed as a ratio of hospital charges for per infant compared to hospital charges for a term infant provided care in the normal nursery (mean ± SD). GA: Gestational age; LOS: Length of stay.

a complicated newborn born prematurely and requiring several days in a Neonatal Intensive Care unit. Nor are families necessarily prepared for all the implications of a multiple-birth and the associated short- and long-term costs. If a pregnancy has a lower than normal probability of success or more potential complications how extensive should physicians explain these risks? How much do intended parents need or want to know regarding potential complications in the newborns and the added financial costs associated with a premature infant or multiple births? These questions are central to the ethical debate that has surrounded surrogacy. Kissin *et al*[13] has stressed that outcomes of assisted reproductive technologies should properly be assessed on the basis of the number of singleton infants born at term not simply based on live births.

An extension of the "moral hazard" concerns with surrogacy has been the misunderstandings that arise between intended parents and surrogates, and unforeseen events during such a pregnancy. Intended parents-surrogates disputes have arisen when the intended parents demand that the surrogate terminate a pregnancy when a significant fetal malformation is identified, or intended parents change their mind mid-gestation, *e.g.,* by initiating divorce proceedings, or when an intended parent dies. Surrogates may make greater demands on intended parents when multi-fetal gestations occur, or they may wish to engage in behaviors forbidden in their contract, or they may wish to parent the infant themselves. As noted by Andrew W. Vorzimer, a prominent attorney in arranging such contracts in Los Angeles, of 118 surrogacy cases in which a dispute arose 82 were cases in which the intended parents changes their mind and the remainder were by women serving as surrogates (many of whom were traditional surrogates providing her eggs and also carrying the infant) (Andrew W. Yorzimer, J.D., personal communication July 18, 2013).

Margalit[14], an attorney, argues that surrogacy contracts are both desirable and necessary to ensure fairness and enforceability to the benefit of all parties involved. To increase the likelihood that these dual goals of fairness and enforceability are achieved, Margalit[14] further argues that all parties should have independent legal representation from the start of the process as well as thorough, precise, medical guidance as to the risks and probabilities of various outcomes, including catastrophic outcomes. In addition, the paper argues that both sides should receive social and psychological support, and the contract should comprehensively deal with all possible outcomes, including unhealthy newborn(s), premature birth, complications/chronic diseases, and the divorce/death of the intending parents. Finally, every effort should be made to ensure that the disparity in economic strength between the parties to the contract does not interfere with the parties decision to enter into the contract nor "interferes with their free will". Additional legal/ethical risk may arise when prospective parents turn to off-shore surrogacy agencies (primarily in India, Thailand and Mexico) in an effort to cut costs. While these agencies often charge approximately half of what United States agencies do, some are not as reputable and engage in unethical practices and sometimes out-right fraud[15].

Finally, what is the insurance company's piece of this puzzle? By and large, families have borne the expense of the surrogacy, but the infant is now covered under the family's insurance plan even though the parents have voluntarily assumed more than the usual risk. The health insurance industry has thus far been slow to adjust premiums to risk profiles. However, as responsibility for payment continues to shift over to patients through high-deductible plans and cost-sharing, it's reasonable to expect that voluntary assumptions of greater risk will be looked at more critically by the insurance industry and by state health exchanges that must assume even greater risk.

A potential game-changer to the surrogacy moral hazard is an ongoing shift in how hospitals contract with insurers. Historically, they have been paid on a fee-for-service basis where they are paid a percentage of charges or a per diem rate. As their usage increases so does their payment. Medicare saw tremendous opportunity for abuse under their cost-plus reimbursement in the 70s and switched to a DRG-based case rate that also affects Medicaid (MediCal) hospital payment in California. Recently a number of state Medicaid programs followed suit with All Patient Refined DRG-based case rate payments. However, by and large, providers are still financially incentivized to increase rather than decrease the cost of care.

Increasingly health insurance policies are requiring consumers to be more accountable for their healthcare or they are charged larger premiums.

Another aspect of the "moral hazard" of surrogacy is that voluntary risk acceptance could come increasingly under extreme scrutiny. If a medical center stood not to gain, and rather potentially to lose a great

deal in the care of surrogate women and the infants from these pregnancies (as may occur in some cases of international prospective patents counting on reimbursement from their countries national health plan, especially countries that deem surrogacy illegal) how might this impact the market for the care of women surrogates, or their infants? All of these dynamic considerations make it imperative that prospective parents and medical providers have a full understanding of the risks and frequently unforeseen costs associated with surrogacy decisions.

In conclusion, data from California indicate that gestational surrogacy is increasing, and data highlight the substantial increase in multiple births, often born prematurely in California. We document at our single site the extensive requirement for neonatal intensive care and associated increased hospital charges for medical services for both surrogate (both gestational and traditional) and infants from surrogate pregnancies. In a value-based health care system, the "moral hazard" associated with promotion of surrogacy and the higher charges associated with maternity and infant care raises important issues in an area of health care services lacking regulation.

COMMENTS

Background

Surrogate pregnancies result in increased maternity costs in spite of pre-selected for maternal reproductive health primarily associated with an increase in multiple gestations that are associated with increased cesarean section rates, more preterm deliveries, increased neonatal intensive care with added neonatal morbidities.

Research frontiers

Surrogate pregnancies are permitted in several United States states, but the outcomes of these pregnancies have not been rigorously evaluated in terms of maternity or neonatal complications or hospital associated charges.

Innovations and breakthroughs

California has more surrogate pregnancies of any United States states and the impact on health economics is imperative for healthcare value with significantly greater multiples births than occur have natural conception.

Applications

Health economists and insurance providers are focused on health care value. Given the increased charges associated with surrogate pregnancies and the infants born thereof, surrogacy may come under additional scrutiny because of the moral hazard created by these gestations and the impact on health care resources.

Terminology

In this paper surrogacy includes both traditional and gestational surrogacy.

Peer-review

The authors have performed a good study, the manuscript is interesting.

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Ovarian simple cysts in asymptomatic postmenopausal women detected at transvaginal ultrasound: A review of literature

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Abstract

AIM: To answer some questions related to the problem of ovarian simple cysts in asymptomatic postmenopausal women.

METHODS: A literature search and systematic review using MEDLINE (PubMed) database from 1980 to 2014 was performed using the following terms: "simple cyst", "postmenopause", "postmenopausal", "ultrasound", "ovary", "ovarian", "asymptomatic". Papers not related to the topic, reviews, letters to editor, opinion letter, commentaries and studies published in non-English language were excluded. Two authors then reviewed the full paper of all the studies initially selected. This review does not claim to be a meta-analysis. Therefore, meta-analysis statistics were not applied and PRISMA guidelines were not strictly followed. Simple descriptive statistics were used providing absolute numbers and corresponding percentages as well as range.

RESULTS: Nine papers were ultimately included in this review, accounting for 98899 postmenopausal women. We have found that ovarian simple cysts are relatively common in asymptomatic postmenopausal women (prevalence: 8.7%). The risk of malignancy is very low (0.19%). More than 90% of these cysts were smaller than 5 cm. Bilaterality rate ranged from 3.7% to 15%. Histologically, most cysts are serous cystadenomas (61%). When managed conservatively, a significant number resolve spontaneously (46.1%) or remain unchanged (39%).

CONCLUSION: According to these data, conservative management should be the first option to offer to these women.

Key words: Ovary; Simple cyst; Diagnosis; Cancer; Management

Core tip: The problem of ovarian simple cysts in asymptomatic postmenopausal women remains a controversial issue in gynecological practice. We have performed a literature review about this topic. We have found that ovarian simple cysts are relatively common in asymptomatic postmenopausal women. The risk of malignancy is very low. Histologically, most cysts are serous cystadenomas. When managed conservatively, a significant number resolve spontaneously or remain unchanged.

Alcazar JL, Martinez N, Juez L, Caparros M, Salas A, Errasti T. Ovarian simple cysts in asymptomatic postmenopausal women detected at transvaginal ultrasound: A review of literature. *World J Obstet Gynecol* 2015; 4(4): 108-112 Available from: URL: http://www.wjgnet.com/2218-6220/full/v4/i4/108.htm DOI: http://dx.doi.org/10.5317/wjog.v4.i4.108

INTRODUCTION

The management of adnexal simple cysts in asymptomatic postmenopausal women remains a controversial issue in gynecological practice. Albeit management of these lesions has certainly evolved from a systematic surgical removal to a more conservative approach[1], many gynecologist are still reluctant to advise such a conservative management.

Some issues regarding to this entity remains unresolved such as the lesion's size above which surgery should be advised, the risk of torsion, what factors are associated with the appearance of this lesion and for how long follow-up should be performed in case of conservative management and which control periodicity should be advised, what factors are associated to the appearance of this lesion. Most importantly, if the lesion is left *in situ,* which is the actual risk for developing a malignancy?

For this reason we decided to conduct a systematic review on the literature in an attempt to answer the following questions: (1) What should be considered an ovarian simple cyst? (2) What is the prevalence of ovarian simple cysts in asymptomatic postmenopausal women? (3) Which are the characteristics of these lesions? (4) What are the factors associated to the appearance of these lesions? (5) What is the natural history of these lesions if conservative management is chosen? (6) What is the most frequent histology if surgery is performed? (7) What is the risk of developing a malignancy? (8) What are the features associated with malignancy? and (9) What is the role of CA-125?

MATERIALS AND METHODS

A systematic review of the literature in MEDLINE (PubMed) database was performed by one of the authors (JLA) from 1980 to 2014 was performed using the following terms: "simple cyst", "postmenopause", "postmenopausal", "ultrasound", "ovary", "ovarian", "asymptomatic". This author screened titles and abstracts and excluded those papers not related to the topic, reviews, letters to editor, opinion letter, commentaries and studies published in non-English language.

Two authors (JLA, NM) then reviewed the full paper of all the studies initially selected according to the following criteria:

Inclusion criteria: (1) Study design: prospective cohort study; (2) Patients: postmenopausal asymptomatic women.

Exclusion criteria: (1) Sample size < 50 women; (2) Incomplete data about patients characteristics, follow-up, histology and malignancy rate; and (3) Ultrasound performed by transabdominal route. We decided exclude these studies because of the possibility of missing papillary projections using this route is higher and some not truly simple cysts could be as such. Disagreement between reviewers was solved by consensus.

This review does not claim to be a meta-analysis. Therefore, meta-analysis statistics were not applied and PRISMA guidelines[2] were not strictly followed. Simple descriptive statistics were used providing absolute numbers and corresponding percentages as well as range.

Additionally, since this is a review Institutional Review Board was waived.

RESULTS

A total of 79 papers were found after database search. First screen excluded 62 papers for the following reasons: paper not related to the topic (n = 42), review paper (n = 12), letter to editor or commentary paper (n = 4) and non-English language (n = 4).

Full paper was reviewed for 17 studies. Eight papers were excluded for the following reasons: retrospective design (n = 3), ultrasound evaluation performed by transabdominal route (n = 2), incomplete data (n = 3).

Therefore, nine papers were ultimately included in this review[3-11].

What should be considered as an ovarian simple cyst?

The vast majority of studies selected defined as simple cyst the presence of an oval or rounded anechoic thin-walled cyst without any irregularity in the internal wall (Figure 1)[1]. The presence of any wall irregularity or echogenic cyst's content should not be considered as a simple cyst (Figure 2).

What is the prevalence of ovarian simple cysts in asymptomatic postmenopausal women?

The nine studies included accounted for 98899

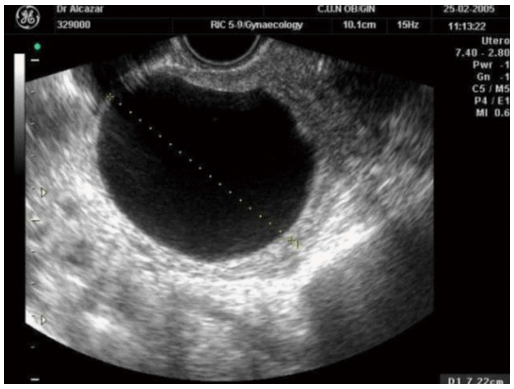

Figure 1 Transvaginal ultrasound showing an ovarian simple cyst as a round anechoic thin-walled cyst with no irregularity on the internal wall.

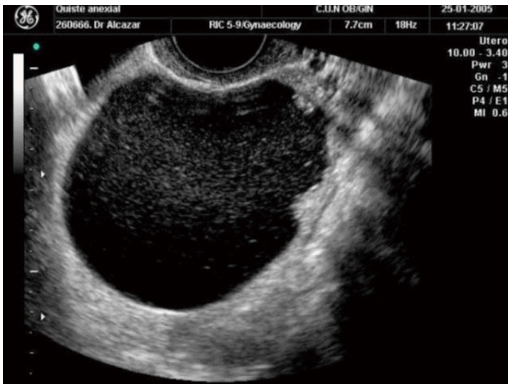

Figure 2 Transvaginal ultrasound showing a lesion that should not be considered as simple cyst, since papillary projection can be seen arising from the wall.

postmenopausal women. Two studies included only women with simple cysts[3,7]. Mean age reported in six studies, ranged from 55.5 years old to 65 years old.

The total number of simple cysts reported was 8600. Therefore, overall prevalence was 8.7% (95%CI: 8.5% to 8.9%). However, prevalence varied among different studies, ranging from 2.5% to 14.1%. Two studies with long term follow-up reported that the annual incidence of simple cysts ranged from 3% to 8%[10,11].

Which are the characteristics of ovarian simple cysts?

Most studies included cysts smaller than 5 cm in size[3-5,10,11]. However, three studies included cysts up to 10 cm[6,8,9] and one even up to 20 cm[7]. According to data derived from these studies more than 90% of these cysts were smaller than 5 cm.

Bilaterality rate was reported in three studies[8-10], ranging from 3.7% to 15%. Moreover, three or more cysts in the same woman has been reported in 5% of the cases[10].

What are the factors associated with the appearance of simple cysts?

Three studies have evaluated factors that could be associated to with the appearance of ovarian simple cysts in postmenopause.

Modesitt *et al*[8] reported that these lesions appeared more frequently in women under hormone replacement therapy (either estrogens alone or estrogens plus progestins) and in women with recent menopause.

Greenlee *et al*[10] performed a more exhaustive analysis in a large population. They found that ovarian simple cysts were associated with age (the younger the woman the higher the risk for simple cyst), non-smoking, previous history of surgery for benign gynecologic condition, previous history of ovarian benign cyst, higher education, precocious menopause and first pregnancy before 20 years old. No association was found with use of hormone replacement therapy, use of hormonal contraception, parity or history of infertility.

Sharma *et al*[11] found that previous hysterectomy, age and weight were significantly associated with simple cysts.

What is the natural history of these lesions if conservatively managed?

Data about the natural history of ovarian simple cysts in asymptomatic postmenopausal women on follow-up has been reported in eight studies[3-6,8-11]. The mean follow-up ranged from 18 to 71 mo.

Ovarian simple cysts resolved spontaneously in almost half of the cases (46.1%). Spontaneous resolution rate ranged from 0% to 69.4%, depending on the study.

Factors associated to resolution were age (the younger the woman the higher the probability of resolution)[6,9], cyst's size (the smaller the size the higher the probability of resolution)[5], the number of cysts (more than 2 cysts was associated to a lower probability of resolution)[10].

Among those cysts that have not resolved, many number did not change in size or aspect (39%; range 23% to 96%[3,4,5,8,9,11]). Among those that changed in appearance most of them developed septations or solid areas[5,8].

What is the most frequent histology in those surgically removed?

Detailed data about histology of surgically removed cysts were reported on six studies[3,5-9]. The total number of cysts removed was 525. Three hundreds and thirty-six were removed after diagnosis and 189 during follow-up. Only on study reported median time elapsed from diagnosis up to removal (27 mo)[9].

Simple cyst and serous cystadenoma were the most frequent histologies (61%, 155/254) followed by cystadenofibroma (8%, 20/254) and para-ovarian cyst (5%, 14/254).

Other reported histologic diagnoses were endometrioma, dermoid cyst, hydrosalpinx and mucinous

cystadenoma.

What is the risk of developing a malignancy?

Overall 16 malignancies among 8600 simple cysts were reported[7,9-11]. This represents a malignancy rate of 0.19%.

Greenlee *et al*[10] reported on 15735 women enrolled in the PLCO (Prostate, Lung, Colorectal and Ovarian Cancer) Screening Trial. Nine women (0.41%) among those 2217 diagnosed as having a simple cyst developed an ovarian cancer. Whereas, 55 out of 12638 (0.44%) without a simple cyst developed an ovarian malignancy. The relative risk for those with ovarian simple cyst was 0.93 (95%CI: 0.46-1.88).

Sharma *et al*[11] reported on 48230 women enrolled in the UKCTOCS (United Kingdom Collaborative Trial of Ovarian Cancer Screening) study. At first scan, 22194 women had normal ovaries visualized, 21943 women had non-visualized one or both ovaries, 1234 women had an inclusion cyst (simple cyst < 10 mm) and 197 had an inclusion cyst and a simple cyst (simple cyst > 10 mm). After a median follow-up of 3 years, and considering only women with both ovaries visualized and those with inclusion and/or simple cyst, three women (0.2%) with only inclusion cyst and one woman (0.5%) with inclusion and simple cyst developed an ovarian malignancy. Twenty nine out of 22194 women in whom both ovaries were visualized and were normal developed an ovarian cancer. The relative risk for women with inclusion cyst was 1.92 (95%CI: 0.62-5.92) and relative risk for women with inclusion cyst and simple cyst was 4.01 (95%CI: 0.69-22.92).

What are the features associated to malignancy?

This is probably the most interesting question. However, the information provided in the studies evaluated for this mini-review is scanty. From data reported from three studies[7,9,11] including eight ovarian cancers, we observed that most of them (6/8) were stage Ⅰ and in 5/8 the size of the cyst was < 5 cm in 5 out of 8 cases.

What is the role of CA-125?

Tumor marker CA-125 was used in all studies but two[7,11]. Some studies established normal CA-125 level (< 35 UI/mL) as an inclusion criteria[4,8-10] and most of them assessed CA-125 during follow-up[3-6,9,10]. However, three studies did not report specific information about CA-125 levels[6,8,10].

Those studies that reported data about CA-125 during follow-up reported no significant changes of this tumor marker during follow-up, even in those cases with sonographic changes in the ovarian simple cyst[3-5]. In the study by Castillo *et al*[9], there was one case of ovarian malignancy. In this case CA-125 raised during follow-up (45 UI/mL).

DISCUSSION

In this systematic review, we have addressed the issue of ovarian simple cysts in asymptomatic postmenopausal women.

We have found that this type of lesions is common in this group of women. This finding is in agreement with data from autopsy studies[12,13]. Another finding in agreement with autopsy studies is that most cysts are small and that the most common histology is simple cyst or serous cystadenoma.

Transvaginal ultrasound route is essential for ascertaining that the lesion is actually a simple cyst, since small papillary projections might be missed using transabdominal ultrasound[14].

Patient's characteristics consistently associated with the presence of a simple ovarian cyst in postmenopausal women are age and previous history of pelvic or gynecologic surgery. This, in fact, is not surprising since younger postmenopausal women may have residual ovarian activity and many of these simple cysts could be "functional cysts" developed from this residual activity. This fact may also explain why the probability of spontaneous resolution is higher in women with recent menopause. On the other hand, previous pelvic surgery increases the risk of peritoneal cysts that may resemble ovarian simple cysts.

No study reported data about complications such as torsion or rupture. Therefore, albeit it is not possible to draw definitive conclusions regarding these complications. However, it seems that they are uncommon in ovarian simple cysts during the menopause.

Additionally no study reported information about when and for how long follow-up should be performed in those cysts that had not resolved spontaneously.

Regarding surgery in most studies there is no data reported about the surgical approach. The authors think that laparoscopic cyst removal should be the best approach when surgery is decided[15].

Probably one of the most important findings in our review is that related to the risk of malignancy of these lesions. Actually, in the two largest studies published so far the relative risk of developing an ovarian malignancy in postmenopausal women with ovarian simple cyst was not increased as compared with those postmenopausal women without ovarian simple cyst.

COMMENTS

Background

Postmenopausal simple cysts in asymptomatic women management remains as an unsolved issue in gynecological practice. This is relevant because of surgery has been traditionally performed. However, recent data challenge this concept showing that conservative management should be offered to these women.

Research frontiers

A number of papers have been published addressing this question in the last fifteen years. However, adequate systematic reviews are lacking.

Innovations and breakthroughs

The paper offers an updated systematic review about this topic. According to the authors' findings in this mini-review, it could be assumed that ovarian simple cysts are common in asymptomatic postmenopausal women. The risk of malignancy is very low and having a simple ovarian cyst does not seem to increase the risk of developing an ovarian cancer. Many of them will resolve spontaneously or will remain unchanged during follow-up. The role of CA-125 at diagnosis and for following-up remains to be determined. Data about the use of CA-125 are scanty in the studies reviewed. Most studies used CA-125 as inclusion criteria and during follow-up. In most cases CA-125 did not change, even in those cysts that changed during follow-up. Only in one case of carcinoma the value of CA-125 was reported as elevated. In the authors' opinion, the role of routine CA-125 assessment in the follow-up of postmenopausal ovarian simple cyst would be arguable. The authors' review has some limitations. First, the authors did not perform a formal meta-analysis. Therefore, conclusions based on statistical inferences cannot be drawn. Second, the number of studies included is small. Third, studies are considerably heterogeneous in design and data reported. However, some questions remain unanswered and future research is needed to try to answer these questions: (1) Which cysts are at higher risk for developing a malignancy? (2) Is follow-up needed for these lesions? (3) For how long these cysts should be followed-up? and (4) Which follow-up protocol should be implemented?

Applications

The results of the analysis may have a relevant clinical impact since the authors have shown that ovarian simple cysts are common in asymptomatic postmenopausal women and the risk of malignancy is very low. These findings should modify the traditional surgical management of these lesions for a more conservative one. However, there is still a room for research since some questions remain unanswered (see above).

Peer-review

This is an interesting manuscript.

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META-ANALYSIS

Use of hyaluronic acid for sperm immobilisation and selection before intracytoplasmic sperm injection: A systematic review and meta-analysis

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Author contributions: Craciunas L designed the review protocol; Craciunas L and Tsampras N performed the literature search and identified studies; Craciunas L and Kollmann M extracted the data from included studies; Stirbu L verified the extracted data; Craciunas L performed analysis in Review Manager; Craciunas L wriote the initial draft; Tsampras N, Kollmann M, Stirbu L and Raine-Fenning NJ contributed with comments and agreed the final version of the paper.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Data sharing statement: Review manager file with statistical code available from the corresponding author at lcraciunas@doctors.org.uk.

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Abstract

AIM: To appraise critically the published randomised controlled trials (RCTs) reporting on the effectiveness of using hyaluronic acid (HA) for sperm immobilisation and selection before intracytoplasmic sperm injection (ICSI).

METHODS: Two authors used the PICO Method in order to perform a comprehensive literature search of the standard medical databases in June 2015. Data from the included studies was extracted independently by two authors using a predefined pro-forma. Review Manager (RevMan) was used to calculate the combined outcomes where multiple studies contributed with their results. Risk ratio (RR) with a 95%CI using the Mantel-Haenszel method was calculated for binary data variables. Heterogeneity was measured using the χ^2 test and quantified using I^2. In case of substantial heterogeneity ($P < 0.10$ for χ^2 test or $I^2 > 50\%$) the combined outcome was calculated using the random effects model. The results from the meta-analysis were displayed as forest plots. The guideline of the Cochrane Collaboration was used to assess the risk of bias and it was illustrated as a risk of bias graph.

RESULTS: The systematic literature search identified 166 different studies related to sperm immobilisation and selection for ICSI. Eleven RCTs involving 13719 oocyte intracytoplasmatic injections with sperm immobilised and selected using HA or polyvinylpyrrolidone (PVP)

were included in this systematic review and meta-analysis. There was low heterogeneity among the included trials (χ^2 = 16.86, df = 11, P = 0.11; I^2 = 35%). There was no statistical difference between HA and PVP groups in terms of fertilisation rate (RR = 1.01; 95%CI: 0.99-1.03; z = 0.75; P = 0.45), good embryos rate (RR = 1.01; 95%CI: 0.96-1.06; z = 0.30; P = 0.76), live birth rate (RR = 1.15; 95%CI: 0.86-1.54; z = 0.92; P = 0.36), clinical pregnancy rate (RR = 1.04; 95%CI: 0.92-1.17; z = 0.62; P = 0.53) and implantation rate (RR = 1.17; 95%CI: 0.94-1.46; z = 0.40; P = 0.16). The quality of most of the included studies was moderate to poor because of unclear randomisation technique, inadequate allocation concealment and blinding.

CONCLUSION: This systematic review and meta-analysis provides evidence of similar efficiency between using HA or PVP for sperm immobilisation and selection before ICSI.

Key words: Hyaluronic acid; Sperm; Intracytoplasmic sperm injection

Core tip: Hyaluronic acid (HA) has been proposed as a physiological alternative to polyvinylpyrrolidone (PVP) for use as a selection medium to reduce sperm motility as a solution for the reported toxicity and unknown long term effects of PVP. We performed a systematic review and meta-analysis of eleven randomised controlled trials involving 13719 oocyte intracytoplasmatic injections with sperm immobilised and selected using HA or PVP. There was no difference between HA and PVP groups in terms of fertilisation, embryo quality, clinical pregnancy, implantation and live birth rates.

Craciunas L, Tsampras N, Kollmann M, Stirbu L, Raine-Fenning NJ. Use of hyaluronic acid for sperm immobilisation and selection before intracytoplasmic sperm injection: A systematic review and meta-analysis. *World J Obstet Gynecol* 2015; 4(4): 113-123 Available from: URL: http://www.wjgnet.com/2218-6220/full/v4/i4/113.htm DOI: http://dx.doi.org/10.5317/wjog.v4.i4.113

INTRODUCTION

The success of intracytoplasmic sperm injection (ICSI), as a bypass of the natural selection processes taking place in the female reproductive tract, would be impossible without advances in the laboratory preparation and identification of sperm for use with ICSI. Several methods (ultramorphology, surface electric charge, apoptotic *vs* nonapoptotic, chromatin structure assay) have been recently proposed for optimising the sperm selection in order to reduce the risk of chromosomal anomalies associated with poor ICSI outcome[1,2].

Hyaluronic acid (HA) is found naturally in the women's reproductive tract and it forms a component of the cumulus-oocyte complex. It has been proposed as a physiological alternative to polyvinylpyrrolidone (PVP) for use as a selection medium to reduce sperm motility as a solution for the reported toxicity and unknown long term effects of PVP[3,4].

Furthermore, it has been shown that sperm's capacity to bind HA is a biochemical marker of maturity and function, suggesting the selection of sperm by HA binding to be an alternative to microscopic assessment of motility and morphology[3].

Several descriptive reviews of the current advanced sperm selection methods support the use of HA for sperm immobilisation and selection for ICSI, but none of them report a quantitative measure of the effect it has on the ICSI outcome[5-8].

The objective of this study is to appraise critically the published randomised controlled trials (RCTs) reporting on the use of HA for sperm immobilisation and selection before ICSI.

MATERIALS AND METHODS

Literature search

We used the PICO Method[9] to formulate a specific and answerable clinical question following which we performed a comprehensive literature search based on a predefined protocol. The medical subject headings (MeSH) "sperm injections, intracytoplasmic", "semen", "hyaluronic acid", "infertility", "fertilization" and "live birth" were combined with free terms "hyaluronan", "sperm", "ICSI", "PICSI", "SpermCatch", "SpermSlow", "polyvinylpyrrolidone", "PVP", "embryo quality", "pregnancy", "implantation", "costs", "adverse events" in order to search Medline/PubMed/PMC, Cochrane Central Register of Controlled Trials (CENTRAL), EBSCOhost, ClinicalTrials.gov and Google Scholar from inception until June 2015. The "Related citations" function and hand search of references were used for all relevant studies in order to identify additional RCTs.

Study selection

We set our inclusion criteria as RCTs evaluating sperm immobilisation and selection using HA before ICSI with no filter for date, country or hospital of origin, publication language, sample size or blinding. For studies presented in more than one publication, we only included the most extensive and recent version in order to avoid overlapping data.

Endpoints

The primary endpoints of the present meta-analysis were defined as: fertilisation rate, embryo quality and live birth rate. Secondary endpoints were: clinical pregnancy and implantation rates, adverse events and costs.

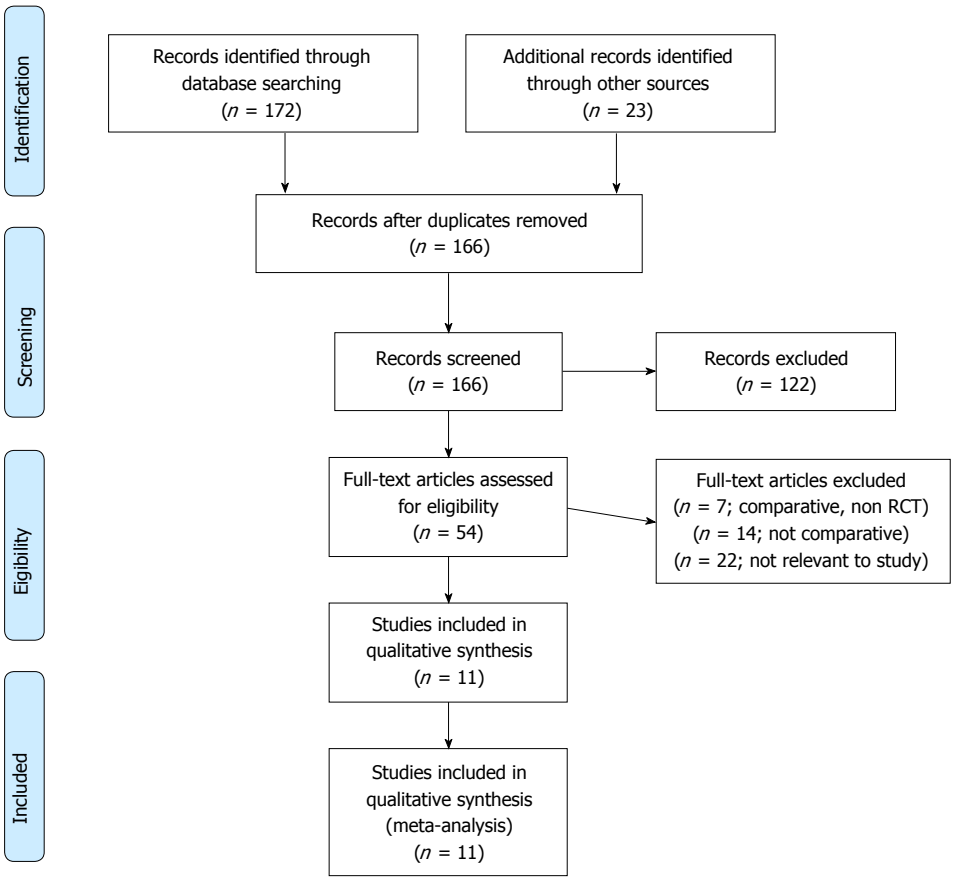


Figure 1 PRISMA flow chart showing trial selection methodology. RCT: Randomised controlled trial.

Data extraction

Two authors extracted the data following the literature search and study selection using predefined tables. Information related to first author, year of publication, country of origin, age of participants, inclusion criteria, the day of embryo transfer, number of embryos transferred, publication type, intervention protocols, number of participants, fertilisation rate, embryo quality, clinical pregnancy rate, implantation rate, live birth rate, adverse events, costs, randomisation technique, allocation concealment, blinding and data reporting was retrieved for each of the included studies. We contacted the study authors in order to obtain more data where it was required.

Statistical analysis

The software package RevMan 5.2.11[10], provided by the Cochrane Collaboration, was used for statistical analysis. We calculated the risk ratio (RR) with a 95%CI using the Mantel-Haenszel method[11] for binary data variables.

We measured the heterogeneity using the χ^2 test and quantified it[12] using I^2. In case of substantial heterogeneity ($P < 0.10$ for χ^2 test or $I^2 > 50\%$) we reported the combined outcome calculated using the random effects model[13]. Forest plots were used for the visual display of the results from the meta-analysis.

The guideline of the Cochrane Collaboration[14] was used to assess the risk of bias and it was illustrated as a risk of bias graph. GradePro (Version 3.2 for Windows) provided by the Cochrane Collaboration[15] was used to generate the summary of the evidence.

We performed subgroup analysis based on publication type (full text *vs* abstract) and type of reporting of the results (per women *vs* per cycle) for each of the variables with summated outcome.

RESULTS

The systemic literature search identified 166 different studies related to sperm immobilisation and selection for ICSI. The PRISMA flow chart to explain the RCTs selection is shown in Figure 1. The summary of the evidence is presented in Figure 2. Eleven RCTs[5,16-25] evaluating 13719 oocyte intracytoplasmatic injections with sperm immobilised and selected using HA or PVP were included in this systematic review and meta-analysis. There were 6926 injections in the HA group and 6793 injections in the PVP group. The characteristics of the included RCTs are shown in Table 1, and the procedure protocols used for the women in all of the RCTs are shown in Table 2. Variables used to achieve a combined outcome are shown in Table 3. One RCT[17] included four arms and we analysed the data as for two studies. Seven RCTs[5,16,18,20,22,23,25] were

Outcome	Illustrative comparative risks[1] (95%CI)		Relative effect (95%CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk Polyvinylpyrrolidone (PVP)	Corresponding risk Hyaluronic acid (HA)				
Fertilisation rate RR Follow-up: mean 8 wk	Study population 740 per 1000 Moderate 745 per 1000	747 per 1000 (733 to 762) 752 per 1000 (738 to 767)	RR 1.01 (0.99 to 1.03)	13719 (12 studies)	⊕⊕⊕⊖ moderate[1]	
Good embryos rate RR Follow-up: mean 8 wk	Study population 466 per 1000 Moderate 367 per 1000	471 per 1000 (447 to 494) 371 per 1000 (352 to 389)	RR 1.01 (0.96 to 1.06)	5834 (8 studies)	⊕⊕⊕⊖ moderate[1]	
Clinical pregnancy rate RR Follow-up: mean 8 wk	Study population 417 per 1000 Moderate 400 per 1000	433 per 1000 (383 to 487) 416 per 1000 (368 to 468)	RR 1.04 (0.92 to 1.17)	1367 (9 studies)	⊕⊕⊕⊖ moderate[1]	
Implantation rate RR Follow-up: mean 8 wk	Study population 150 per 1000 Moderate 164 per 1000	175 per 1000 (141 to 219) 192 per 1000 (154 to 239)	RR 1.17 (0.94 to 1.46)	1637 (4 studies)	⊕⊕⊕⊖ moderate[1]	
Liver birth rate RR Follow-up: mean 37 wk	Study population 253 per 1000 Moderate 263 per 1000	291 per 1000 (218 to 390) 302 per 1000 (226 to 405)	RR 1.15 (0.86 to 1.54)	473 (3 studies)	⊕⊕⊕⊖ moderate[1]	

[1]Selection, performance and detection bias due to inadequate concealment technique, blinding of personnel and outcome assessment. RR: Risk ratio.

Figure 2 Summary and strength of the evidence from trials analysed on GradePro®.

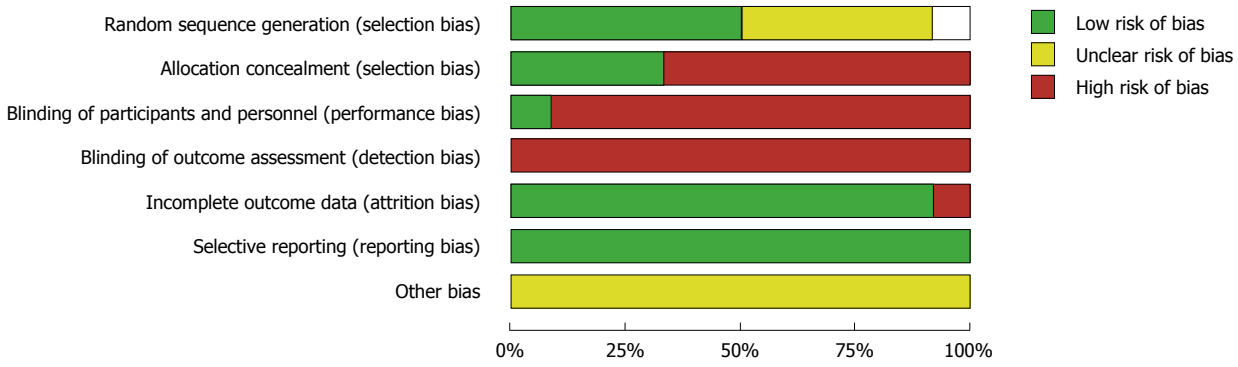


Figure 3 Risk of bias generated using cochrane risk of bias assessment tool.

published as full articles and four RCTs were published as abstracts[17,19,21,24]. Two RCTs[5,22] reported the results per ICSI cycle not per woman randomised. There was complete agreement between authors in terms of included studies and extracted data.

Methodological quality of included studies

Based upon the guidelines suggested by the Cochrane Collaboration, the quality of most of the included studies was moderate to poor because of unclear randomisation technique, inadequate allocation concealment and blinding (Figure 3).

The combined outcome of all of the variables is given below.

Fertilisation rate per oocyte injected

All the included RCTs reported on this outcome with low heterogeneity [χ^2 = 16.86, df = 11 (P = 0.11); I^2 = 35%] among them. The fertilisation rate was similar (RR = 1.01; 95%CI: 0.99-1.03; z = 0.75; P = 0.45; Figure 4A) in the HA group compared to the PVP group. The result did not change when we excluded the two RCTs[5,22] reporting the outcomes per ICSI cycle (P = 0.47) or when we used the random effects

Table 1 Characteristics of included trials

Trial	Country	Mean age	Inclusion criteria	Day of embryo transfer	Number of embryos transferred	Publication type
Balaban *et al*[16]						
HA	Sweden	NA	Male factor	Day 2-3	3.1	Full text
PVP					3.1	
Barak *et al*[5]						
HA	Israel	31.0	Male factor	Day 2-3	3.8	Full text
PVP		30.9			3.8	
Castillo-Baso *et al*[17]						
HA	Mexico	35.1	NA	Day 2-3 or day 5-6	NA	Abstract
PVP		35.3				
Choe *et al*[18]						
HA	Korea	35.4	Previous low fertilization rate Multiple IVF failures	Day 3 or day 5	NA	Full text
PVP						
Gandhi *et al*[19]						
HA	Spain	NA	NA	NA	NA	Abstract
PVP						
Majumdar *et al*[20]						
HA	India	31.7	Unexplained infertility, normal semen analysis	Day 2-3	2.49	Full text
PVP		31.5			2.39	
Moon *et al*[21]						
HA	Korea	NA	NA	Day 3 or day 5	NA	Abstract
PVP						
Parmegiani *et al*[22]						
HA	Italy	37.5	Sperm number > 10^6 and sperm motility > 5%	Day 2-3	2.25	Full text
PVP		37.1			2.15	
Van Den Bergh *et al*[23]						
HA	Switzerland	NA	Women younger than 38 yr with at least four metaphase Ⅱ oocytes	Day 2-3	NA	Full text
PVP						
Worrilow *et al*[24]						
HA	United States	NA	NA	NA	NA	Abstract
PVP						
Worrilow *et al*[25]						
HA	United States	33.3	Women younger than 40 years with at least four metaphase Ⅱ oocytes; Men with sperm count > 10000/mL	Day 2-3 or day 5-6	NA	Full text
PVP		33.7				

HA: Hyaluronic acid; PVP: Polyvinylpyrrolidone; NA: Not available.

model for calculation (P = 0.83).

Good embryos rate per oocyte injected

Eight RCTs reported on this outcome with low heterogeneity [χ^2 = 12.08, df = 7 (P = 0.10); I^2 = 42%] among them. There were 2714 good quality embryos obtained from 5835 oocytes injected. No statistically significant difference was found between the HA and PVP groups (RR = 1.01; 95%CI: 0.96-1.06; z = 0.30; P = 0.76; Figure 4B). Similar results were obtained when we excluded the RCT[22] reporting the outcomes per ICSI cycle (P = 0.56) or when we used the random effects model for calculation (P = 0.59).

Live birth rate per cycle

Three RCTs reported on this outcome with no heterogeneity [χ^2 = 0.67, df = 2, (P = 0.73); I^2 = 0%] among them. The live birth rate was similar in the HA group compared to the PVP group (RR = 1.15; 95%CI: 0.86-1.54; z = 0.92; P = 0.36; Figure 4C). Same results were obtained after excluding the RCT[22] reporting the outcomes per ICSI cycle (P = 0.68) or when we calculated using the random effects model (P = 0.41).

Clinical pregnancy rate per transfer

Nine RCTs reported on this outcome with no heterogeneity [χ^2 = 5.50, df = 8, (P = 0.70); I^2 = 0%] among them. The clinical pregnancy rate was similar between HA group and PVP group (RR = 1.04; 95%CI: 0.92-1.17; z = 0.62; P = 0.53; Figure 5A). No difference was found by excluding the two RCTs[5,22] reporting the outcomes per ICSI cycle (P = 0.98) or by calculating the combined outcome using the random effects model (P = 0.68).

Implantation rate per embryo transferred

Four RCTs reported on this outcome with no heterogeneity [χ^2 = 1.12, df = 3, (P = 0.77); I^2 = 0%] among them. Similar implantation rates were obtained in the HA and PVP groups (RR = 1.17; 95%CI: 0.94-1.46; z = 0.40; P = 0.16; Figure 5B). Excluding the two RCTs[5,22] reporting the outcomes per ICSI cycle (P = 0.67) or using the random effects model for calculation (P = 0.17) did not change the result.

Table 2 Treatment protocol adopted in included trials

Ref.	HA group	PVP group
Balaban *et al*[16]	Oocytes were injected with sperm exposed to, SpermCatch (NidaCon International, Gothenburg, Sweden), a viscous liquid containing hyaluronate and human serum albumin	Oocytes were injected with sperm exposed to the PVP-containing product
Barak *et al*[5]	Sperm suspension which contained motile spermatozoa was introduced into the center of viscous suspension of hyaluronic acid. Motility of the sperm cells was slowed down. After breaking the sperm tail with the injection pipette, the spermatozoa were injected into the oocytes	MII oocytes were injected with sperm cells which were immobilized and aspirated for injection in 10% PVP solution
Castillo-Baso *et al*[17]	Sperm selected by PICSI (Mid Atlantic Diag. Inc.)	Sperm selected by conventional ICSI
Gandhi *et al*[19]	2 μL droplet with suspension of spermatozoa was placed to a 5 μL droplet of HA-containing medium (SpermSlow; MediCult, Jyllinge, Denmark) and incubated for 15 min at 37 ℃ under oil. Spermatozoa bound to HA in the junction of the two droplets were identified and carefully detached by injecting pipette (ICSI Micropipette; TPC, Thebarton, Adelaide, South Australia) and subsequently injected into a MII oocyte	Before injection, 3 μL of sperm suspension was transferred to 7 μL of 7% polyvinylpyrrolidone (PVP; SAGE) solution to remove debris and get better control. Spermatozoa with best morphology were selected for injection into a MII oocytes using inverted microscope equipped with micromanipulators
Gandhi *et al*[19]	Donated oocytes to avoid female infertility as a bias factor, randomly carried out with SpermSlow for sperm selection	Donated oocytes to avoid female infertility as a bias factor randomly carried out with PVP for sperm selection
Majumdar *et al*[20]	Sterile PICSI dishes (Origio MidAtlantic Devices, United States) with three hyaluronan microdots attached to the interior bottom, were used. 10 μL droplets of culture medium (GMOPS, Vitrolife) were placed over the hyaluronan microdots and an elongated 10 μL drop of PVP was made below the drops, before covering the dish with oil. 1-2 μL of sperm suspension was then added to the hyaluronan microdot containing droplets. After 5 min of incubation at 37 ℃, HA bound sperm with normal morphology were removed with an injecting micropipette (TPC, Australia) to the adjacent PVP droplet, immobilized and subsequently injected	An elongated 10 μL poly vinyl pyrolidone drop (PVP, Medicult, Denmark) under oil, was used to select spermatozoa with normal morphology for subsequent injection
Moon *et al*[21]	ICSI were performed with husband spermatozoa which immobilized in 2.5 mg/mL hyaluronic acid	ICSI were performed with husband spermatozoa which immobilized in 5% PVP
Parmegiani *et al*[22]	Spermatozoa were selected for their ability to bind to HA: A 2-mL droplet with suspension of spermatozoa was connected with a pipette tip to a 5-mL droplet of HA-containing medium (SpermSlow; MediCult) and allowed to incubate for 15 min at 37 ℃ under oil (Liquid Paraffin; MediCult). Spermatozoa bound to HA in the junction zone of the two droplets were selected and easily detached by injecting pipette (ICSI Micropipette; Humagen Fertility Diagnostics) and subsequently injected into oocytes	Conventional PVP-ICSI procedure
Worrilow *et al*[24]	At the time of injection, drops were prepared in the lid of a Falcon Petri dish (353004; Becton Dickinson, Franklin Lakes, United States). In the middle of the dish a 10 μL drop of Spermslow (Medicult) and a 10 μL Flushing Medium drop (Medicult) were connected by a 3-4 mm junction bridge of medium and consecutively encircled by five 10 μL drops of Flushing medium. This setup was covered with liquid paraffin (Medicult). A 2 μL volume of prepared semen was added to the medium part of the Spermslow/Flushing medium central mixture. The spermatozoa were allowed to migrate towards the junction for a period of 15-20 min at 37 ℃. Spermatozoa were carefully selected near the junction between the sperm droplet and the SpermSlow droplet	Non-bound, forward-moving spermatozoa were taken from the SpermSlow droplet
Worrilow *et al*[24]	PICSI embryos created using Hyaluron Bond-sperm	Standard sperm selection criteria
Worrilow *et al*[25]	The final sperm suspension of HYAL patients was placed upon microdots of hyaluronan in the PICSI Sperm Selection Device (Biocoat, Inc., Horsham, PA) and overlaid with oil. Following a 5-10 min incubation period, HB sperm were selected following the manufacturer's instructions	The final sperm suspension of patients in the control group was placed into standard ICSI dishes for selection

PVP: Polyvinylpyrrolidone; ICSI: Intracytoplasmic sperm injection; HA: Hyaluronic acid.

Adverse events and costs

None of the studies reported on these outcomes.

DISCUSSION

Main findings

This systematic review and meta-analysis based on eleven moderate to low quality RCTs provides evidence of similar efficiency between using HA or PVP for sperm immobilisation and selection before ICSI in terms of fertilisation, embryo quality, clinical pregnancy, implantation and live birth rates. None of the studies reported on costs hence we could not perform a cost-effectiveness analysis.

Strengths and limitations

By performing a comprehensive literature search of the standard medical databases and grey literature

Table 3 Variables used for systematic review and meta-analysis *n* (%)

Trial	Transfers (*n*)	Fertilisation rate	Good embryos	Clinical pregnancy	Implantation rate	Live birth
Balaban *et al*[16]	Women					
HA	48	360 (72.14)	226 (50.33)	20 (41.66)	27 (18.12)	19 (39.58)
PVP	44	337 (75.05)	211 (46.99)	19 (43.18)	27 (19.14)	18 (40.90)
Barak *et al*[5]	Cycles					
HA	58	525 (72.61)	NA	29 (50.00)	41 (18.55)	NA
PVP	65	484 (74.57)		25 (38.46)	35 (14.00)	
Castillo-Baso *et al*[17]	Women					
HA	30	143 (49.14)	87 (29.89)	16 (53.33)	-34	NA
PVP	30	134 (50.95)	84 (31.93)	12 (40.00)	-24	
Castillo-Baso *et al*[17]	Women					
HA	30	140 (56.91)	105 (42.68)	14 (46.66)	-25	NA
PVP	30	163 (61.97)	99 (37.64)	13 (43.33)	-22	
Choe *et al*[18]						
HA	18 women	81 (75.70)	13 (12.14)	NA	NA	NA
PVP		93 (83.03)	15 (13.39)			
Gandhi *et al*[19]	Women					
HA	77	909 (82.33)	675 (61.14)	41 (53.24)	NA	NA
PVP	77	923 (82.48)	698 (62.37)	47 (61.03)		
Majumdar *et al*[20]	Women					
HA	71	353 (64.65)	154 (43.62)	25 (35.21)	39 (22.03)	22 (30.98)
PVP	80	371 (65.66)	170 (45.82)	28 (35.00)	36 (18.84)	21 (26.25)
Moon *et al*[21]						
HA	1 woman	18 (81.81)	11 (50.99)	NA	NA	NA
PVP		22 (78.57)	10 (35.71)			
Parmegiani *et al*[22]	Cycles					
HA	125	304 (91.56)	101 (30.42)	31 (24.80)	35 (12.41)	29 (23.20)
PVP	105	236 (85.81)	55 (20.00)	22 (20.95)	23 (10.17)	19 (18.09)
Van Den Bergh *et al*[23]						
HA	44 women	154 (75.49)	NA	NA	NA	NA
PVP		142 (69.95)				
Worrilow *et al*[24]	Women					
HA	7	77 (61.11)	NA	4 (57.14)	NA	NA
PVP	8	98 (66.66)		2 (25.00)		
Worrilow *et al*[24]	Women					
HA	237	2105 (77.21)	NA	112 (47.25)	NA	NA
PVP	245	2024 (74.41)		117 (47.75)		
Total						
HA		5169 (74.63)	1372 (46.44)	292 (42.75)	142 (17.12)	70 (28.68)
PVP		5027 (74.00)	1342 (46.59)	285 (41.66)	121 (14.97)	58 (25.32)

HA: Hyaluronic acid; PVP: Polyvinylpyrrolidone; NA: Not available.

with no filters for date, country or hospital of origin, publication language, sample size or blinding we were able to identify eleven RCTs including conference abstracts in order to calculate the combined outcomes. Where the reported data was insufficient we contacted the study authors to gain extended reports.

The heterogeneity was low among the included RCTs and lead to consistent results by using both fixed effect and random effects models for calculations.

Our study is limited by the moderate to poor methodological quality of the included studies because of unclear randomisation technique, inadequate allocation concealment and blinding.

Some might argue in relation to the inclusion of the two RCTs reporting the results per ICSI cycle and not per women randomised, but we performed calculations excluding them for each of the primary and secondary outcomes, without identifying any significant difference.

Comparison with other studies and further research

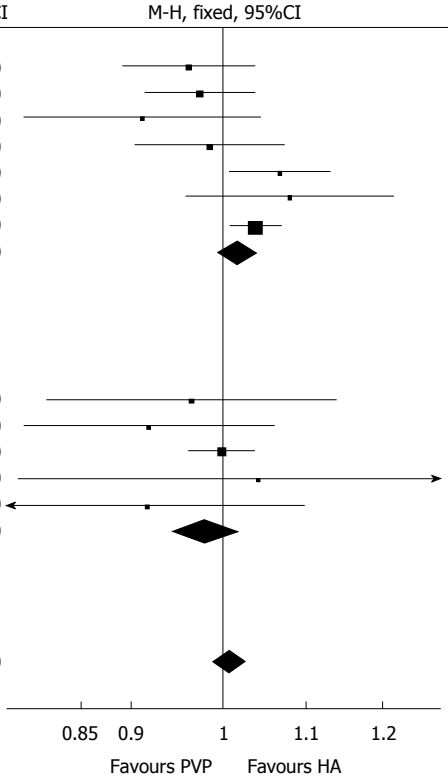
To our knowledge this is the first meta-analysis comparing HA with PVP for sperm immobilisation and selection before ICSI. By observing the forest plot for each end point one can easily notice the similarities between the RCTs as most of them cross the vertical line meaning there is no statistical difference between the groups.

Future trials should be conducted according to the CONSORT guidelines. Due to the nature of the intervention, it would be difficult to achieve blinding of the embryologist performing the sperm selection, but the risk of bias could be reduced by blinding the outcome assessors and the personnel performing the embryo transfers.

It is important to report on the possible adverse events related to less physiological immobilisation and selection of the sperm and to set live birth rate as a primary outcome for the comparisons.

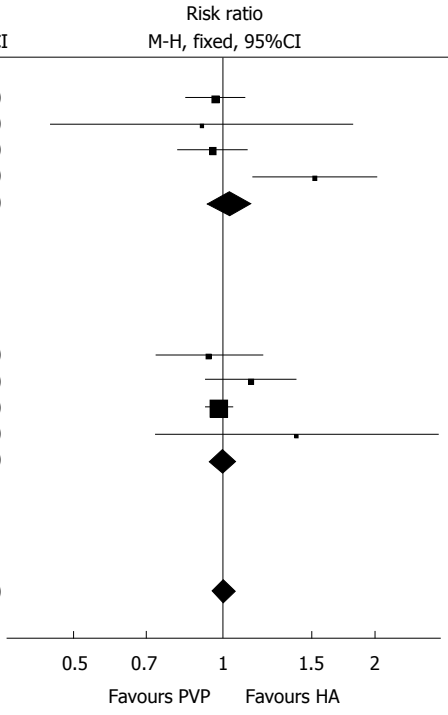
A

Study or subgroup	HA Events	HA Total	PVP Events	PVP Total	Weight	Risk ratio M-H, fixed, 95%CI
1.1.1 Full texts						
Balaban 2003	360	499	337	449	7.0%	0.96 (0.89, 1.04)
Barak 2001	525	732	484	649	10.1%	0.97 (0.91, 1.04)
Choe 2012	81	107	93	112	1.8%	0.91 (0.80, 1.04)
Majumdar 2013	353	546	371	565	7.2%	0.98 (0.90, 1.07)
Parmegiani 2010	304	332	236	275	5.1%	1.07 (1.01, 1.13)
Van Den Bergh 2009	154	204	142	203	2.8%	1.08 (0.96, 1.22)
Worrilow 2013	2105	2726	2024	2720	39.9%	1.04 (1.01, 1.07)
Subtotal (95%CI)		5137		4973	73.9%	1.02 (0.99, 1.04)
Total events	3882		3687			
Heterogeneity: Chi^2 = 12.23, df = 6 (P = 0.06); I^2 = 51%						
Test for overall effect: z = 1.48 (P = 0.14)						
1.1.2 Abstracts						
Castillo-Baso 2011 a	143	291	134	263	2.8%	0.96 (0.82, 1.14)
Castillo-Baso 2011 b	140	246	163	263	3.1%	0.92 (0.79, 1.06)
Gallego 2013	909	1104	923	1119	18.1%	1.00 (0.96, 1.04)
Moon 2000	18	22	22	28	0.4%	1.04 (0.79, 1.37)
Worrilow 2006	77	126	98	147	1.8%	0.92 (0.77, 1.10)
Subtotal (95%CI)		1789		1820	26.1%	0.98 (0.94, 1.02)
Total events	1287		1340			
Heterogeneity: Chi^2 = 2.40, df = 4 (P = 0.66); I^2 = 0%						
Test for overall effect: z = 1.03 (P = 0.30)						
Total (95%CI)		6926		6793	100.0%	1.01 (0.99, 1.03)
Total events	5169		5027			
Heterogeneity: Chi^2 = 16.86, df = 11 (P = 0.11); I^2 = 35%						
Test for overall effect: z = 0.75 (P = 0.45)						
Test for subgroup differences: Chi^2 = 2.67, df = 1 (P = 0.10); I^2 = 62.6%						



B

Study or subgroup	HA Events	HA Total	PVP Events	PVP Total	Weight	Risk ratio M-H, fixed, 95%CI
1.2.1 Full texts						
Balaban 2003	226	499	211	449	16.5%	0.96 (0.84, 1.11)
Choe 2012	13	107	15	112	1.1%	0.91 (0.45, 1.82)
Majumdar 2013	154	353	170	371	12.3%	0.95 (0.81, 1.12)
Parmegiani 2010	101	332	55	275	4.5%	1.52 (1.14, 2.03)
Subtotal (95%CI)		1291		1207	34.3%	1.03 (0.93, 1.14)
Total events	494		451			
Heterogeneity: Chi^2 = 9.02, df = 3 (P = 0.03); I^2 = 67%						
Test for overall effect: z = 0.59 (P = 0.55)						
1.2.2 Abstracts						
Castillo-Baso 2011 a	87	291	84	263	6.5%	0.94 (0.73, 1.20)
Castillo-Baso 2011 b	105	246	99	263	7.1%	1.13 (0.92, 1.40)
Gallego 2013	675	1104	698	1119	51.4%	0.98 (0.92, 1.05)
Moon 2000	11	22	10	28	0.7%	1.40 (0.73, 2.68)
Subtotal (95%CI)		1663		1673	65.7%	1.00 (0.94, 1.06)
Total events	878		891			
Heterogeneity: Chi^2 = 2.96, df = 3 (P = 0.40); I^2 = 0%						
Test for overall effect: z = 0.11 (P = 0.91)						
Total (95%CI)		2954		2880	100.0%	1.01 (0.96, 1.06)
Total events	1372		1342			
Heterogeneity: Chi^2 = 12.08, df = 7 (P = 0.10); I^2 = 42%						
Test for overall effect: z = 0.30 (P = 0.76)						
Test for subgroup differences: Chi^2 = 0.31, df = 1 (P = 0.57); I^2 = 0%						



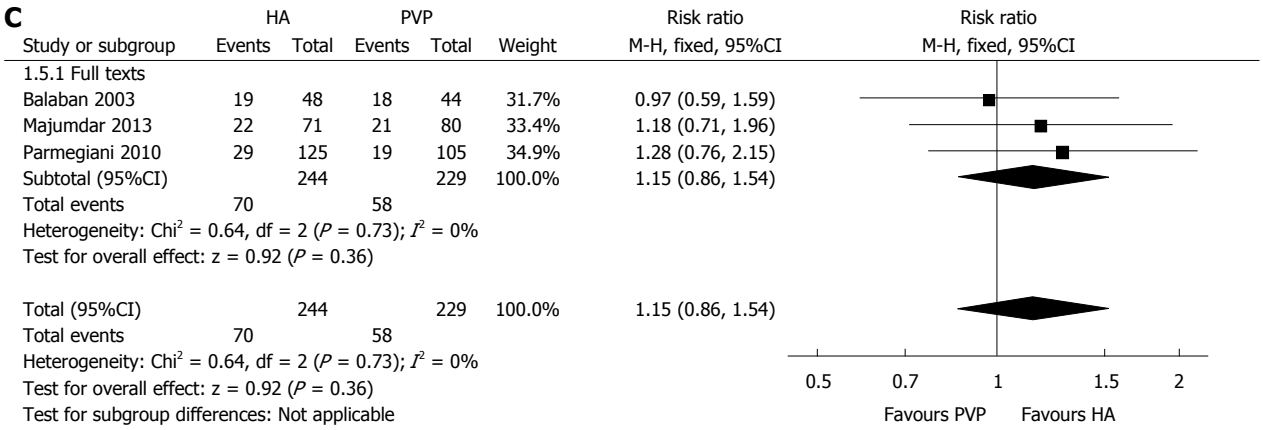


Figure 4 Primary endpoints. A: The forest plot showing fertilisation rate per oocyte injected. Risk ratio is shown with 95%CI; B: The forest plot showing good embryos rate per oocyte injected, risk ratio is shown with 95%CI; C: The forest plot showing live birth rate per cycle. Risk ratio is shown with 95%CI. HA: Hyaluronic acid; PVP: Polyvinylpyrrolidone.

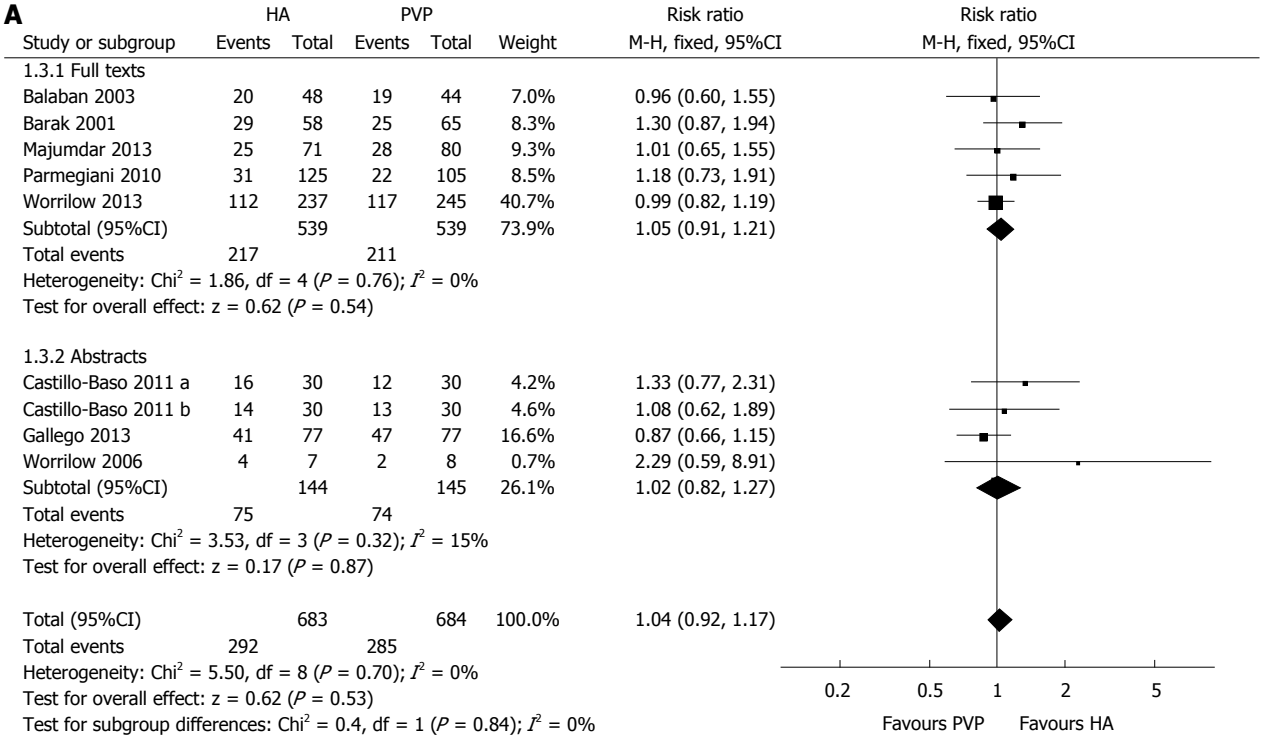


Figure 5 Secondary endpoints. A: The forest plot showing clinical pregnancy rate per transfer. Risk ratio is shown with 95%CI; B: The forest plot showing implantation rate per embryo transferred. Risk ratio is shown with 95%CI. HA: Hyaluronic acid; PVP: Polyvinylpyrrolidone.

Sub-group analysis considering the quality of the sperm, previous failed ICSI cycles, infertility cause, number and quality of transferred embryos should be considered in order to eliminate confounding factors.

Until then, this review may provide reassurance of non inferiority of one method over another for embryologists and laboratory staff involved in the acquisition of laboratory materials.

COMMENTS

Background

The majority of patients undergoing intracytoplasmic sperm injection (ICSI) treatment will reach the stage of embryo transfer due to important improvements of ovarian stimulation protocols and laboratory technology, but only a small proportion of transferred embryos implant leading to an overall success rate of 10%-40%.

Research frontiers

Several methods (ultramorphology, surface electric charge, apoptotic *vs* nonapoptotic, chromatin structure assay) have been recently proposed for optimising the sperm selection in order to reduce the risk of chromosomal anomalies associated with poor ICSI outcome.

Innovations and breakthroughs

Hyaluronic acid has been proposed as a physiological alternative to polyvinylpyrrolidone (PVP) for use as a selection medium to reduce sperm motility as a solution for the reported toxicity and unknown long term effects of PVP. Several studies investigated this method, but this is the first meta-analysis to assess the effect of using hyaluronic acid compared to PVP.

Applications

The results of this meta-analysis combining the outcomes from 11 randomised controlled trials concluded that there is no difference between hyaluronic acid and PVP for sperm immobilisation and selection before ICSI in terms of fertilisation, embryo quality, clinical pregnancy, implantation and live birth rates.

Terminology

Sperm immobilisation and selection is an important step in the ICSI process and refers to the use of a medium in the laboratory for reducing the speed of sperm in order to allow its manipulation in the ICSI process.

Peer-review

The peer-reviewers appreciated the completeness of this meta-analysis. The manuscript was assessed as being well prepared, interesting, clear and well defined.

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