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Original Article

Early pregnancy

Development of a single-visit protocol for the management of pregnancy of unknown location following *in vitro* fertilization: a retrospective study

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ABSTRACT

STUDY QUESTION: Can women with pregnancy of unknown location (PUL) following *in vitro* fertilization (IVF) be risk-stratified regarding the subsequent need for medical intervention, based on their demographic characteristics and the results of serum biochemistry at the initial visit?

SUMMARY ANSWER: The ratio of serum hCG to number of days from conception (hCG/C) or the initial serum hCG level at ≥5 weeks' gestation could be used to estimate the risk of women presenting with PUL following IVF and needing medical intervention during their follow-up.

WHAT IS KNOWN ALREADY: In women with uncertain conception dates presenting with PUL, a single serum hCG measurement cannot be used to predict the final pregnancy outcomes, thus, serial levels are mandatory to establish a correct diagnosis. Serum progesterone levels can help to risk-stratify women at their initial visit but are not accurate in those taking progesterone supplementation, such as women pregnant following IVF.

STUDY DESIGN, SIZE, DURATION: This was a retrospective study carried out at two specialist early pregnancy assessment units between May 2008 and January 2021. A total of 224 women met the criteria for inclusion, but 14 women did not complete the follow-up and were excluded from the study.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We selected women who had an IVF pregnancy and presented with PUL at ≥5 weeks' gestation.

MAIN RESULTS AND THE ROLE OF CHANCE: A total of 30/210 (14.0%, 95% CI 9.9–19.8) women initially diagnosed with PUL required surgical intervention. The hCG/C was significantly higher in the group of women requiring an intervention compared to those who did not ($P = 0.003$), with an odds ratio of 3.65 (95% CI 1.49–8.89, $P = 0.004$). A hCG/C <4.0 was associated with a 1.9% risk of intervention, which accounted for 25.7% of the study population. A similar result was obtained by substituting hCG/C <4.0 with an initial hCG level <100 IU/l, which was associated with 2.0% risk of intervention, and accounted for 23.8% of the study population ($P > 0.05$)

LIMITATIONS, REASONS FOR CAUTION: A limitation of our study is that it is retrospective in nature, and as such, we were reliant on existing data.

WIDER IMPLICATIONS OF THE FINDINGS: A previous study in women with PUL after spontaneous conception found that a 2% intervention rate was considered low enough to eliminate the need for close follow-up and serial blood tests. Using the same 2% cut-off, a quarter of women with PUL after IVF could also avoid attending for further visits and investigations.

STUDY FUNDING/COMPETING INTEREST(S): No external funding was required for this study. No conflicts of interest are required to be declared.

TRIAL REGISTRATION NUMBER: N/A.

Keywords: pregnancy complications / spontaneous abortion / threatened abortion / ectopic pregnancy / tubal pregnancy

Introduction

Pregnancy of unknown location (PUL) is the term used when it is not possible to visualize a pregnancy on transvaginal ultrasound scan (TVUS) in a clinically stable woman with a positive pregnancy test (Banerjee *et al.*, 1999). This concept was developed in recognition of the high diagnostic accuracy of TVUS, which enables a skilled operator to detect any pregnancy large enough to potentially cause significant complications.

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The key principles underpinning the management of women with PUL are that no medical intervention should be initiated without a clear diagnosis, and that expectant management should be considered regardless the location of pregnancy (Hahlin et al., 1995). The aims of PUL protocols are to provide effective and safe care to women presenting with suspected early pregnancy complications by avoiding unnecessary medical interventions and by minimizing the number of follow-up visits (Day et al., 2009; Nadim et al., 2020).

In clinical practice, however, the term PUL is often used synonymously with the term suspected ectopic pregnancy (Zee et al., 2014; Fistouris et al., 2022). Management algorithms which adopted this concept are based on the fact that 17–26% of clinically significant ectopic pregnancies cannot be detected on the initial ultrasound scan in dedicated early pregnancy units (Kirk et al., 2007; Dooley et al., 2019). They tend to rely on changes in serum human chorionic gonadotropin (hCG) to define the location of a pregnancy, which is the main outcome of interest (Bobdiwala et al., 2019; Christodoulou et al., 2021). Lastly, some advocate treatment based on the results of biochemical tests without seeking ultrasound confirmation of the pregnancy site (Barnhart et al., 2011a).

A large prospective UK study reported that the average rates of PUL in women attending early pregnancy assessment units (EPAUs) was 11.3% (Memtsa et al., 2020), but the rates vary widely and can be as high as 42% when TVUS are performed by residents on-call (Mol et al., 1998; Bottomley et al., 2009; Barnhart et al., 2011b).

Repeated follow-up visits, TVUS and blood tests create a significant workload and costs for providers of early pregnancy care and efforts have been made to develop clinical algorithms which may help to reduce this burden, without compromising patient safety (Cordina et al., 2011; Bobdiwala et al., 2020).

In women with uncertain conception dates, a single hCG measurement cannot differentiate between normally and abnormally developing pregnancies and most protocols stipulate that another measurement is taken, usually 2 days later, to assess the changes in the hormone levels (Fistouris et al., 2016; National Institute for Health and Care Excellence (NICE), 2019).

Women who become pregnant following *in vitro* fertilization (IVF), however, are a very different population from those who conceive spontaneously. Their date of conception is known and all normally sited healthy pregnancies should be visible on ultrasound scan at ≥5 week's gestation, unless the uterus is abnormally large due to distortion by fibroids or adenomyosis. In view of that, it could be deducted that it is extremely unlikely that the pregnancy could be healthy if not seen on TVUS more than 3 weeks after the date of conception. In addition, they are routinely given progesterone supplementation and may have multiple corpora lutea, which limits the value of progesterone measurements for the assessment of early pregnancy behaviour (Banerjee et al., 1999; Bobdiwala et al., 2020).

We hypothesized that with a known conception date, a single serum hCG measurement at presentation may be enough to assess the pace of pregnancy growth and the potential risk to women's health with an abnormally developing pregnancy. In addition, a ratio of the hCG level to the number of days since conception should provide information of similar quality as serial hCG measurements in women with spontaneous pregnancies.

The aim of this study was to explore the potential of using the hCG/days from conception ratio (hCG/C) at the initial visit to identify women with PUL following assisted conception who are at low risk of needing medical intervention and may not require additional follow-up visits.

Materials and methods

This was a retrospective study which was carried out at a two specialist EPAUs, at University College Hospital (UCH) and Kings College Hospital (KCH), London. We searched our databases to identify all consecutive women presenting with PUL between May 2008 and January 2021. We included only women who had an IVF pregnancy, who presented with PUL at ≥5 weeks' gestation and who had completed follow-up.

The majority of ultrasound examinations were carried out by clinical fellows, who are Level 2 ultrasound operators, under supervision of consultant gynaecologists, who were expert, Level 3 ultrasound operators (European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB), 2006).

Women attended with symptoms of pain and/or vaginal bleeding. In addition, a reassurance TVUS were offered to asymptomatic women with history of ectopic pregnancy or miscarriage.

In all cases one of the following diagnoses were made, after the initial TVUS examination: (i) live, normally sited (eutopic) pregnancy: defined as a gestational sac located within the uterine cavity, which contained a live embryo; (ii) normally sited (eutopic) pregnancy of uncertain viability: defined as an empty gestational sac, measuring <25 mm, or an embryo with a crown-rump length (CRL) of <7 mm, without visible cardiac activity; (iii) early embryonic demise: defined as an empty, normally sited gestational sac measuring ≥25 mm or an embryo with a CRL of ≥7 mm with no cardiac activity; (iv) incomplete miscarriage, defined as the presence of trophoblastic tissue within the uterine cavity, with no visible gestational sac; (v) ectopic pregnancy: defined as a pregnancy seen to be implanted partially or completely outside the uterine cavity ('Probable ruptured ectopic pregnancy' was defined when a patient in severe pain had an TVUS with no visible pregnancy but evidence of significant haemoperitoneum); and (vi) PUL, defined as the absence of a visible pregnancy on TVUS in a clinically stable patient.

In all cases of PUL, blood tests were taken to check levels of serum hCG and progesterone and further follow-up was arranged based on these results, in accordance with our previously published protocol (Cordina et al., 2011).

We only included in this study women presenting with PUL who had undergone IVF and fresh embryo transfer. The date of egg retrieval was recorded as the conception date. In those who had frozen embryo transfer, the conception date was determined by taking into account the date of embryo transfer and the age of embryo at the time of freezing. We then calculated the hCG/C ratio by dividing the initial hCG level in IU/l by the number of days from the conception date to the date of presentation to the EPAUs.

All women were followed up until either serum hCG had reduced to non-pregnant levels or the location of the pregnancy had been determined on TVUS, based on the above definitions. Spontaneous resolution of pregnancy (SRP) was defined as a decrease of hCG levels to non-pregnant levels, without the need for any medical or surgical intervention, where the location of the pregnancy was not determined. We defined an intervention as the need for medical or surgical treatment in women diagnosed with miscarriage or ectopic pregnancy on follow-up TVUS.

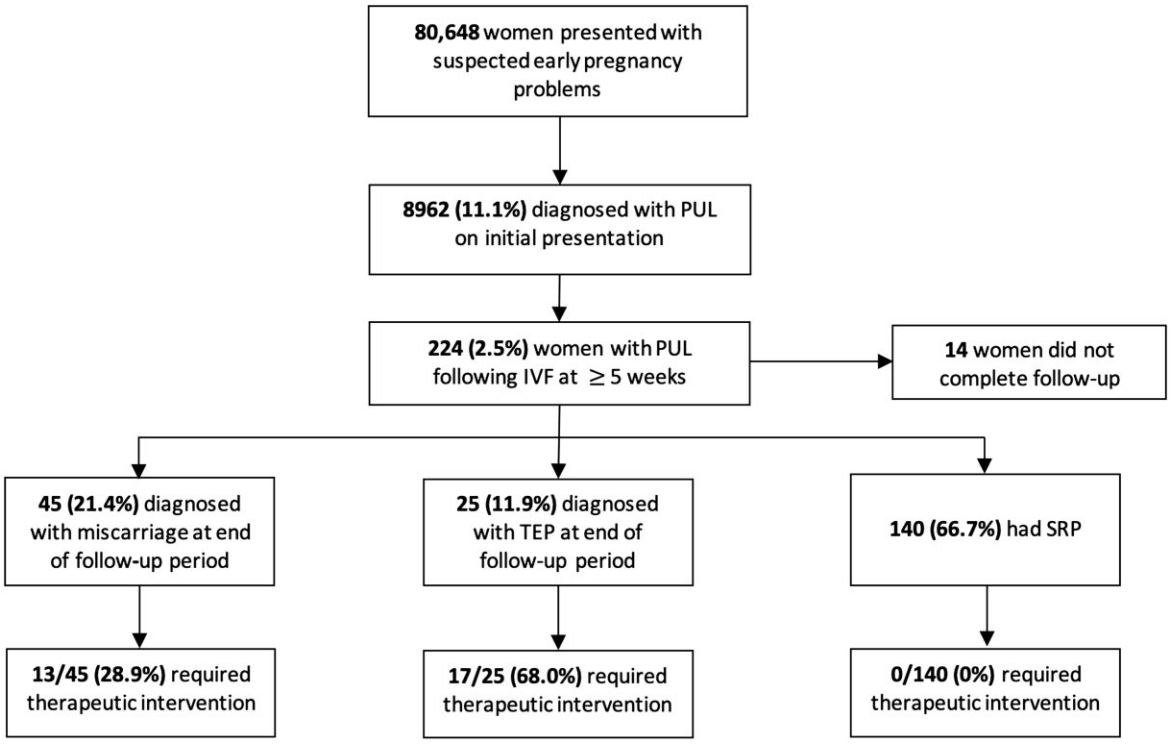

Figure 1. Flowchart of women through the study. n = 80 648. PUL, pregnancy of unknown location; TEP, tubal ectopic pregnancy; SRP, spontaneous resolution of pregnancy.

Statistical Package for Social Sciences (SPSS, version 25; IBM Corp., Armonk, NY, USA) was used for statistical analysis of the data. The baseline variables were tested for normality of distribution using the Shapiro–Wilk test. The averages of parametric and non-parametric data were expressed as mean/SD and median/interquartile range, respectively. A *P*-value of <0.05 was considered to be statistically significant throughout.

Univariate analysis was used to identify factors that correlated with the risk of requiring medical or surgical intervention. Backward selection logistic regression was used to produce the protocol. We have previously devised a clinical algorithm for the management of PUL following spontaneous conception which identifies 37% as being at low risk of intervention. The aim of this study was to propose a protocol for the management of PUL in IVF pregnancies, which would reduce the need for follow-up by 25%, with a risk of intervention rate of 3% or less in the group not requiring follow-up.

The sample size calculation was based on the assumption that the 25% figure was within ±5% of the population figure, which would be sufficiently accurate. Using a 95% confidence level, we calculated that 203 women would be required for the study.

We were advised by the Joint Research Office of University College London and UCH that formal ethical approval was not required for this study, given its retrospective nature and that the data were anonymized and analysed within the clinical care team. The study was registered on the Research Registry (reference number 8871).

Results

A total of 210 women presented at ≥5 weeks' gestation following IVF and were subsequently found to have a PUL on their initial TVUS and completed follow-up. A flowchart of women through the study is shown in Figure 1. The patient demographics are shown in Table 1.

Table 1. Demographic data of patients included in the study (n = 210).

Characteristic	
Age (years)	
Mean (SD)	37.7 (5.1)
Gravidity	
Median (min, max)	2 (1, 8)
Parity	
Median (min, max)	0 (0, 3)
Previous ectopic pregnancy	
Median (min, max)	0 (0, 2)
Number of embryos transferred	
Single embryo transfer, n (%)	125 (59.5)
Multiple embryos transferred, n (%)	85 (40.5)

On the completion of follow-up, there were no cases of a healthy normally sited pregnancies. The majority had SRP and the location of the pregnancy remained unknown. A comparison of the different patient characteristics with regards to the final diagnosis is summarized in Table 2. The results suggested significant differences between the three diagnosis groups for initial hCG, initial progesterone and hCG/C with the significantly higher levels seen for those later diagnosed with miscarriage (early embryonic demise) compared with those with tubal ectopic pregnancy (TEP) or SRP. A similar proportion of women presented with bleeding only in all three groups. The miscarriage group had a higher proportion of women who were asymptomatic compared to the TEP and SRP groups.

A total of 30/210 (14.0%, 95% CI 9.9–19.8) eventually required surgical intervention. Surgical evacuation of retained pregnancy

Table 2. Summary of final diagnosis following completion of follow-up, n = 210.

	Miscarriage n = 45	TEP n = 25	SRP n = 140	P-value
Maternal age (years)	38.9 ± 3.9	36.5 ± 3.3	38.7 ± 5.4	0.16
Gestational age (weeks)	6.1 [5.4, 6.7]	5.9 [5.3, 6.4]	6.0 [5.6, 7.0]	0.59
Symptoms				
Bleeding	16/45 (35.6%)	7/25 (28.0%)	43/140 (30.7%)	0.007
Pain	10/45 (22.2%)	3/25 (12.0%)	9/140 (6.4%)	
Pain and bleeding	10/45 (22.2%)	14/25 (56.0%)	70/140 (50.0%)	
Asymptomatic	9/45 (20.0%)	1/25 (4.0%)	18/140 (12.9%)	
Initial hCG (IU/l)	1320 [424, 2968]	370 [154, 1356]	279 [64, 1138]	<0.001
Initial progesterone (nmol/l)	64 [26, 154]	56 [24, 104]	30 [9, 53]	<0.001
Days from conception	29 [24, 33]	27 [23, 31]	28 [25, 35]	0.59
hCG/C	40 [15, 111]	17 [7, 53]	11 [2, 42]	0.001

The values are given as mean (±SD) and median [interquartile range].
TEP: tubal ectopic pregnancy; SRP: spontaneous resolution of pregnancy; hCG/C: hCG to days from conception ratio.

Table 3. Indications for the cases of surgical intervention in the study, n = 30.

Indication	Overall n/N (%)	TEP cases	Miscarriage cases
Rising hCG levels	10/30 (33)	10	0
Patient choice	10/30 (33)	3	7
Incomplete miscarriage	4/30 (13)	N/A	4
Haemoperitoneum/ haematosalpinx	3/30 (10)	3	0
Pain/bleeding	2/30 (7)	1	1
Suspected molar pregnancy	1/30 (3)	0	1

TEP: tubal ectopic pregnancy; N/A: Not applicable.

tissue was carried out in 13 cases, whilst 17 had surgical management of TEP, with 15 requiring laparoscopic salpingectomy, one requiring laparoscopic salpingotomy and one requiring laparotomy. The proportion of women requiring intervention was significantly higher in those diagnosed with TEP [17/25 (68.0%)] compared with those diagnosed with miscarriage (13/45 (28.9%) ($z = 3.16$, $P = 0.0016$)). Histology confirmed the diagnosis in all cases requiring surgical intervention.

The indications for interventions are shown in Table 3. There were 2/210 (1.0%) women who experienced complications. One presented at 53 days after conception and had an unusually high initial hCG level of 28 339 IU/l, thus her hCG/C was 534. After an initial scan was PUL, she was later confirmed to have a TEP and required a laparotomy for a ruptured TEP. The second woman presented 23 days after conception with a hCG of 3014 IU/l, thus her hCG/C was 131. Her first scan was PUL, but she presented 2 days later with bleeding and on the follow-up scan was found to have an incomplete miscarriage with products of conception seen within the cervix. The products of conception removed were confirmed on histology to be a molar pregnancy. She was referred to the local regional trophoblastic disease centre and her hCG levels declined to non-pregnant levels without the need for any further intervention. She did not suffer a significant harm, but we categorized this case as a complication bearing in mind the risk of recurrent trophoblastic disease.

We examined the factors recorded at initial visit and their association with interventions. The results are summarized in Table 4. We found that the initial hCG at ≥5 weeks' gestation and hCG/C were significantly higher in the group of women requiring an intervention compared to those not requiring an intervention.

The second stage of the analysis examined the joint association between the patient characteristics and the need for intervention. Only variables showing any evidence of an association with intervention from the first stage of the analyses were considered. There was a strong correlation between initial hCG and hCG/C which meant that only one could be included in the multivariable analysis.

The hCG/C had an odds ratio of 3.65 (95% CI 1.49–8.89, $P = 0.004$), where a one unit increase on the log scale was associated with a 3.7 times higher risk of intervention. This relationship is demonstrated further in Figure 2.

We then replaced hCG/C ratio with the initial hCG level at ≥5 weeks' gestation and performed a second multivariate analysis. This showed a significant association between initial hCG and requirement for intervention. The initial hCG level had an odds ratio of 3.58 (95% CI 1.47–8.71, $P = 0.005$). The results suggested that a one-unit increase on the log scale (equivalent to a 10-fold increase) resulted in the odds of an intervention being 3.6 times higher, as demonstrated in Figure 3.

The occurrences of intervention in our study above or below different hCG/C and initial hCG levels are shown in Tables 5 and 6, respectively. There was one patient who required an intervention who had a hCG/C of below 4, as well as an initial hCG <100 IU/l. Her initial hCG was 96 IU/l with an hCG/C ratio of 2.9 and she was confirmed to have a TEP on follow-up TVUS. She required a laparoscopic salpingectomy for pain symptoms. The procedure was uncomplicated, and the histology confirmed the diagnosis of an ectopic pregnancy.

The proportions of patients who could be potential candidates for a single visit using cut-offs for hCG/C <4 and initial hCG <100 IU/l were similar (25.7% vs 23.8%, P 0.65). The two patients who experienced complications had very high hCG/C and initial hCG levels and neither would have fulfilled the criteria for a single visit.

Discussion

The results of our study show that both hCG/C and the initial hCG level at ≥5 weeks' gestation could be used to estimate the risk of medical intervention in pregnant women diagnosed with PUL following IVF. Only one woman (1.9%) presenting with hCG/C <4.0 eventually required laparoscopic surgery for unruptured TEP. This risk is low enough not to warrant further routine follow-up visits and women presenting with low hCG/C ratio

Table 4. Comparison of cases requiring and not requiring intervention, n = 210.

Outcome	Category	No intervention n/N (%)	Intervention n/N (%)	P-value
Maternal age (years)		37.7 ± 5.1	37.5 ± 4.6	0.84
Gestation (weeks)		6.0 [5.4, 6.7]	6.1 [5.4, 6.7]	0.89
Symptoms	Bleeding only	57/180 (31.7)	9/30 (30.0)	0.83
	Pain only	20/180 (11.1)	2/30 (6.7)	
	Bleeding + pain	80/180 (44.4)	14/30 (46.7)	
	Asymptomatic	23/180 (12.8)	5/30 (16.7)	
Initial hCG (IU/l)		374 [88, 1440]	1148 [370, 2995]	0.003
Initial progesterone (nmol/l)		35 [11, 63]	56 [24, 98]	0.06
Days since conception		28 [24, 33]	29 [24, 33]	0.89
hCG/C		12 [3, 48]	38 [12, 116]	0.003

The values given are mean ± SD, median [interquartile range], or number (percentage).
hCG/C: hCG to days from conception ratio.

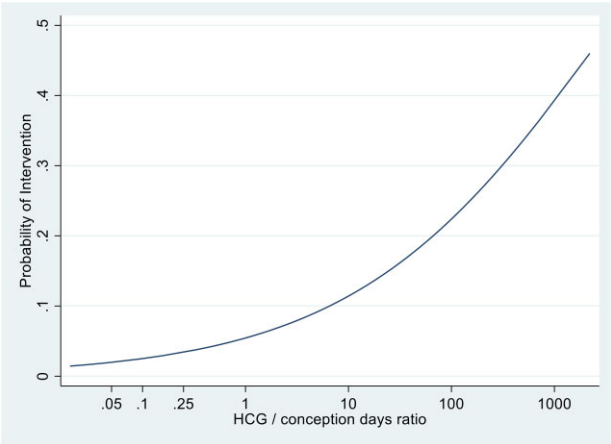

Figure 2. Graph showing the fitted relationship between hCG to days from conception ratio and the probability of an intervention. n = 210.

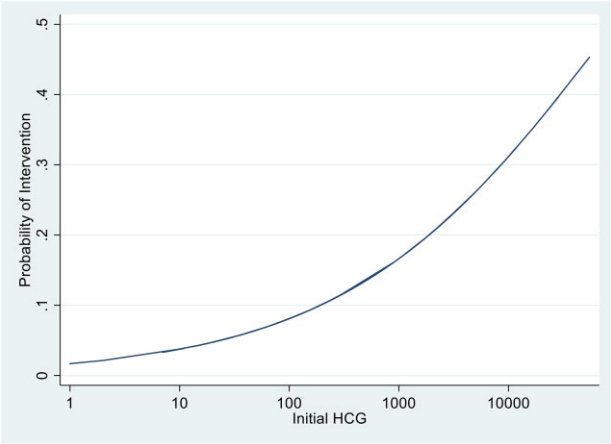

Figure 3. Graph showing the fitted relationship between initial hCG level at ≥5 weeks' gestation and the probability of an intervention. n = 210.

could be advised only to perform a urine pregnancy test in 2 weeks to confirm the resolution of the pregnancy. They accounted for 25% of all post-IVF PUL patients and implementation of a single-visit protocol for this subgroup of women could reduce significantly the number of follow-up visit in this population of women with PUL. However, a single-visit protocol does not imply that pregnant women are denied access to further care. They are encouraged to return to the clinic if their symptoms worsen and they are routinely contacted by the nursing team 2 and 6 weeks later to ensure that they are well and that their urine pregnancy test is negative.

Table 5. Occurrence of intervention at different levels of hCG/days from conception ratios, n = 210.

hCG/C	Cases below this level n (%)	Cases requiring intervention below this level n/N (%)	Cases requiring intervention above this level n/N (%)
<4.0	54 (25.7)	1/54 (1.9)	24/156 (18.6)
<6.0	66 (31.4)	3/66 (4.6)	27/144 (18.8)
<15.0	106 (50.4)	9/106 (8.5)	21/104 (20.2)
<55.0	158 (75.2)	20/158 (12.7)	10/52 (19.2)

hCG/C: hCG to days from conception ratio.

Table 6. Occurrence of intervention for selected initial hCG cut-offs at ≥5 weeks' gestation (n = 210).

Initial hCG cut-off (IU/l)	Cases below this level n (%)	Cases requiring intervention below this level n/N (%)	Cases requiring intervention above this level n/N (%)
<100	50 (23.8)	1/50 (2.0)	29/160 (18.1)
<200	73 (34.8)	4/73 (5.5)	26/137 (19.0)
<500	114 (54.3)	11/114 (9.7)	19/96 (19.8)

Similarly, women presenting with very low initial hCG levels at ≥5 weeks' gestation were also at low risk of requiring intervention. A potential single-visit protocol based on the initial hCG level <100 IU/l was marginally less effective, compared with hCG/C, but the difference was not statistically significant.

Our study is novel and a separate protocol for the management of women with PUL post-IVF has never previously been designed. Although the sample size is relatively small, data have been extracted from two units which contained data of more than 80 000 women presenting with suspected early pregnancy complications.

A limitation of our study is that it is retrospective in nature, and as such, we were reliant on existing data. In view of that, further prospective studies will be required to confirm the optimal hCG/C or initial hCG level to risk stratify women presenting with PUL post-IVF.

The results of our study confirmed that the final pregnancy outcomes in women presenting initially with PUL differ between women who have conceived spontaneously and those who have had IVF. In women with spontaneous pregnancies, approximately 21–25% are eventually diagnosed with healthy normally sited pregnancies (Banerjee *et al.*, 1999; Bobdiwala *et al.*, 2022),

whilst in our study, as expected, all pregnancies following IVF presenting initially as PUL >5 weeks' gestation were abnormal. The proportion of women diagnosed with miscarriages was therefore higher compared to those who conceived spontaneously, but the proportion of ectopic pregnancies was similar (10–12% vs 14%) (Banerjee *et al.*, 1999; Bobdiwala *et al.*, 2022). A higher proportion of abnormal pregnancies is associated with a higher risk of intervention, hence the proportion of PUL identified as low risk in this study population was lower compared to that following spontaneous conception (25% vs 37%).

Assisted conception provides a known date of conception. This allows for a calculation of the hCG/C, which provides information about the behaviour of functional trophoblast and potential of an abnormal pregnancy to grow and cause harm. Calculating hCG/C could thus remove the need for a repeat blood tests to measure hCG, which is main component of various algorithms for the initial management of PUL (Fistouris *et al.*, 2016; National Institute for Health and Care Excellence (NICE), 2019). hCG/C also removes the need to measure serum progesterone which is used in some PUL protocols to determine whether non-visualized pregnancies are likely to progress or whether they are already in regression (Day *et al.*, 2009; Bobdiwala *et al.*, 2022). The main outcome of interest was spontaneous pregnancy resolution and therefore our protocol was applicable to all post-IVF conceptions regardless of the number of embryos transferred.

Our results show that the measurement of serum hCG at the initial visit at ≥5 weeks' gestation could also be used to assess the risk of intervention in women with PUL following IVF. Such a protocol would be easy to implement into clinical practice, as it would not require medical staff to perform any mathematical calculations. However, a single hCG measurement may be more prone to variability than the hCG/C ratio and its value will need to be checked in further prospective studies.

Our previous prospective study in women presenting with PUL, following a spontaneous conception used a 2% risk of intervention rate as low enough to advise patients that close follow-up and serial blood tests are not required. This facilitated implementation of a single-visit strategy covering 37% of women presenting with PUL (Cordina *et al.*, 2011). Using the same cut-off of 2%, a quarter of women with PUL post-IVF could be advised that they do not require further blood tests or scans. Although this is less compared to population of women with PUL following spontaneous conception, a 25% reduction in follow-up visits is still significant in terms of reducing the burden of follow-up visits on busy clinical services and the social and professional inconvenience to patients.

With a hCG/C <6, the risk of intervention was <5%. Although this would be too high to discharge women from close follow-up, it indicated that pregnancies in this subgroup of patients were developing very slowly. In view of that, it would be reasonable in these cases to schedule the next blood test or urine pregnancy test in 7 days rather than in 48 h as stipulated by hCG-based protocols (National Institute for Health and Care Excellence (NICE), 2019). This would reduce the need for 48-h hCG measurements by nearly a third.

In conclusion, our study has demonstrated that the novel marker of hCG/C taken at the initial visit could be used to assess the risk of needing medical intervention in women diagnosed with PUL following IVF. Women with hCG/C <4.0 are at particularly low risk of intervention and a single-visit protocol could be implemented in these cases. This could lead to a modest, but significant reduction in the workload required to manage these women, without compromising their safety. Similarly, women presenting with an initial hCG <100 IU/l at ≥5 weeks' gestation were also at a low risk of intervention and further work is needed to show which of these two protocols is better suited for the use in routine clinical practice. The proportion of women presenting with PUL after IVF is relatively low in general early pregnancy units, thus our proposed protocol may be more helpful to fertility specialists who tend to primarily look after women who are pregnant following assisted conception. Further prospective studies, however, are needed to confirm our findings before the single-visit protocol is implemented into routine clinical practice.

Data availability

The data underlying this article will be shared upon reasonable request to the corresponding author.

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Authors' roles

W.M.D. was involved in the acquisition and analysis of the data, and in the drafting and revision of the article. L.V.d.B. and S.P. were involved in the acquisition of the data. M.W. was involved in the analysis of the data. J.A.R. was involved in the acquisition of data and in the revision of the article. D.J. conceived the study and was involved in the interpretation of data and in the revision of the article. All authors have approved the revisions and the final article.

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Conflict of interest

No conflicts of interest are required to be declared for this study.

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