While lifestyle factors have been investigated during the fertility workup, patient behaviours can change so it is recommended to review these and their optimization when RIF is encountered.

There are insufficient data to recommend the routine measurement of vitamin D levels or treatment of vitamin D deficiency.

Screening for genetic factors: karyotyping of the female and male partner

In a survey of clinical practice, 67% of clinicians reported considering chromosomal disorders as a potential risk factor for RIF and most clinicians assess both the female and male karyotypes (Cimadomo *et al.*, 2021). Embryonic chromosomal disorders represent the major cause of (early) pregnancy loss in humans (Papas and Kutteh, 2021). Aneuploid blastocysts have a significantly reduced developmental capacity during the preimplantation stage (Rubio *et al.*, 2007; Martín *et al.*, 2021) and negligible implantation potential (Grati *et al.*, 2018; Capalbo *et al.*, 2022). However, most embryonic chromosomal aneuploidies are of maternal meiotic origin.

In line with these observations, case–control studies have shown that karyotype anomalies are more frequent in patients with RIF, even if the absolute prevalence (2.1%) is low (Stern *et al.*, 1999; Raziel *et al.*, 2002; De Sutter *et al.*, 2012). These figures are within the prevalence range of chromosomal abnormalities described in infertile couples undergoing ART, ranging from 2.8% to 12% in males and from 3.0% to 15% in females (Meschede *et al.*, 1998). With regards to the type of karyotype abnormalities in couples with RIF (8 females and 5 males), autosomal abnormalities, sex chromosome aberrations, and chromosomal mosaicism were found in 6, 2, and 1 females and 4, 0, and 1 males, respectively (De Sutter *et al.*, 2012).

The contribution of abnormal parental karyotype to predispose to chromosomal embryonic errors is plausible (Insogna *et al.*, 2021; Yuan *et al.*, 2021).

Despite the low prevalence, karyotyping can be considered to confirm the absence of a chromosomal abnormality in parents.

If a chromosomal abnormality is detected, genetic counselling and, where relevant, preimplantation genetic testing (PGT), is recommended.

Anatomical investigations

Eighty-five per cent of clinicians have been reported to take anatomical and gynaecological investigations into account in diagnosing the cause of RIF (Cimadomo *et al.*, 2021). Asherman's syndrome, hydrosalpinx, endometriosis/adenomyosis, uterine malformations, endometrial atrophy, as well as uterine fibroids, are widely considered relevant. Ovarian cysts were considered relevant by 23% of clinicians. Hysteroscopy is the most widely used technique for anatomical investigations, followed by 3D and 2D transvaginal ultrasound (Cimadomo *et al.*, 2021).

Assessment of the uterine cavity

Transvaginal ultrasound is considered to be performed as part of the fertility workup.

Given the general diagnostic accuracy attributed to 3D transvaginal ultrasound, it has been proposed as an alternative non-invasive procedure for the diagnosis of uterine anomalies and a good practice approach (Grimbizis *et al.*, 2016). Currently, there are no studies evaluating whether 3D transvaginal ultrasound improves the outcomes in patients with RIF. Given the limited cost and non-invasiveness, it can be considered a routine diagnostic tool during fertility workup, when available. If not performed at the start of the ART treatment, it may be of benefit when assessing the patient presenting with RIF.

If 3D ultrasound has not been performed at fertility workup, it can be considered.

While assessment of the presence of adenomyosis, endometriosis, and submucosal fibroids should be carried out before ART, if there is renewed suspicion owing to emerging clinical signs or ultrasound features noted after RIF, then further investigations including MRI or diagnostic laparoscopy should be considered.

The use of hysteroscopy is often proposed when uterine pathology has been detected by transvaginal ultrasound and further diagnostics are indicated (e.g. submucous fibroids, uterine adhesions). A meta-analysis focussing on patients with RIF reported a significantly higher LBR after hysteroscopy compared to those that did not have hysteroscopy (Risk Ratio (RR) 1.29; 95% CI 1.03–1.62; 4 studies; $n = 2247$; $P = 0.046$) (Cao *et al.*, 2018). The analysis by Cao *et al.* included a large randomized controlled trial (RCT) (the TROPHY study) that reported a similar LBR after ART in patients with RIF (two to four failed IVF cycles) without a previously recognized pathology ($n = 702$) when comparing those undergoing hysteroscopy versus those proceeding to ART without hysteroscopy (29% versus 29%, RR 1.0; 95% CI 0.79–1.25; $P = 0.96$) (El-Toukhy *et al.*, 2016). Sonohysterography is another technique to diagnose uterine pathologies, but it is less well studied in RIF (Negm *et al.*, 2012; Reda *et al.*, 2016).

Other uterine cavity anomalies can be treated by established interventions including endometrial polypectomy, surgical removal of submucous fibroids, uterine septum resection, or removal of intrauterine adhesions. While the interventions are established for the treatment of symptoms, their impact on pregnancy or LBRs has, to our knowledge, not been evaluated in patients with RIF. Similarly, the effect of treatment of adenomyosis on pregnancy or LBR in women with RIF has not been evaluated.

Hysteroscopy can be considered, especially when there is a suspicion of a uterine anomaly visualized on transvaginal ultrasound.

There is a lack of studies evaluating hysterosalpingography (HSG) in the context of RIF, but HSG or other means of imaging of the fallopian tubes can be considered if there is a doubt about hydrosalpinx after ultrasound.

Endometrial function and receptivity tests

During anatomical and gynaecological investigations to explore possible causes of RIF, 59% of clinicians reported considering the window of implantation (WOI) (endometrial biopsy) (Cimadomo *et al.*, 2021).

However, this assesses just one element and the mechanisms underlying human endometrium receptivity are complex. Given the numerous endometrial functions that can collectively be considered to represent 'receptivity', it is unlikely that a single test