Small supernumerary marker chromosomes (SMCs) are found at a frequency of approximately 0.14–0.72 per 1000 newborns (Hamerton et al. 1975; Jacobs et al. 1997; Wandstrat and Schwartz 2000; Eggermann et al. 2002). Inverted duplication of chromosome 15 [inv dup (15)] is the most common SMC, representing 50% or more of all cases (Webb 1994; Huang 1997; Shim et al. 2001; Eggermann et al. 2002). Inv dup (15) chromosomes are generally classified into two groups according to their length (Grammatico et al. 1994; Crolla et al. 1995; Huang et al. 1997; Shim et al. 2001). The small ones are those who usually do not contain the Prader-Willi (PW)/ Angelmann syndrome (AS) critical region at 15q11-q13. The second group includes larger markers containing the PW/AS critical region (Huang et al. 1997).

The chromosomes in these two groups are commonly pseudodentric and bisatellited with two copies of the short (p) arm (Wandrat and Schwartz 2000). Their phenotypic effects are variable and depend on the amount of euchromatin (Eggermann et al. 2002). Carriers of a large inv dup (15) usually have mental and growth retardation, seizures and dysmorphic features (Huang et al. 1997). Individuals carrying a small inv dup (15) are generally normal, but they may present some features described in patients with PW or AS due to uniparental disomy or deletion of one chromosome 15 homolog (Robinson et al. 1993; Huang et al. 1997; Shim et al. 2001). In addition, males with oligospermia or azoospermia without other abnormalities have a high frequency of inv dup (15) (Eggermann et al. 2002).

In this report, a man with infertility without other clinical features, presenting a 47,XY,+mar karyotype, was studied by spectral karyotyping (SKY) and fluorescence in situ hybridization (FISH) using a chromosome-15-specific probe (LSI SNRPN). By these techniques, the marker chromosome was identified as a small inv dup (15). Possible causes for male infertility in this case are discussed.

**Abstract.** We report on a phenotypically normal man with infertility, whose 47,XY,+mar karyotype was studied by spectral karyotyping (SKY) and fluorescence in situ hybridization (FISH) using a chromosome-15-specific probe (LSI SNRPN). By these techniques, the marker chromosome was identified as a small inv dup (15). Possible causes for male infertility in this case are discussed.

**Key words:** cytogenetics, fluorescence in situ hybridization, inv dup (15), male infertility, spectral karyotyping.

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due to infertility. The husband, 33 years old, was affected by unilateral cryptorchidism, which was surgically repaired in childhood; a sperm analysis revealed oligospermia. His wife, 34 years old, was phenotypically normal, including gynecologic examinations. After repeated attempts to achieve pregnancy over many years, they decided for an artificial insemination, which was unsuccessful. Subsequently, there were two natural conceptions ending in spontaneous miscarriage, both before 8 weeks of gestation. Family history was unremarkable except for multiple congenital anomalies in a nephew of the wife; that child had been evaluated by a clinical geneticist, who did not reach a diagnosis but considered his clinical picture to be a sporadic condition.

Physical examination of the couple did not reveal a dysmorphic picture. Chromosomal analysis with GTG banding of the husband showed 47,XY,+mar (Figures 1a,e); his parents could not be investigated. The karyotype of his wife was normal (46,XX).

Cell culture and cytogenetic study on peripheral blood lymphocytes and GTG-banding were performed according to standard protocols. A SKY assay was performed to identify SMC origin and then FISH assay was performed to elucidate its structure.

The SKY assay was performed with a SKY Paint probe (Applied Spectral Imaging Inc. Vista, CA). Fourteen metaphases were karyotyped with ASI Image Acquisition Software (Spectral Imaging 2.6) and analyzed with ASI Analysis Software (SKYView 2.1). The FISH probe set, LSI SNRPN (Vysis/Abbott), consisted of one centromeric probe D15Z1 at the 15p11.2 (spectrum green), LSI SNRPN at 15q13-15 (spectrum orange) and LSI PML at 15q22 (spectrum orange). Vectashield DAPI (0.15 µg µL⁻¹) was applied as chromatin counterstain. Twenty metaphase spreads and 500 interphases were analyzed with FISH – Genus (CytoVision 3.1).

SKY showed that the SMC was derived from chromosome 15 (Figures 1c,d). Using FISH, the marker was twice positive for the probe D15Z1, but negative for the probes LSI SNRPN (15q13-15) and LSI PML (15q22) (Figures 1b,f). Altogether, the analyses identified the extra chromosome as a der(15) inv dup(15) (pterq11.2). These findings indicate that the SMC is an inv dup (15) composed of two short (p) arms, two centromeres and the pericentric region, but excluding the PWS/AS critical region.

The detection of a SMC in a routine karyotyping of a man with infertility reinforces the importance of clinical and cytogenetic evaluation before reproductive options are offered to a couple, because this marker can reflect an increased risk of abnormal progeny. In the present case, unsuccessful assisted reproduction techniques had been performed before the genetic assessment was done.

Figure 1. Whole and partial karyotypes in G-banded cells (a,e) and in cells submitted to spectral karyotyping (SKY) (d,c). In SKY analysis, pink and green colors report chromosome 15 centromeric and euchromatin region, respectively. Dual color FISH assay with the LSI SNRPN probe is illustrated in a metaphase spread (b) and a partial karyotype (f). In FISH assay, green signals indicate hybridization with the centromeric probe D15Z1 at 15p11.2 and orange signals indicate hybridization with LSI SNRPN at 15q13-15 and LSI PML at 15q22. Altogether, the analyses identified the extra chromosome as a der(15) inv dup(15)(pterq11.2).