Reproductive BioMedicine Online (2013) **26**, 360–367

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ARTICLE

Anti-Müllerian hormone as an independent predictor of twin versus singleton pregnancy in fresh cycles

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Abstract This study evaluated anti-Müllerian hormone (AMH) as a possible predictor of twin pregnancy in women undergoing fresh cycles who had more than one embryo transferred. A retrospective study was performed of 139 patients undergoing fresh non-donor cycles which resulted in either singleton or twin pregnancy between 2009 and 2010 in this fertility clinic. Random serum AMH and other clinically relevant variables were compared. For further analysis, the population was stratified by age (<34 and ≥34 years). Random serum AMH concentrations were 1.4-times greater in women conceiving twins compared with singletons ($P = 0.03$). In women aged ≥34, the AMH concentration in twins was 1.8-fold greater than singletons ($P = 0.001$). Multivariate analysis demonstrated that AMH was an independent predictor of twins. ROC curve analysis showed that AMH had a significant predictive ability for twin pregnancy in women aged ≥34 (AUC 0.67, $P = 0.01$). In contrast, in women aged <34, AMH was not different between twin and singleton pregnancies. In summary, random serum AMH is an independent predictor of twin gestation when more than one embryo is transferred in women aged ≥34. Considering a woman's AMH before transferring more than one embryo may assist in reducing the incidence of twins.

KEYWORDS: anti-Müllerian hormone, assisted reproduction technology, single-embryo transfer, twin pregnancy

Introduction

Transferring multiple embryos to increase the chance of pregnancy and live birth rate is a common practice among assisted reproduction providers although it often results in a high proportion of multiples. In fact, assisted reproduction accounts for only 1% of total births in the USA, but is responsible for 17% of twins, as couples undergoing assisted repro-

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2012.12.002

duction treatment are 27 times more likely to have twins than couples conceiving spontaneously (Practice Committee of the Society for Assisted Reproductive Technology and the American Society for Reproductive Medicine, 2004). High-order multiple (three or more fetuses) rates in the USA have significantly declined due to an emphasis on minimizing the number of embryos transferred. However, the twinning rate has plateaued (Martin et al., 2007). Multiple-birth infants are at significant risk for a number of adverse outcomes, including preterm birth, low birthweight, congenital anomalies, infant death and disability among survivors (Kallen et al., 2010; Martin et al., 2007). Maternal complications include haemorrhage, pregnancy-induced hypertension and anaemia, as well as maternal mortality (Martin et al., 2007).

In an effort to minimize the twin pregnancy rate, a policy of elective single-embryo transfer (eSET) has been adapted in many countries for specified patient groups (Cutting et al., 2008; De Neubourg et al., 2006; Nyboe Andersen et al., 2009). The introduction of the eSET policy has been shown to lead to a dramatic reduction in multiple pregnancies and improvement in both maternal and neonatal outcomes, although at the cost of slightly reduced pregnancy rates (Kallen et al., 2010; Luke et al., 2010). However, despite revised American Society of Reproductive Medicine (ASRM) guidelines recommending consideration of eSET in favourable prognosis women under the age of 35 years, eSET has not been readily adopted in the USA and accounts for only approximately 10% of all transfers in this age group in 2009 (Practice Committee of the Society for Assisted Reproductive Technology and Practice Committee of the American Society for Reproductive Medicine, 2012).

Until eSET becomes common practice, identifying factors predictive of multiple pregnancies is of paramount importance for the successful development of strategies to minimize multiple gestations. A number of previous studies have analysed risk factors for multiple pregnancies in IVF cycles following transfer of two or more embryos. Age, number of embryos transferred and stage of embryo transfer were each shown to be associated with risk of multiple pregnancies following transfer of more than one embryo in an IVF cycle (Bustillo, 1997; De Jonge and Wolf, 1997; Karaki et al., 2002; Milki et al., 2000; Schieve et al., 1999; Templeton and Morris, 1998). Maternal age varies inversely with a woman's chances of achieving pregnancy (Bustillo, 1997; De Jonge and Wolf, 1997) and younger age has been consistently associated with higher rates of multiple pregnancies following IVF (Schieve et al., 1999; Templeton and Morris, 1998). Several large observational studies demonstrated that the number of embryos transferred in IVF cycles correlates with multiple pregnancy rates (Schieve et al., 1999; Templeton and Morris, 1998). Blastocyst transfer when available allows selection of embryos that have increased viability and proven developmental capacity, resulting in higher pregnancy rates compared with cleavage-stage transfer (Karaki et al., 2002; Milki et al., 2000). It has been reported that, although blastocyst transfer was associated with decreased number of embryos transferred compared with cleavage-stage transfer, the risk of multiple birth was still higher following blastocyst transfer (Kissin et al., 2005).

Anti-Müllerian hormone (AMH), also known as Müllerian-inhibiting substance, belongs to the transforming growth factor β superfamily and is considered a local growth factor and a cellular differentiation factor (Lee et al., 1996). In women, AMH is exclusively secreted by ovarian granulosa cells surrounding pre-antral and small antral follicles and its concentrations become undetectable in the serum of menopausal women (Weenen et al., 2004). AMH has been shown to be closely correlated with antral follicle count and is an excellent predictor of pre-ovulatory oocyte supply in response to ovulation induction (Nardo et al., 2009; Seifer and Maclaughlin, 2007; Seifer et al., 2002). Aside from the strong association of serum AMH concentration with quantitative ovarian response, serum AMH concentration has been suggested to be indicative of qualitative aspects of assisted reproduction technology such as embryo quality and pregnancy rates. It has been demonstrated that serum AMH on the day of human chorionic gonadotrophin (HCG) correlated with quality of embryos obtained and subsequently embryo implantation potential (Silberstein et al., 2006). However, other studies did not confirm these findings (Lie Fong et al., 2008). Although still controversial, accumulating evidence suggests that AMH may play a role as a predictor of IVF pregnancy and live birth rates independent of age (Blazar et al., 2011; Eldar-Geva et al., 2005; Elgindy et al., 2008; Gleicher et al., 2010; La Marca et al., 2011; Nelson et al., 2007). In addition, age exhibits a strong inverse correlation with both serum AMH (Seifer et al., 2011) and multiple pregnancy rates (Schieve et al., 1999; Templeton and Morris, 1998). Therefore, the present study postulated that serum AMH, independently of age, may be associated with twin pregnancy rates for women having more than one embryo transferred in an IVF cycle. Accordingly, the specific aim was to examine if random serum AMH concentrations were associated with assisted reproduction outcome for a singleton versus twin gestation.

Materials and methods

This study identified all fresh non-donor assisted cycles in the clinic which resulted in either a singleton or twin pregnancy in which more than one embryo were transferred between 2009 and 2010. This study excluded cycles in which embryos were derived from an egg donor ($n = 107$) and cycles in which the embryos transferred had been retrieved and fertilized at an earlier date, frozen via cryopreservation and thawed for use in the current cycle ($n = 224$). As this study was aimed at identifying predictors of twin pregnancy following assisted reproduction, we further excluded single-embryo transfers ($n = 64$) as well as cycles in women over age 40 ($n = 111$) as these cycles carry a lower risk of multiple gestation. The final sample included 374 fresh non-donor assisted cycles, of which 224 did not result in a pregnancy and the rest of the cycles resulted in 79 singleton pregnancies, 60 twin pregnancies and 11 higher-order pregnancies. The study was approved by the Institutional Review Board of Maimonides Medical Centre on 21 November 2012 (reference number 12/12/XA04).

A total of 139 cycles resulting in singleton ($n = 79$) or twin pregnancy ($n = 60$) were examined. Only one cycle per patient was included. Reasons for infertility included male factor, endometriosis, hormonal factor, tubal factor and unexplained infertility. The following variables were assessed as