Reproductive BioMedicine Online (2014) **28**, 64–69

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Probability of live birth in women with extremely low anti-Müllerian hormone concentrations

Krzysztof Łukaszuk [a,b,c,d], Michał Kunicki [e,*], Joanna Liss [b], Alicja Bednarowska [e], Grzegorz Jakiel [e,f]

[a] *Department of Perinatology, Medical University, Gdańsk, Poland;* [b] *INVICTA Fertility and Reproductive Center, Gdańsk, Poland;* [c] *Department of Obstetrics and Gynecological Nursing, Faculty of Health Sciences, Medical University of Gdansk, Poland;* [d] *Department of Obstetrics and Gynecology, Faculty of Medical Sciences, University of Varmia and Masuria, Olsztyn, Poland;* [e] *INVICTA Fertility and Reproductive Center, Warszawa, Poland;* [f] *Department of Obstetrics and Gynaecology, Medical Center of Postgraduate Education, Warsaw, Poland*

[*] Corresponding author. *E-mail address:* michal.kunicki@invicta.pl (M Kunicki).

Krzysztof Lukaszuk is professor of Obstetrics and Gynaecology at Medical University of Gdansk, Poland, and a head of the Fertility Clinics Invicta in Warsaw and Gdansk. He obtained his medical degree from the Medical University of Gdansk in 1993 and his PhD from the same institution in 1998. He completed his basic training in 2000 and gained his habilitation degree in 2003. He has been a professor in Medical University of Gdansk since 2008 and he completed his training in endocrinology in 2012. Krzysztof has been developing preimplantation genetic diagnosis in Poland since 2005.

Abstract The aim of the present study was to investigate the clinical pregnancy and live birth rates in women with extremely low (≤0.4 ng/ml) anti-Müllerian hormone (AMH) concentrations. The study included 101 women (188 cycles) with extremely low AMH concentrations undergoing IVF cycles and compared the number of live births in women with low AMH. Moreover, the study compared the number of live births in women with or without endometriosis stage III/IV. Fourteen clinical pregnancies and 14 live births (including one pair of twins) were recorded; one woman miscarried. Significantly higher clinical pregnancy ($P = 0.046$) and live birth rates ($P = 0.018$) were found in women aged <35 years compared with older women. AMH concentration did not differ significantly between women with or without endometriosis and there were six live births in women with endometriosis. This was not significantly different from the rate in healthy women. It is concluded that live births are possible in women with extremely low AMH concentrations. The presence of endometriosis stage III/IV did not affect live birth rates in women with extremely low AMH concentrations although an important limitation of the study is the small number of women included who were affected by that disease.

KEYWORDS: AMH, endometriosis, inhibin B, ovarian reserve, DHEA, IVF

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2013.09.017

Introduction

Anti-Müllerian hormone (AMH) is an established marker of ovarian reserve (La Marca et al., 2010; Nelson et al., 2009) and predicts both high and low responses in ovarian stimulation cycles (Eldar-Geva et al., 2005; Nardo et al., 2009; Nelson et al., 2007). Presently, AMH helps clinicians counsel patients prior to IVF treatment (La Marca et al., 2011), despite the fact that it fails to predict who will become pregnant (Lamazou et al., 2011; Riggs et al., 2011). It has been demonstrated that poor responders can achieve both pregnancy and live birth (Weghofer et al., 2011). There are few studies regarding extremely low AMH concentrations and live births (Fraisse et al., 2008; Tocci et al., 2009; Weghofer et al., 2011) and they present either a small number of patients or limited data describing the groups of investigated patients.

Another factor affecting pregnancy rates is endometriosis, a chronic gynaecological disease characterized by the presence of functional endometrial tissue outside the uterine cavity (Koninckx et al., 1991). Many studies have reported that pregnancy rates are lower in women with endometriosis than in controls (Gupta et al., 2008; Koninckx et al., 1991 Pellicer et al., 2000). Lower AMH serum concentrations are associated with endometriosis severity (Shebl et al., 2006).

The primary objective of the present study was to assess live birth rates in women with extremely low AMH concentrations with respect to age. Additionally, another objective was to determine live birth rates in women with both extremely low AMH concentrations and endometriosis stage III/IV.

Materials and methods

Selection of subjects

This study retrospectively analysed a computer database of women with extremely low AMH concentrations treated with intracytoplasmic sperm injection (ICSI) in the (IVF) unit (invicta private fertility Centre) between May 2007 and January 2011. Serum AMH assays were included as a standard measure in the IVF program. A cut-off AMH value of $\leq$0.4 ng/ml was chosen according to Gnoth et al. (2008) and Weghofer et al. (2011). AMH concentrations were measured prior to the start of each cycle.

Women were divided into three age categories — $<$35, 35—39 and $>$39 years — according to data presented by Mosher and Pratt (1991).

Endometriosis staging was performed according to the revised classification of the American Society for Reproductive Medicine, ranging from moderate to severe (American Society For Reproductive Medicine, 1997). None of the patients had medical treatment for endometriosis within 3 months prior to laparoscopy. The interval between laparoscopy and IVF was 3.36 ± 2.8 years. Moreover, none of the participants had been taking any hormonal treatment for at least 3 months before entering the study. This study received expedited Institutional Review Board approval (reference no. 4/2011, approved 6 October 2011). Written informed consent was obtained from each included couple to perform ICSI on at least some of the retrieved oocytes. All data were de-identified and analysed anonymously. Moreover, informed consent to present our data in any publication was obtained as long as confidentiality was maintained.

Specimen collection and preparation and hormone analysis

Fasting venous blood samples (7 ml) were collected aseptically without any additives from 08:00 to 12:00 h between days 1 and 3 of the menstrual cycle prior to the beginning of stimulation. Blood was allowed to clot at room temperature and serum was separated by centrifugation (10 min at 1500 *g*). The samples were stored at −20°C until analysis. Serum FSH concentrations were measured using a standard chemiluminescence immunoassay (Immulite; DPC, Los Angeles, CA, USA) according to the manufacturer's instructions. The lower detection concentration was 0.1 mIU/ml. Serum AMH and inhibin B concentrations were measured by ELISA (Diagnostic Systems Laboratories, Webster, TX, USA). The assay limit of detection was 0.06 ng/ml for AMH and 7 pg/ml for inhibin B. The intra- and interassay coefficients of variation were $<$10% for all parameters.

Stimulation protocol

All women underwent a long protocol of pituitary suppression with the gonadotrophin-releasing hormone agonist Diphereline at a dose of 0.1 mg/day (Pharmacia Upjohn, Kalamazoo, MI, USA), beginning on day 14 of the oral contraception cycle. Fourteen days later (i.e. 7 days after the end of oral contraceptive administration and after menses), the administration of urinary gonadotrophins (Gonal-F; Serono, Feltham, UK; or Fostimon; Genevrier, Sophia-Antipolis, France) for ovarian stimulation was initiated (300 IU/day). Gonadotrophin treatment was initiated if no follicles were larger than 10 mm in diameter and oestradiol concentrations were $<$50 pg/ml. The FSH dose was based on the woman's age and AMH concentration. In this unit, 300 IU/day is routinely given to patients with extremely low AMH concentrations.

Follicular growth was monitored using a day-8 ultrasonographic scan and a serum oestradiol assay. Ovulation was induced by administration of 5000 IU human chorionic gonadotrophin (Pregnyl, Organon, Oss, Netherlands) when at least one leading follicle had reached a diameter of 17 mm and oocyte retrieval was performed 36 h later.

Embryo transfer was performed on cleavage-stage day 5 in all cases using a soft catheter. The number of embryos transferred was determined by the available number and quality of embryos and by the guidelines of the institution and ASRM (Practice Committee. Society for Assisted Reproductive Medicine and the American Society for Reproductive Medicine, 2004).

All patients were given supplementation with natural micronized progesterone (Luteina; Adamed, Czosnów, Poland), given vaginally in three divided doses of 200 mg/day, beginning on the day of oocyte retrieval. The