Anti-Müllerian hormone and inhibin B as predictors of pregnancy after treatment by in vitro fertilization/ intracytoplasmic sperm injection

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Objective: To evaluate anti-Müllerian hormone (AMH) as a marker of reproductive outcome after IVF/intracytoplasmic sperm injection (ICSI).
Design: Longitudinal study.
Setting: University hospital.
Patient(s): Two hundred seventy-six consecutive women undergoing IVF/ICSI.
Intervention(s): Ovarian stimulation, oocyte retrieval, IVF, ICSI, embryo transfer, AMH, and inhibin B determinations in serum and follicular fluid (FF).
Main Outcome Measure(s): The AMH and inhibin B concentrations in 276 matched FF/serum pairs have been determined. Different outcome groups have been compared and set in relation to the oocyte count, morphological parameters, and steroid hormone levels.
Result(s): The concentrations of AMH and inhibin B in both serum and FF were significantly higher in the group of women who became pregnant in the corresponding treatment cycle than in those who did not conceive. Positive correlations were observed between serum inhibin B concentrations and embryo morphology (r = 0.126, 95% confidence interval 0.026–0.284). Serum and FF AMH or inhibin B correlated positively with the oocyte count and negatively with the pretreatment cycle day 3 FSH level and the total administered gonadotropin dose.
Conclusion(s): The AMH and inhibin B levels on the day of oocyte retrieval are correlated to reproductive outcome. (Fertil Steril® 2008;90:2203–10. ©2008 by American Society for Reproductive Medicine.)

Key Words: Anti-Müllerian hormone, inhibin B, reproductive outcome, IVF/ICSI

Anti-Müllerian hormone (AMH), also called Mullerian inhibiting substance, is a dimeric glycoprotein, belonging to the transforming growth factor-β (TGF-β) superfamily, such as activins and inhibins, and is produced exclusively in the gonads, as shown more than two decades ago in animals (1) and later in humans (2, 3). In men, it originates from the Sertoli cells and is responsible for the regression of the Müllerian ducts in the early embryonic life (hence its name).

In women, AMH is produced in the ovary by the granulosa cells (GC) surrounding preantral and small antral follicles (4, 5). Even when using ultrasensitive assays AMH is barely detectable in the serum at birth, but it reaches higher levels after puberty (6, 7) and then declines with advancing female age, until becoming undetectable again at the time of the menopause (8). Although its physiological actions and regulations are still unclear, recent studies point out the interest of AMH as an attractive marker for the assessment of ovarian activity. Anti-Müllerian hormone is produced by the GCs of recruited follicles before they become sensitive to FSH (9). It has been suggested that AMH might regulate follicular recruitment; it would inhibit the initiation of primordial follicle growth and FSH-induced follicle growth, and prevent the depletion of the primordial follicle pool (10).

Because serum AMH levels are likely to reflect both the quantity and the quality of the ovarian follicle pool, the clinical usefulness of its measurement in women has no longer been restricted to the diagnosis and follow-up of GC-derived cancers (11, 12). In patients with the polycystic ovary syndrome (PCOS) higher serum AMH levels before the beginning and during stimulation with gonadotropins were detected as the result of an abnormal activity of the GCs. In addition, it was found that hyperandrogenism was correlated with an additional increase in AMH (13). Higher serum AMH levels have also been observed in obese when compared to normal weight women (14).

Basal AMH, determined before stimulation (usually cycle day 3), was found to be a better measure for the assessment of a decreased ovarian reserve when compared to the classic

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parameters such as an increase in FSH, the decrease of inhibin B, or the antral follicle count (15–17). This observation has recently been confirmed by several other groups (18–20). It has also been shown that AMH was inversely correlated, in addition to age, to basal FSH values (21). The association between the ovarian response and the basal AMH production was confirmed by other studies, showing an increased yield of mature oocytes after controlled stimulation in women presenting high serum AMH levels (19, 22–27). Furthermore, more recent studies demonstrated that basal AMH had a marginally better correlation with the number of collected oocytes and the antral follicle count than did basal FSH or inhibin B (18, 28).

Regarding AMH levels in the serum during FSH stimulation, the literature is much less abundant, and only one longitudinal study reaching into the period after the embryo transfer is currently available (29). During the controlled ovarian hyperstimulation (COH) phase the circulating AMH concentrations were found to differ from those observed in spontaneous cycles (30). This may, however, not be the case in the early luteal phase, as the serum AMH pattern observed (29) is in agreement with a recent study from our laboratory on normal ovulatory cycles (31). The AMH concentrations cross-sectionally determined in the early stimulation phase were suggested to be better predictors of the ovarian response than the measurements of its basal level (14).

Concerning the relation of AMH serum levels and the pregnancy rates (PR) after reproductive therapies such as IVF, however, data from literature are not very clear. Some investigators (14, 26, 32, 33) could not find a correlation between basal AMH levels and prediction of pregnancy, whereas others (23, 28, 34) observed higher basal serum AMH levels to be associated with higher clinical PRs. Similarly, a positive correlation between the embryo score and the serum AMH level at the time of hCG administration was found in one study from 2006 (35), whereas in another study, an even more recent investigation (33), no correlation between serum AMH at day 3 of a control cycle and the embryo morphology was found. A recent study reported a significant association between serum AMH, taken on the first day of a COH cycle, and the outcome of the treatment cycle (pregnancy) using a cutoff level for a negative predictive value (27). Comparisons between the published data are therefore difficult to make due to their heterogeneity.

The concentration of AMH in the follicular fluid (FF) has been analyzed in a small number of studies. It was found to be higher in small than in large follicles, and negatively correlated to the FF P level but not to E_2 (36). Similar to basal serum AMH, FF AMH was positively correlated to the number of retrieved oocytes and negatively to the total dose of FSH administered during the ovarian stimulation (36). In addition, and in spite of the strong variation between individual follicles, AMH concentrations in the FF correlated with serum AMH levels taken on the same day (day of oocyte retrieval). In a report involving fewer patients (n = 31), FF AMH concentrations were positively associated with the fertilization rate (37).

The aim of the present study was to investigate and to compare the relations of AMH, inhibin B, and the female hormones (FSH, E_2, and P), in serum and FF, with the prognostic parameters and the outcome of assisted reproductive treatment (ART). To the best of our knowledge such an investigation has not been performed to date.

MATERIALS AND METHODS

Patient Characteristics and IVF/Intracytoplasmic Sperm Injection Procedure

For the present study, AMH, inhibin B, FSH, and the gonadal hormones E_2 and P have been determined in serum and FF (serum was taken on the day of retrieval; see also the next section "Collection of serum and follicular fluid"). The relations of these hormones have been compared with the prognostic parameters and IVF outcome data.

Serum and FF were obtained from 276 consecutive women undergoing IVF/intracytoplasmic sperm injection (ICSI) in our unit. Criteria for inclusion were a written consent for the treatment by IVF/ICSI, a patient age of 42 years maximum, normal pretreatment hormonal values, gynecological ultrasound results, and cervical smears. Exclusion criteria for an IVF/ICSI treatment were acute or chronic infectious diseases of the woman or her partner, severe psychiatric illnesses, or being a carrier of severe genetic diseases. Most couples presented for a male cause of infertility and were treated by ICSI. The present study was approved by the ethical committee of the University of Berne.

The long stimulation protocol was used for COH in all cases as previously described (38). Thirty-five to 36 hours after hCG administration, the FF with the oocytes was retrieved from all follicles by needle aspiration. Oocyte pick-up (OPU) was performed with transvaginal ultrasound guidance and under routine IV sedation. The quality of the retrieved cumulus–oocytes complexes was morphologically scored (39). The oocytes were denuded by hyaluronidase treatment and assessed for maturity. Only metaphase II (MII) oocytes, identified by the presence of the first polar body, were chosen for insemination or ICSI. Three to 6 hours after OPU ICSI was performed by using described techniques and instrumentation (40). Seventeen hours after the ICSI procedure, fertilization was assessed using an established pronuclei (PN) scoring system (41). From the normally fertilized oocytes (containing 2PN and two polar bodies) two or, exceptionally, one or three with the highest PN score and with the best morphological grade were chosen for embryo transfer and cultured at 37°C in fresh CO_2 equilibrated IVF medium (Vitrolife, Göteborg, Sweden). The remaining normally fertilized oocytes had to be cryopreserved at the PN stage according to the Swiss law. Two or 3 days after OPU, the embryos obtained in culture were assessed