General and Comparative Endocrinology 214 (2015) 56–61

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

An assessment of anti-Müllerian hormone in predicting mating outcomes in female hamsters that have undergone natural and chemically-accelerated reproductive aging

Kristen A. Roosa *, Devin A. Zysling, Ned J. Place

Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

ARTICLE INFO

Article history:
Received 21 January 2015
Revised 9 March 2015
Accepted 15 March 2015
Available online 21 March 2015

Keywords:
Anti-Müllerian hormone
Reproductive aging
Hamster
4-Vinylcyclohexene diepoxide

ABSTRACT

In mammals, female fertility declines with age due in part to a progressive loss of ovarian follicles. The rate of follicle decline varies among individuals making it difficult to predict the age of onset of reproductive senescence. Serum anti-Müllerian hormone (AMH) concentrations correlate with the numbers of ovarian follicles, and therefore, AMH could be a useful predictor of female fertility. In women and some production animals, AMH is used to identify which individuals will respond best to ovarian stimulation for assisted reproductive technologies. However, few studies have evaluated AMH's predictive value in unassisted reproduction, and they have yielded conflicting results. To assess the predictive value of AMH in the context of reproductive aging, we prospectively measured serum AMH in 9-month-old Siberian hamsters shortly before breeding them. Female Siberian hamsters experience substantial declines in fertility and fecundity by 9 months of age. We also measured serum AMH in 5-month-old females treated with 4-vinylcyclohexene diepoxide (VCD), which selectively destroys ovarian follicles and functionally accelerates ovarian aging. Vehicle-treated 5-month-old females served as controls. AMH concentrations were significantly reduced in VCD-treated females yet many females with low AMH reproduced successfully. On average, both young and old hamsters that littered had higher AMH concentrations than females that did not. However, some females with relatively high AMH concentrations failed to litter, whereas several with low AMH succeeded. Our results suggest that mean AMH concentration can predict mating outcomes on a population or group level, but on an individual basis, a single AMH determination is less informative.

1. Introduction

Female fertility declines with age due in part to a progressive decrease in the number of ovarian follicles and the quality of oocytes therein (Gosden, 1987; Navot et al., 1991). The rate of ovarian senescence is variable among individuals because of the many environmental and genetic factors that can influence the process (te Velde and Pearson, 2002). Consequently, in many instances chronological age alone is a poor predictor of female reproductive potential (te Velde and Pearson, 2002), and biomarkers that can accurately and reliably predict the likelihood that a female will reproduce are needed. Because serum concentrations of anti-Müllerian hormone (AMH) correlate with the size of the ovarian follicular reserve in several mammalian species (Appt et al., 2009; Hansen et al., 2011; Kevenaar et al., 2006), AMH has the potential to be a good biomarker of female fertility.

Anti-Müllerian hormone is a homodimeric glycoprotein of the transforming growth factor β family (Cate et al., 1986). First recognized for its role in regression of the Müllerian ducts during male fetal development (Jost, 1953), AMH was originally named Müllerian inhibitory substance. AMH was subsequently identified in adult ovaries (Hutson et al., 1981; Vigier et al., 1984), where it is produced by the granulosa cells of pre-antral and small antral follicles (Baarends et al., 1995; Vigier et al., 1984). AMH regulates the rate at which primordial (non-growing) follicles leave the resting state and initiate growth, which in turn affects the rate of follicle depletion and ovarian senescence (Durlinger et al., 2002). AMH is detected in blood serum, and its concentration correlates well with the numbers of antral follicles (de Vet et al., 2002) and primordial follicles (i.e., the ovarian reserve) (Appt et al., 2009; Hansen et al., 2011; Kevenaar et al., 2006). Serum concentrations decline with age in adulthood, which reflects a progressive decline

* Corresponding author at: College of Veterinary Medicine, Cornell University, S2-072 Schurman Hall, Ithaca, NY 14853, USA.
E-mail address: Kar277@cornell.edu (K.A. Roosa).

http://dx.doi.org/10.1016/j.ygcen.2015.03.007
0016-6480/

in the number of ovarian follicles (Kevenaar et al., 2006). AMH also declines in the circulation of female cancer patients after treatment with chemotherapeutic agents that are toxic to oocytes and follicles (Peigne and Decanter, 2014).

Anti-Müllerian hormone is a promising predictor of female reproductive potential in several situations, and it has been studied most extensively in women. It can be helpful in estimating the time to menopause in women (Hansen et al., 2011; Kevenaar et al., 2006), and it has been extensively used to determine how individual women will respond to ovarian stimulation for the purposes of assisted reproductive technologies (Gleicher et al., 2010; Lukaszuk et al., 2014). Generally, women with low AMH levels are least likely to respond to gonadotropin treatment (Broer et al., 2013b), whereas those with very high levels may be at risk for ovarian hyperstimulation syndrome (Broer et al., 2013a). Similarly, serum AMH concentration can predict the success of ovarian stimulation and embryo retrieval in some production animals, e.g., ovine, caprine, and bovine (Lahoz et al., 2012; Monniaux et al., 2011; Rico et al., 2012). AMH might also be useful in estimating the extent of ovarian damage after exposure to radiation, chemotherapy, or other ovarian toxicants (Visser et al., 2012). However, few studies have investigated the ability of AMH to predict spontaneous pregnancy in healthy, aging females. The utility of AMH as a predictor of mating outcomes has only been studied in humans to date, and the value of AMH for this purpose remains controversial (Casadei et al., 2013; Gleicher et al., 2010; Murto et al., 2013; Streuli et al., 2014). This is because some women with extremely low or even non-detectable AMH values have conceived spontaneously (Casadei et al., 2013; Fraisse et al., 2008; Streuli et al., 2014).

The goal of this study was to determine the predictive value of AMH for spontaneous mating outcomes in the context of ovarian aging in a non-human species. To that end, we measured serum AMH in female Siberian hamsters (*Phodopus sungorus*) that had experienced either natural or accelerated ovarian aging prior to breeding. In our first experiment we measured serum AMH in 9-month-old Siberian hamsters, an age at which females of this species demonstrate significantly reduced fertility and fecundity (Place and Cruickshank, 2010; Place et al., 2004). In the second experiment we measured AMH in young females during the prime of their reproductive lifespan (Place and Cruickshank, 2010) following treatment with the ovarian toxicant 4-vinylcyclohexene diepoxide (VCD) or vehicle. VCD selectively accelerates the natural process of follicle death in primordial and primary ovarian follicles (Springer et al., 1996), which reduces follicle numbers and fertility in hamsters after repeated daily exposure (Roosa et al., 2015). VCD has this effect on the ovary at dosages that do not cause generalized toxicity or accelerated somatic aging (Sahambi et al., 2008). Collectively, the two experiments were meant to test the hypothesis that a determination of serum AMH concentration can predict the outcome of spontaneous matings in hamsters that have undergone natural or chemically-accelerated reproductive (ovarian) aging.

2. Materials and methods

2.1. Animal procedures

Experimental procedures were approved by Cornell University's Institutional Animal Care and Use Committee. Food (Teklad 8626, Madison, WI) and water were available *ad libitum*. Ambient temperature and relative humidity were held constant at 21 ± 5 °C and 50 ± 10% respectively. The time of lights off was synchronized for all animals to 1800 Eastern Standard Time (EST). Hamsters from our breeding colony were originally derived from wild-bred stock obtained from Dr. K. Wynne-Edwards, Queens University (Kingston, Ontario, Canada).

2.1.1. Experiment 1: AMH as a predictor of mating outcomes in naturally-aged females

Twenty-eight virgin female hamsters were held in 14 h light/-day from birth to 9 months of age. For each female, a small blood sample (~100 μL) was collected from the retro-orbital sinus under isoflurane anesthesia 2 weeks before she was paired with an adult male. Pairings were maintained for 10 days, which allowed for at least two estrous cycles. Females were inspected daily for the presence of a postcopulatory vaginal plug. After separation from the male, litter checks were performed twice daily. The number of live pups was recorded on the date of birth (DOB) and at weaning on postnatal day 18 (PD18). Mass of each live weanling was also recorded on PD18, and each dam was sacrificed by CO_2 inhalation at this time. The uterus was removed, examined for fetoplacental tissue, and stained for implantation sites with ammonium sulfide and potassium hexacyanoferrate (Salewski, 1964). Because gestation length is 18 days, hamsters that failed to litter 20 days after separation from the male were sacrificed at this time and the uterus was inspected and stained as described above.

2.1.2. Experiment 2: AMH as a predictor of mating outcomes after accelerated ovarian aging

As part of a larger study to evaluate the toxic effects of VCD under long and short photoperiodic conditions (Roosa et al., 2015), separate cohorts of hamsters from our colony were paired in long days (16 h light/day) or short days (8 h light/day) ($n = 20$ per photoperiod) to generate experimental females that would be mated at 5 months of age. Experimental females were weaned on PD18 and placed individually in polypropylene cages. Females were maintained in their natal photoperiod and assigned to control and VCD-treated groups at 10 weeks of age. At this age, 240 mg/kg/d intraperitoneal VCD (Sigma Aldrich, St. Louis, MO) or vehicle (1:1 mixture of 0.9% saline and DMSO) injections were administered over 10 days. The VCD dosage was based on previous studies in mice (Sahambi et al., 2008) and a pilot study in hamsters in which substantial reductions in ovarian follicle numbers were observed. All injections were given during the light phase of the light–dark cycle between 10:00 and 12:00 EST.

Sexual maturity is delayed when female hamsters are reared in short days (Adam et al., 2000); therefore animals in short photoperiod were transferred to long days 1 week following the final injection to induce sexual maturation. After 7 weeks in long days, blood was collected from the retro-orbital sinus for serum AMH determination. After 8 weeks, each virgin female was transferred to a clean cage and paired with an adult male for 15 days. Animals that were reared and treated in long days remained in this photoperiod, and the timing of blood collection and male pairing were identical to that of females previously held in short days. After pairing, the experimental procedures were the same as those described in Experiment 1. Because photoperiod did not modulate VCD ovarian toxicity (Roosa et al., 2015), the long- and short-day groups were combined for the assessments of AMH as a predictor of mating outcomes.

2.2. Serum AMH measurements

Whole blood was clotted on ice for 1 h, and serum was removed after centrifugation (1000*g*) and frozen at −80 °C until assayed. AMH was measured by a commercially available enzyme-linked immunosorbent assay kit (Ansh Labs, Webster, TX) that we had validated for Siberian hamsters. Each sample was run in duplicate, according to the manufacturer's instructions. Serial dilutions of pooled Siberian hamster serum samples yielded concentrations