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Article

Lower follicular fluid vitamin D concentration is related to a higher number of large ovarian follicles

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KEY MESSAGE

The lowest concentration of 25OHD3 in the first follicle fluid aspirated in patients submitted to ovarian stimulation indicates a greater response based on the number of larger follicles and higher serum oestradiol concentrations.

A B S T R A C T

Vitamin D receptor-knockout mice fail to produce mature oocytes, indicating vitamin D is crucial for folliculogenesis in mice. However, the actions of vitamin D during folliculogenesis remain unknown. This prospective study aimed to assess whether follicular fluid (FF) vitamin D (25OHD3) concentrations are related to specific responses to ovarian stimulation. Women undergoing ovarian stimulation for IVF participated in the study. FF 25OHD3 concentrations were assessed in the first follicle aspirate on oocyte retrieval day. Oestradiol and progesterone concentrations were assessed on the trigger day. K-means grouping analysis showed that 25OHD3 FF concentrations clustered into a higher and lower group (mean ± SEM 17.4 ± 6.61 ng/ml and 35.5 ± 7.17 ng/ml, respectively, $P < 0.001$). The clusters were analysed according to the oestradiol and progesterone concentrations, follicle number and size and resulting oocyte number and maturity. The FF 25OHD3 concentrations were no different among the infertility diagnoses. The lower 25OHD3 group had more follicles (≥16.0 mm, $P = 0.009$) and higher serum oestradiol concentrations ($P < 0.03$) on the day of HCG administration. In this study, lower follicular 25OHD3 concentrations predicted a better response to ovarian stimulation shown by a greater production of larger follicles and higher serum oestradiol concentrations.

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Introduction

A low concentration of vitamin D is considered a public health problem in several countries, including Brazil. Recently, vitamin D deficiency has been considered a pandemic issue, affecting every continent (Palacios and Gonzalez, 2014). One important aspect is how this deficiency affects women of reproductive age. In humans, 80–90% of vitamin D is synthesized in the skin after exposure to ultraviolet rays, and the remainder is obtained through diet. Importantly, low vitamin D concentrations have been associated with an increased risk of several diseases in humans (Rosen et al., 2012), although the data reporting a causal relationship between vitamin D deficiency and those diseases are limited.

The predominant form of vitamin D found in the bloodstream is 25-hydroxyvitamin D (25OHD3), whereas the active form, 1,25OHD3, is produced in the kidney or locally in several tissues (Irani and Merhi, 2014a). The classic vitamin D pathway involves the binding of 1,25OHD3 to its intracellular receptor, vitamin D receptor (VDR). The VDR–1,25OHD3 complex crosses into the nucleus and binds DNA as a transcription factor (Rosen et al., 2012).

Classical actions of vitamin D were originally described in relation to calcium–phosphate homeostasis. However, regardless of their origin, active forms of vitamin D may have distinct functions (Irani and Merhi, 2014a), as VDR expression and signalling have been detected in several tissues such as the pituitary, testis, ovary, uterus, pancreas and muscle (DeLuca, 2004; Ozkan et al., 2010). In particular, the presence of VDR in gonadal tissues and other reproductive organs in humans, as well as the existence of several animal models showing adverse reproductive outcomes related to the lack of VDR signalling (Du et al., 2005; Johnson and DeLuca, 2001; Panda et al., 2001), have raised a pertinent discussion about the effects of vitamin D on reproductive outcomes in humans.

Although conflicting evidence suggests that follicular fluid (FF) concentrations of vitamin D have an impact on clinical outcomes of in vitro fertilization (IVF) such as implantation rates, clinical pregnancy rates and live birth rates (Anifandis et al., 2010; Farzadi et al., 2015; Lv et al., 2016; Ozkan et al., 2010; Paffoni et al., 2014), no studies have determined whether the intrafollicular concentrations of pro-vitamin D (25OHD3) were correlated to ovarian response and follicular dynamics in patients undergoing IVF cycles. Therefore, the present study aimed to investigate whether intrafollicular 25OHD3 concentrations are related to the type of ovarian response in patients undergoing IVF – more specifically, to investigate the relationship between FF concentrations of 25OHD3 and several outcomes of ovarian stimulation, such as number and final size of follicles stimulated per cycle, concentrations of progesterone and oestradiol and number of metaphase II oocytes retrieved. Through examining these outcomes, it was possible to evaluate the direct role of intrafollicular vitamin D on the oocyte and follicle itself, avoiding clinical outcomes that could easily be misleading due to the considerable number of influential variables that cannot be controlled. This type of approach is the first of its kind to try to determine the actual influence of intrafollicular vitamin D on follicular dynamics and hormonal production as well as oocyte maturation in IVF cycles.

Materials and methods

The current study had a prospective design. All patients eligible for IVF/intracytoplasmic sperm injection (ICSI) were considered as potential subjects. Women who underwent ovarian stimulation for IVF/ICSI cycles from September 2013 to September 2015 were recruited to the study. The exclusion criteria were use of vitamin D supplementation or an aromatase inhibitor during the ovarian stimulation and cycle cancellation.

All subjects signed an informed consent during their first medical evaluation in the centre. None of the patients were categorized by ethnicity due to the mixed genetic heterogeneity of the Brazilian population (Lins et al., 2011). The project was revised and approved by the Ethics Committee on Research at Maternidade Escola, Universidade Federal do Rio de Janeiro (project number 02213812.4.0000.5275, approval date 6 September 2012).

Ovarian stimulation protocols

All patients used combinations of FSH alone (Gonal-F®; Merck-Serono, Italy); or FSH and LH (Pergoveris®; Merck-Serono, Italy); or Menopur® alone (Ferring Pharmaceutical, Germany); or Menopur® plus Bravelle® (Ferring Pharmaceutical, Germany); or recombinant FSH (Gonal-F®; Merck-Serono, Italy) plus Menopur® (Ferring Pharmaceutical, Germany). Pituitary suppression was achieved with the gonadotrophin-releasing hormone (GnRH) antagonist analogue cetrorelix acetate (Cetrotide® 0.25 mg; Merck-Serono, Italy), and the final maturation trigger was recombinant human chorionic gonadotrophin (HCG) (Ovidrel® 250 µg; Merck-Serono, Italy).

Ovarian stimulation began at day 2 or 3 after the start of menses. Based on the clinician criteria, the FSH dosage varied from 150 IU/day to 300 IU/day, and the LH dosage ranged from 75 IU/day to 300 IU/day.

Pituitary suppression with cetrorelix acetate began when the first follicle reached 14 mm in diameter. From this point forward, daily 0.25 mg injections were given until Ovidrel® was administered. Recombinant HCG was administered when at least one follicle reached 18 mm or two follicles reached 16 mm at their largest diameter. Oocyte pick-up was performed 35 h post Ovidrel® administration.

FF collection

FF aspiration was performed transvaginally using a transvaginal ultrasound probe as a guide (Medison X8®). A 17G oocyte aspiration needle (Wallace®) connected to a closed vacuum system under 90 mmHg of negative pressure was used to empty the follicles.

Before follicle aspiration was initiated, one follicle equal or larger than 16 mm at its largest diameter was selected and placed into a sterile container. After fluid from the chosen follicle was collected, the aspiration needle was removed from the ovary, and the tubing contents were emptied into a sterile receptacle. The fluid was immediately checked for the presence of an oocyte. When present, the oocyte was placed in culture medium, and the FF was immediately frozen in liquid nitrogen (–196°C). This technique allowed the storage of fluid from a single follicle equal or larger than 16 mm in diameter without contamination by fluid from other follicles.