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SHORT COMMUNICATION

The effect of human follicular fluid on bovine oocyte developmental competence and embryo quality


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Abstract In this study, the hypothesis that embryo development during routine IVF procedures is determined by the pre-ovulatory follicular fluid composition was tested. Follicular fluid from women with obesity ('obese') and a 'positive' or 'negative' IVF outcome was added during the in-vitro maturation of bovine oocytes. 'Negative' and 'obese' follicular fluid reduced bovine embryo development, compared with laboratory control embryo development ($P < 0.05$ or $P < 0.1$). The addition of follicular fluid also altered bovine blastocyst gene expression. Furthermore, *LDHA* and *PPARGC1B* gene expression differed between follicular fluid groups. Data suggest that pre-ovulatory follicular fluid can potentially affect oocyte developmental competence and embryo quality. Furthermore, the bovine model may be used as a screening tool. 

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KEYWORDS: embryo quality, follicular fluid, in-vitro maturation, IVF, oocyte developmental competence

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Introduction

Maternal metabolic disorders, such as obesity and type 2 diabetes, are associated with hormonal and biochemical changes in the serum, which are reflected in the micro-environment of the maturing oocyte, the follicular fluid (Leroy et al., 2012; Valckx et al., 2012). Such changes, such as follicular fluid free fatty acid (FFA) concentrations, hamper in-vitro bovine oocyte developmental competence as well as embryo quality and metabolism (Van Hoeck et al., 2011). Moreover, adding human follicular fluid high in triglycerides and FFAs from obese patients impaired murine oocyte maturation and caused endoplasmic reticulum stress (Yang et al., 2012). Furthermore, adding bovine follicular fluid with high concentrations of FFA during bovine in-vitro cumulus-oocyte complex (COC) maturation, resulted in a massive intracellular lipid storage in the cumulus cells, which may be seen as a protective mechanism (Aardema et al., 2013). Other parameters, such as insulin, cholesterol and total protein in the follicular fluid have also been studied (Valckx et al., 2012), but it remains unclear whether and how the composition of the pre-ovulatory follicular fluid may influence the enclosed oocyte's developmental success during in-vitro embryo growth. The aim of this study was to test whether the follicular fluid composition during final maturation *in-vivo* might influence the quality of embryos generated during human IVF procedures. To investigate this, it was essential to uncouple intrinsic oocyte quality from potential effects caused by the follicular fluid. Therefore, human follicular fluid was used in an entirely independent in-vitro setting, with the aim of studying the effect of exposing bovine in-vitro maturing oocytes to human follicular fluid from women with differential IVF outcomes or obesity, on bovine oocyte developmental competence and subsequent embryo quality. The bovine model was chosen because of multiple similarities between human and bovine ovarian physiology, oocyte maturation and early embryo development (Menezo and Herubel, 2002). To our knowledge, this is the first time this model has been implemented as a potential screening tool to study the effect of the composition of pre-ovulatory follicular fluid on oocyte and embryo quality.

Materials and methods

Collection and selection of follicular fluid samples

Samples were collected as previously described by Valckx et al. (2012), with ethical approval (FER-P0905/F18). Because numerous variables may influence IVF outcome, strict exclusion criteria were applied to eliminate known confounders: age over 38 years, body mass index (BMI) less than 18 kg/m², polycystic ovarian syndrome, blood sample contamination, fewer than six oocytes aspirated, less than 50% successfully fertilized oocytes and the male factors cryptozo- or azoospermia. Selected samples consequently originated from women that reacted well to ovarian stimulation, with the exclusion of major male and female subfertility risk factors. Three groups were defined using specific inclusion criteria: 'negative' ($n = 6$): 30% or less day 3 good-quality embryos (criteria as described in Valckx et al., 2012) originating from that oocyte retrieval session, BMI less than 30 kg/m² and the oocyte

originating from the follicular fluid sample did not develop into a good-quality embryo; 'positive' ($n = 6$): 50% or more day 3 good-quality embryos originating from that oocyte retrieval session, BMI less than 30 kg/m² and the oocyte originating from the follicular fluid sample developed into a good-quality embryo; and 'obese' ($n = 6$): BMI over 30 kg/m².

Following these criteria, six samples per treatment group, originating from 15 women, were selected out of a total of 120 samples from 70 women.

Bovine in-vitro embryo production

Immature bovine COCs were isolated from slaughterhouse ovaries and matured in groups of 50 for 24 h in 500 μ l serum-free maturation medium, supplemented with 25% heat-inactivated follicular fluid pools from the different treatments. A routine serum and follicular fluid free laboratory control was run in parallel. Oocytes were fertilized and presumptive zygotes cultured in groups of 25–30 embryos (50 μ l serum-free synthetic oviduct fluid medium droplets under oil), after routine laboratory procedures as in Van Hoeck et al. (2011) with minor modifications.

Bovine oocyte and embryo quality parameters (four replicates)

In each replicate, 100 COCs were cultured in each treatment group. Five or six oocytes and presumptive zygotes per treatment and per replicate were stained in 5 μ g/ml 4',6-diamidino-2-phenylindole (DAPI) (5 min, Sigma-Aldrich, Bornem, Belgium), to determine maturation and fertilization rate, respectively. Denuded mature oocytes were collected after 24-h in-vitro maturation (10-min vortexing to remove cumulus cells) and presumptive zygotes were collected after fertilization (3-min vortexing). Cleavage rate and blastocyst formation, for the remaining presumptive zygotes, were documented on days 2 and 8 post insemination, respectively. Six or seven day-8 expanded blastocysts, per treatment and per replicate were stained with DAPI and TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling (Roche Diagnostics, Vilvoorde, Belgium) as previously described (Van Hoeck et al., 2011) to study total cell number and apoptotic cell ratio, respectively.

Gene expression (four replicates)

An equal number of normal and expanded blastocysts for each treatment was snap frozen (in groups of 10) on day 7.5 of culture, and analysed for the transcript abundance of the genes presented in Figure 1. These genes were particularly chosen for their relevance in embryo development, quality and metabolism. mRNA isolation, retrotranscription and real-time polymerase chain reaction quantification were carried out as described by Van Hoeck et al. (2011), with minor modifications.

Statistical analyses

PASW 18.0 for Windows, Chicago, IL, USA was used for statistical analysis. Binary outcomes (oocyte and embryo

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