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No single way to explain cytoplasmic maturation of oocytes from prepubertal and cyclic gilts

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Abstract

The objective of this study was to evaluate selected aspects of cytoplasmic maturation in oocytes from prepubertal and cyclic crossbred gilts before and after in vitro maturation. For this purpose, cortical granule redistribution, mitochondrial DNA content and mitochondria translocation were analyzed. Moreover, for the first time the fatty acid profiles in follicular fluid (FF) of both gilt categories was evaluated. The nuclear maturation (the percentage of metaphase II oocytes was 83% in prepubertal gilts compared with 87% in cyclic gilts), cortical granule relocation from the cortex to peripheral ooplasm (98.7% vs. 98.8% of oocytes, respectively) and mitochondrial DNA content (227 543 vs. 206 660, respectively) was not affected by sexual maturity of the donor gilt. However, the redistribution of active mitochondria during in vitro maturation was observed only in the oocytes of cyclic gilts. With regard to FF analysis, saturated, unsaturated, and monounsaturated fatty acids were significantly more abundant in the FF of prepubertal females. In particular, stearic (C18:0) and palmitic (C16:0) fatty acids had significantly higher concentrations in the FF of prepubertal gilts. In conclusion, although the oocytes of prepubertal gilts matured in vitro at a rate similar to those of cyclic gilts, they differed with respect to the selected factors attributed to cytoplasmic maturation. We suggest that the higher content of particular fatty acids, which is known to have a negative influence on oocyte maturation, as well as impaired mitochondria redistribution are factors limiting the maturation potential of oocytes from prepubertal gilts.

Keywords: Puberty; Oocyte quality; Cytoplasmic maturation; Follicular fluid; Gilt

1. Introduction

Oocyte quality (competence, viability) is referred to as the ability to resume meiosis, cleave after fertilization and finally develop to term in good health [1]. It is mainly attributed to the intrinsic properties acquired during development in the ovarian follicle [2]. However, because of the complexity of the subject, the current state of knowledge regarding the role of particular factors influencing this trait is ambiguous and incomplete. Several parameters related to oocyte quality have been described, which include both intrinsic (e.g., the diameter of the oocyte) and extrinsic factors (e.g., the maturation environment) [3]. The reduced quality of oocytes and embryos of prepubertal females has been demonstrated in several species, such as pig [4], cattle [5,6], and goat [7]. This reduction is believed to be because of insufficient cytoplasmic maturation, which is reflected in decreased in vitro fertilization (e.g., polyspermy) and embryo culture efficiency.

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The majority of published data compare oocytes of prepubertal females with those of adult animals. Only some experiments have focused on comparing oocytes of the two categories of peripubertal pigs: sexually immature prepubertal gilts (P) and sexually mature cyclic gilts (C). Oocytes of prepubertal gilts showed similar [8,9] or lower [4,10] meiotic competence when compared with those of sexually mature females (cyclic gilts or adult sows). Although Bagg et al. [8] did not observe significant differences, the blastocyst rate has usually been lower for oocytes derived from the P females [9,11,12]. It was hypothesized that this difference is because of the predominance of small follicles on the ovaries of prepubertal gilts (diameter $\leq$ 3 mm [10]) containing oocytes that are not fully competent [11]. However, reduction in oocyte quality has been restricted to P gilts because it has not been noticed in the oocytes derived from small follicles of cyclic gilts and adult sows. Therefore, it may be suggested that P oocytes show variable quality, which in our opinion is related to factors affecting ooplasmic maturation. It has been shown that the cytoplasmic maturation of oocytes from prepubertal goats, sheep, and heifers, defined by the migration of organelles, such as cortical granules and mitochondria, is altered [13–15]. Moreover, it is of particular importance to store a sufficient number of mitochondria because no mitochondrial DNA (mtDNA) replication occurs between the mature oocyte and blastocyst stages [16–19]. The amount of mtDNA has also been correlated with developmental potential of murine, porcine, and bovine oocytes [18,20,21]. To date there are no published data concerning mtDNA content and mitochondria distribution in oocytes in terms of the sexual maturity of the donor.

The beneficial role of follicular fluid in cytoplasmic maturation is especially evident in the pig [22]. Recent studies revealed that the main role of follicular fluid (FF) is to protect the oocyte against oxidative stress [23]. Although FF is routinely used in porcine IVM, relatively little work focuses on the specific factors that may affect oocyte quality. Among the potential factors are fatty acids (FA); however, the data supporting the effect of porcine FF are limited when compared with the data supporting the effect of bovine FF [24–26]. Bovine oocytes with reduced competence originated from follicles with higher C16:0 and lower C18:3 n-3 content (Matoba et al., International Embryo Transfer Society Meeting 2011, poster). Bender et al. [27] observed more C16:0, C18:0, C18:1 n9c, and C18:3 n-3 in the FF of adult cows with reduced fertility when compared with heifers. The addition of C16:0 and C18:0 to IVM media reduced the maturation, cleavage, and blastocyst rates [28,29].

The objective of this study was to evaluate selected factors related to cytoplasmic maturation to identify the causes of reduced oocyte quality in prepubertal gilts. Moreover, we also aimed to investigate whether this phenomenon could be related to differences in the composition of the FF.

2. Materials and methods

Unless stated otherwise, all chemicals and reagents used in this study were purchased from Sigma-Aldrich (Munich, Germany). The ovaries used in our experiment were collected postmortem from commercially slaughtered gilts.

2.1. Recovery of COCs

Cumulus-oocyte complexes (COCs) were collected from the ovaries of commercially slaughtered crossbred gilts at the age of 5 to 6 mo and a weight of approximately 100 to 110 kg. The reproductive tract was removed from each slaughtered gilt, and the ovaries were excised and placed in a separate plastic container for transportation. The ovaries were transported to the laboratory in a thermo isolated flask within 2 h of animal slaughter. Upon arrival, the ovaries were divided into two groups based on their morphology: P (lack of corpus luteum, several 1 to 4 mm follicles) and C (presence of corpus luteum or albicans, 3 to 6 mm follicles) [8]. COCs were aspirated from the 2 to 6 mm follicles with a syringe, placed in HEPES-Tyrode's Albumin-Lactate-Pyruvate (TALP) medium and morphologically evaluated under a stereomicroscope. Only the COCs with evenly granulated cytoplasm and at least three compact cumulus cell layers were selected for the experiment. Half of the collected COCs were analyzed directly after the collection, whereas the other half were subjected to in vitro maturation and analyzed afterward. Consequently, four groups of porcine COCs were generated: (1) P (without IVM), (2) P IVM, (3) C (without IVM), and (4) C (IVM).

Each of the investigated parameters was analyzed in three replicates (three independent pools of oocytes) before IVM and in three replicates after in vitro maturation. The COCs were collected from batches of P and C ovaries and after morphologic evaluation randomly allocated into specific experimental groups. Each batch of collected COCs was analyzed for all parameters.