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Low oxygen tension during in vitro maturation of porcine follicular oocytes improves parthenogenetic activation and subsequent development to the blastocyst stage

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Abstract

To establish a reliable in vitro maturation system for activation and subsequent development as nuclear recipients for the effective production of pig clones, we assessed maturation, activation and parthenogenetic development in response to the following: (1) type of immature oocytes (cumulus–oocyte complexes (COCs) or parietal granulosa plus cumulus–oocyte complexes (GCOCs)); (2) oxygen (O_2) tension (5 or 20%); and (3) maturation period (36–60 h). The rate of nuclear maturation

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to metaphase-II (M-II) in the GCOC group (73.0 ± 3.1%) was higher than that in the COC group ($P < 0.05$, 60.6 ± 3.5%), but the rates did not differ between the 5 and 20% O_2 tension groups. M-II rate increased ($P < 0.05$) to about 70% after 42 h and then remained constant until 60 h of culture. When oocytes were matured under 5% O_2 tension and stimulated, the rate of normal oocyte activation (a female pronucleus formation and emission of the second polar body) was higher ($P < 0.05$, 38.5 ± 3.9%) than when oocytes were matured under 20% O_2 tension (24.5 ± 3.9%). On the other hand, the rate of normal activation was not significantly different between the COC and GCOC groups, and the highest ($P < 0.05$) normal activation rate was obtained in oocytes cultured for 48 and 54 h (48.4 ± 5.5% and 47.9 ± 8.2%, respectively). When COC and GCOC matured for 48 h under 5 and 20% O_2 tension were stimulated and subsequently cultured in vitro for 6 days, the rate of blastocyst formation did not differ between the oocyte types nor between the O_2 tension groups. However, blastocyst quality, as measured by mean total cell number, was significantly higher in the 5% O_2 group ($P < 0.05$, 34.6 ± 2.0 for COC; 33.8 ± 1.8 for GCOC) compared with the 20% O_2 group (25.9 ± 1.8 for COC; 27.0 ± 2.0 for GCOC). In conclusion, low O_2 tension (5%) during in vitro maturation of porcine oocytes promoted their ability to be activated normally and improved the quality of parthenogenetic blastocysts developed in vitro in modified NCSU-37 solutions. This knowledge may be applicable for preparation of in vitro matured oocytes with good quality as recipient oocytes for generating pig clones.

Keywords: Pig oocyte; IVM; Oxygen tension; Activation; Blastocyst

1. Introduction

The success of pig cloning from somatic cells is affected by several factors, such as the source of somatic cells as donor cells, the method of nuclear transfer and the activation protocol. As well as these factors, cytoplasmic ability for development or, in other words, the degree of ''cytoplasmic maturation'' of recipient oocytes, seems to be important [1]. In vivo matured oocytes are of good quality and can be used as recipient oocytes for pig cloning [2–6]. On the other hand, the use of in vitro matured (IVM) oocytes is preferable because it is less costly and time-consuming to prepare large numbers of recipient oocytes and it facilitates timing of collection of matured oocytes. Reports on the success of pig cloning using IVM oocytes [7–9] are encouraging. However, quality control is an important factor for the use of IVM oocytes because they are considered to have poor developmental ability [10]. Many attempts have been made to produce IVM oocytes of good quality [11]. Some IVM oocytes after in vitro fertilization (IVF) can develop into blastocysts of high quality [12]; normal piglets with high farrowing rates were obtained after the transfer of these to recipients [13,14]. Therefore, a population of follicular oocytes seems to have developmental ability after nuclear transfer even when they are matured in vitro. Furthermore, the oocyte selection protocol is very important for obtaining oocytes of good quality. However, before or even after maturation culture, it is difficult to predict whether the oocytes retain their normal activation and developmental ability. The development of a reliable IVM system for generating recipient oocytes with a certain level of developmental competence is essential. Morphological evaluation of oocyte types seems to be the only method to predict this ability. In general, oocytes with multiple layers of cumulus cells are selected and processed for IVM [15,16]. The oxygen (O_2) tension during