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Effects of hexoses on in vitro oocyte maturation and embryo development in pigs

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Abstract

The objective was to determine the effects of supplementing hexoses in oocyte maturation and embryo culture medium on in vitro maturation (IVM) and in vitro fertilization (IVF) of porcine oocytes and in vitro development of in vitro produced (IVP) porcine embryos. In the first experiment, oocytes were matured in vitro in modified North Carolina State University (NCSU)-37 medium, supplemented with hexoses (glucose, fructose or galactose) at various concentrations: 0 (control), 2.5, 5.5 and 10 mM. Supplementing the maturation medium with either glucose or fructose (5.5 mM) increased the percentages of oocytes that matured to metaphase II (79.4 and 70.2%, respectively), as compared with the control group ($P < 0.05$). However, supplementing galactose had no effects on meiotic maturation and fertilization. In the second experiment, cleaved embryos were collected 3 days after IVF of oocytes matured in the maturation medium supplemented with 5.5 mM of glucose; they were cultured for an additional 4 days in modified NCSU-37 medium, supplemented with 5.5 mM of glucose, fructose or galactose. The incidence of blastocyst formation was higher ($P < 0.05$) in the glucose and fructose groups (18.6 and 18.2%, respectively) than in the galactose group and non-supplemented control group (12.9 and 9.2%). Moreover, fructose supplementation increased the total cell number/blastocyst (48.0 versus 37.6) and reduced the index of DNA-fragmented nucleus in the blastocysts (7.6% versus 11.8%), as compared with glucose supplementa-

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tion ($P < 0.05$). In conclusion, fructose was a practical alternative to glucose for supporting IVM of porcine oocytes and fructose was superior to glucose for producing high-quality porcine embryos in vitro.

Keywords: Hexose; Porcine embryo; IVF; Quality

1. Introduction

The environment in which oocytes are cultured during in vitro maturation (IVM) and in vitro produced (IVP) embryos are cultured after in vitro fertilization (IVF), play an important role in in vitro development of embryos. The types and concentrations of energy substrates added to a culture medium alter metabolic profiles, development and quality of embryos [1]. The North Carolina State University (NCSU) medium [2], which contains glucose as a potential energy substrate, has been widely used for IVM of porcine oocytes and subsequent in vitro culture (IVC) of IVP embryos [3,4]. During IVM, the metabolism of glucose, via glycolysis and the pentose phosphate pathway (PPP), not only supplies substrates that contribute to ooplasmic integrity, but is also linked to regulation of meiotic maturation of oocytes [5–7]. Glucose has been known to have a detrimental effect on early-stage development of embryos, but it supplies an important energy substrate to embryos at the compacted morula and blastocyst stages [8,9]. Like glucose, fructose and galactose are monosaccharide hexoses capable of entering the glycolytic pathway. Galactose impaired the development of hamster and bovine embryos [10,11]. However, galactose was present in porcine follicular fluid (pFF) that surrounds oocytes during physiological maturation [12]. Fructose was present in the reproductive tract of many species [13–17] and effectively supported embryonic development in hamster [11], bovine [10,18], mouse [19] and human embryos [20]. Replacement of glucose with fructose in a culture medium supported the development of embryos at the cleavage stage as well as glucose-containing medium, and fructose can increase total cell numbers in both hamster and bovine blastocysts [10,11]. In contrast, fructose can not completely compensate as an energy source in mouse embryos [19], indicating that a certain aspect of embryo metabolism may be species-specific. In pigs, the effects of hexose during maturation and development of embryos are not well understood.

The objectives of the present study were to determine whether fructose and/or galactose are practical alternatives to glucose for supporting meiotic maturation of porcine oocytes and their subsequent development. Furthermore, the quality of embryos produced by each hexose supplementation was evaluated to establish an effective culture system for porcine embryos.

2. Materials and methods

2.1. Recovery and IVM of oocytes

Ovaries from prepubertal crossbred gilts were collected at a local abbatoir and transported to the laboratory in physiological saline (0.85% [w/v] NaCl) at 35 °C. Cumulus