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In vitro maturation and artificial activation of donkey oocytes

Gaoping Zhao[a], Kaifeng Wu[b], Liang Cui[b], Lixia Zhao[b], Yiyi Liu[b], Xiuwen Tan[c], Huanmin Zhou[b,*]

[a] *College of Animal Science and Veterinary Medicine, Inner Mongolia Agricultural University, Hohhot 010018, China*
[b] *College of Life Science, Inner Mongolia Agricultural University, Hohhot 010018, China*
[c] *Academy of Animal Husbandry and Veterinary Medicine, Shandong Academy of Agriculture Science, JiNan 250000, China*

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Abstract

Three media were evaluated for their ability to support *in vitro* maturation of donkey (*Equus asinus*) oocytes and their development after parthenogenetic activation. The basal medium for Medium 1 (M1) and Medium 2 (M2) was M199 and DMEM/F12 respectively, whereas, Medium 3 (M3) consisted of equal parts (v/v) of M199 and DMEM/F12. All three media were supplemented with 10% (v/v) fetal calf serum, 0.01 units/mL porcine FSH, 0.01 units/mL equine LH, 200 ng/mL insulin-like growth factor 1(IGF-I), 10 μl/mL insulin-transferrin-selenium (ITS), 0.1 mg/mL taurine, 0.1 mg/mL L-cysteine, 0.05 mg/mL L-glutamine, 0.11 mg/mL sodium pyruvate, and 25 mg/mL gentamycin. There were no significant differences among the three maturation media for oocyte maturation. Maturation rate of donkey oocytes in M1 was 53% for compact (Cp) cumulus-oocyte complexes and 75% for expanded (Ex) cumulus-oocyte complexes; in M2 these were 55 and 77%, respectively; and in M3, 58 and 75%. The percentage of cleaved parthenotes and 4- or 8-cell embryos were not significantly different for oocytes matured in the various media (61 and 24% for M1; 66 and 32% for M2; and 67 and 33% for M3). Oocytes matured in M3 tended to yield a higher rate of advanced embryo development (morula) than oocytes matured in M1 (22 vs 9%; $P = 0.07$). In conclusion, donkey oocytes were matured and parthenogenetically activated *in vitro*, using methods similar to those used in the horse.
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Keywords: Oocyte; In vitro maturation; Parthenogenetic activation; Equus asinus

1. Introduction

In recent years, development of assisted reproductive technologies such as *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), and somatic cell nuclear transfer (SCNT) has been rapid; *in vitro* maturation (IVM) of the oocyte was one of the key steps in developing these technologies. At present, offspring have been obtained by the above-mentioned technologies using oocytes matured *in vitro* in a variety of domestic livestock, including sheep [1], cows [2–5], pigs [6–8], mules [9,10], and horses [11–13]. Much research involving IVM has been conducted with respect to the horse oocyte, but there is little known regarding the donkey oocyte. As a source of protein and raw material for production of medications, the commercial value of the donkey tends to attract widespread attention. Therefore, the study of IVM and other assisted reproductive technology for the donkey would have great importance.

In previous studies, *in vitro* maturation in horse oocytes was performed in conditions similar to those used for cattle oocytes, using a basal medium (M199)

* Corresponding author. Tel.: +86 471 4309386; fax: +86 471 4309386.
E-mail address: huanminzhou@263.net (H. Zhou).

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[12,14–19], which resulted in high development rates for equine blastocysts (> 30%) [19]. Recently, it was reported that DMEM/F12-based media [20] supported horse oocyte maturation *in vitro,* and provided higher rates of blastocyst development than M99-based medium in that laboratory (26 vs 12%, respectively).

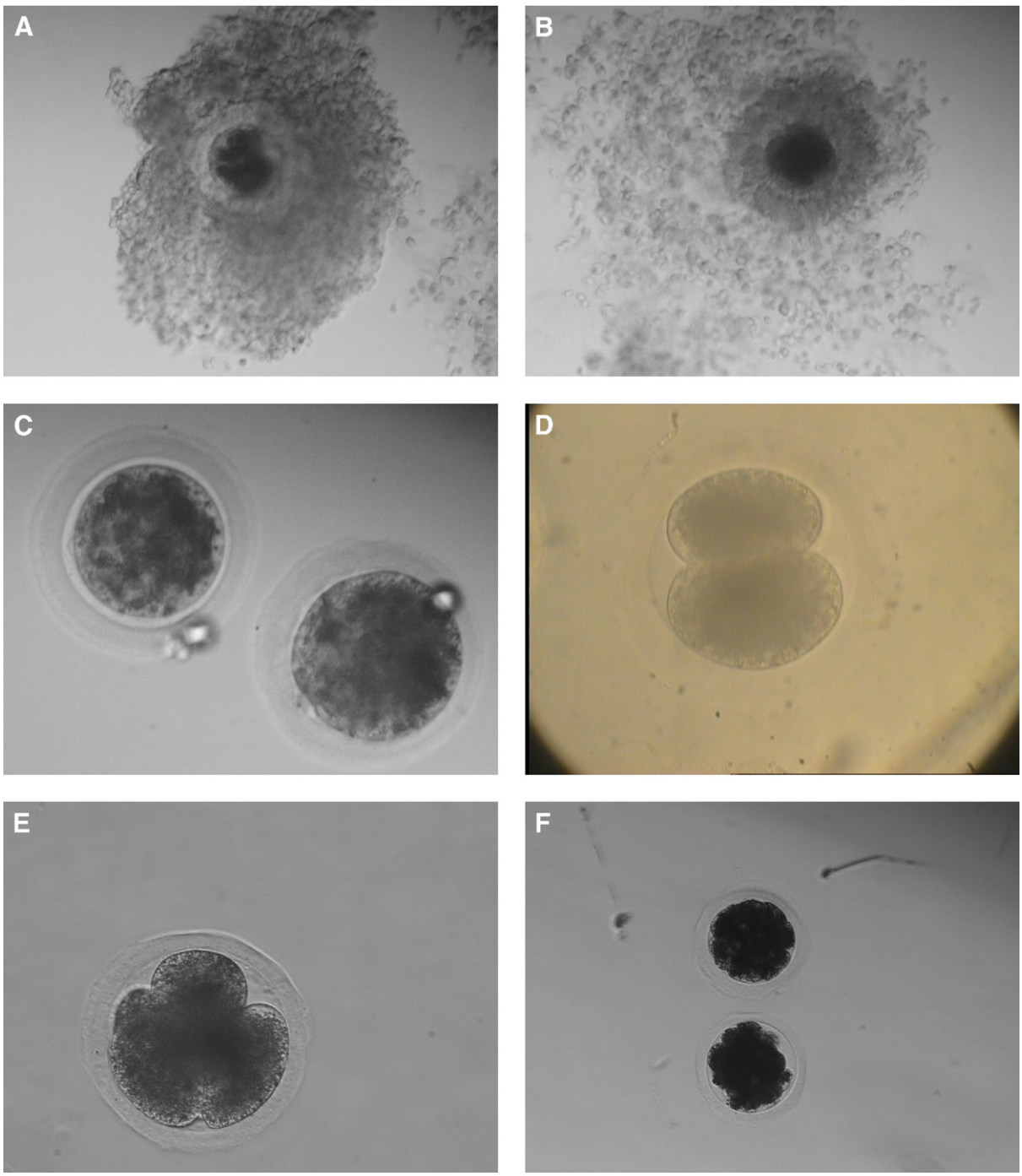

Fig. 1. Donkey oocytes and embryos. (A) Compact (Cp) cumulus-oocyte complexes (100X); (B) Expanded (Ex) cumulus-oocyte complexes (100X); (C) Oocytes with a polar body, as indicated by the arrow (200X); (D) Two-cell stage parthenogenetic embryo (200X); (E) Four-cell stage parthenogenetic embryo (200X); and (F) morulae (100X).

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