Chronology of in vitro maturation of donkey (Equus asinus) oocytes collected by ovum pick up

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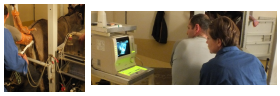
Introduction

Most wild donkey breeds are currently endangered and many domestic donkey breeds are vulnerable to extinction. Embryos cryopreservation allows the preservation of genetics from both male and female and is the fastest method to restore a breed. However, embryo production in vivo is limited in equids. In vitro production of embryos would allow the production of several embryos per cycle, but no technique has yet been developed in the donkey for in vitro production of embryos. The first steps for in vitro embryo production are collection of immature oocytes on a donor female using ovum pick up (OPU) and in vitro maturation of the oocytes. However, OPU has never been reported on jennies and the chronology of IVM of jennies oocytes has never been studied. Our objective was to develop OPU in jennies and to analyze the chronology of in vitro maturation of donkey oocytes.

Materials & Methods

Jennies oocytes collection

Five OPU sessions were performed, with 3 to 6 cyclic jennies per session.



After sedation, analgesia and antispasmodia, donkey cumulus-oocyte complexes (COCs) were collected from 5 to 25mm follicles.

Oocytes maturation for 24, 30, 34 or 38 hours

In vitro maturation of 10 to 30 oocytes in TCM199 supplemented with fetal calf serum and epidermal growth factor in an atmosphere of 5% CO_2 in air at 38.5°C.

Nuclear status

Oocytes are fixed in paraformaldhehyde and stained with Hoechst.

Evaluation of oocytes nuclear status.

Results

A total of 92 COCs was collected out of 193 follicles (48%) with an average of 4.2 COCs per jenny as shown in Table 1.

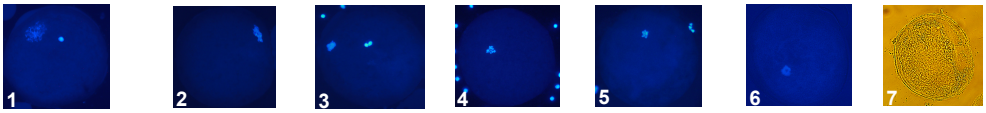
Table 1. Oocyte recovery rate from donkey jennies by ovum pick up

Number of females per puncture session	Number of follicles punctured	Number of oocytes recovered	Recovery rate per follicle	Number of oocytes per female
4	32	11	34%	2.75
5	37	16	43%	3.2
4	31	16	52%	4
3	38	18	47%	6
6	55	31	56%	5.2

All COCs were expanded after over 24 hours in vitro maturation (IVM). At collection, jennies COCs contained a germinal vesicle. After 24 hours IVM, COCs contained either a germinal vesicle or condensed chromatin. Metaphase 1 oocytes were observed after 30 hours IVM and 45% were in metaphase 2 after 34 hours IVM. Data shown in table 2.

Table 2. Nuclear stage of jennies oocytes at collection (0h) or after 24, 30, 34 or 38 hours in vitro maturation

Duration of IVM	filamentous germinal vesicle (fig 1)	partly condensed GV (fig 2)	condensed chromatin (fig 3)	metaphase 1 (fig 4)	metaphase 2 + polar body (fig 5)	metaphase 2 decondensing (fig 6)	degenerated (fig 7)	total
0h	6 (43%)	7 (50%)					1 (7%)	14
24h	4 (45%)	2 (22%)	2 (22%)				1 (11%)	9
30h	4 (44%)			4 (45%)			1 (11%)	9
34h	1 (11%)	2 (22%)	1 (11%)	1 (11%)	4 (45%)			9
38h		2 (25%)	2 (25%)			2 (25%)	2 (25%)	8



Conclusions

In conclusion, we established for the first time conditions for ovum pick up in jennies and the chronology of in vitro maturation of donkey oocytes. We showed that in vitro maturation of donkey oocytes can produce 45% of metaphase 2 oocytes after 34 hours in culture while most equine oocytes reach metaphase 2 after only 30 hours in culture.

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