Repeated Ovum Pick-Up and In-vitro Embryo Production in Buffalo

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ABSTRACT: Transvaginal ultrasound-guided ovum pick-up (OPU) was performed on 40 Nili-Ravi and Murrah buffaloes for consecutive 3 times each, at an interval of 3 days. A total of 936 follicles were aspirated, 643 oocytes were retrieved and 456 oocytes of them were usable. The recovery rate was 68.77% (643/935) and the percentage of usable oocytes was 70.92% (456/643), with an average of 3.80±0.23 oocytes usable per buffalo. The number of usable oocytes for the first, second and third OPU was 3.82±0.50; 3.82±0.35; 4.05±0.43, respectively, and the difference among them was not significant (P<0.05). After in-vitro maturation (IVM), in-vitro fertilization (IVF) and co-incubation with single layer of granular cells, the cleavage rate was 56.36% - 257/456, blastocyst formation rate was 20.39% (93/456). The result proved the feasibility of consecutive OPU at an interval of 3d and produce buffalo embryo via OPU-IVF. Further studies are needed to improve the efficiency of OPU-IVF.

Key words: Buffalo, Ovum pick-up, In vitro fertilization.

INTRODUCTION - In 1988, Pieterse et al. invented transvaginal ultrasound-guided ovum pick-up (OPU), and it has been found a wide application in bovine after decades of development. The adoption of multiple ovulation and embryo transfer (MOET) in buffalo is limited, due to a low MOET efficiency compare to cattle and poor response to hormonal stimulation (Misra et al., 1999). The application of OPU, combined with other reproductive technologies such as in-vitro fertilization (IVF) and embryo transfer (ET), allows repeated collection of oocytes from live donors of high genetic value without any blight to donor’s productivity and reproduction and produce more transferable embryos per buffalo per month (2.0vs0.6) (Gasparini, 2002). Therefore, OPU combining with IVF and ET can enhance the reproductive efficiency of females, reduce generation intervals and accelerate genetic improvement progress. This research aimed to produce well-bred buffalos with specific genealogy by OPU combining with IVF, and to find new approaches to accelerate breeding progress in buffalo.

MATERIAL AND METHODS - Oocyte donor buffalos. Forty empty Nili-Ravi and Murrah buffalos (5-12 years old), weighed 580~720 kg without ovarian abnormity and fed in half-isolated cowsheds, were randomly allocated to 4 groups of 10 buffalos each.

2 OPU - Unless otherwise stated, all chemicals were obtained from Sigma Aldrich USA. HS2000B style ultrasound monitor (FHK, Japan) with a 5 MHZ sector scanner vaginal probe (Aloka, Tokyo, Japan) and 17G 60cm needle for oocyte aspiration. Transvaginal ultrasound-
guided aspiration was adopted for oocyte recovery as described by Huang et al. (2004).

3) **In vitro embryo production procedures.** The oocyte-cumulus-complexes (COCs) were classified into 4 categories: Class A: oocytes with homogeneous cytoplasm enclosed by at least 2 layers of compact cumulus cells; Class B: oocytes with homogeneous cytoplasm enclosed within 1 layer of compact cumulus cells; Class C: denuded oocytes and oocytes with heterogeneous cytoplasm; Class D: oocytes enclosed by diffused cumulus cells. Only the Class A and Class B oocytes were adopted for culture. In vitro embryo production procedure was referred to the former previous study (Liang et al., 2007). Selected oocytes were culture separately for each buffalo. Cleavage was checked at 48 h post fertilization and the blastocysts development was determined on Day 5-9 after fertilization.

**RESULTS AND CONCLUSIONS** -

1) **Effects of OPU Replicates on Oocyte Recovery.** Nili-Ravi and Murrah buffalos underwent OPU for consecutive 3 times in this study, a total of 936 visible follicles were aspirated. Table 1 shows the mean number of follicles aspirated from each buffalo per time was 7.69±0.33, the aspirated follicle of first OPU was higher than those of the second and third OPU, but the usable oocytes recovered per donor and the recover rate from different puncture session were no different.

The result of table 2 show the oocytes recovered by OPU were mainly Grade A and B, the mean percentage of usable oocytes was 68.27%. The percentage of usable oocytes during the first OPU session was only 56.20%, while the data increased to 80.98% and 78.80% during the second and third session, the difference was significant (P<0.01). The results were probably due to that the atretic follicles were excessive during the first OPU session, as the OPU times increased, the number of atretic follicles decreased, and the quality of oocytes were improved. It’s worthy of notice that the percentage of denuded oocytes is 16.33%, but 7.31% of them were empty zona pellucida, probably owing to excess suction power for aspiration during OPU sessions. On the other hand, a high individual variability in follicular recruitment was observed in this experiment, there’s great difference in the number of recovered oocytes per OPU from different individuals (varied from 0 to 15). This point of view is confirmed by Gasparrini (2002).

2) **Effect of OPU Replicate on In-vitro Fertilization.** A total of 462 buffalos oocytes were recovered by OPU and the cleavage rate following IVF was 55.63%. The IVF cleavage rate of the first OPU session (46.67%) was significantly lower than those of the second (58.82%) and third sessions (62.50%), while the blastocyst rates were 18.79%, 16.99% and 25.00%, respectively, which showed no difference (P> 0.05). The average blastocyst rate in this ex-
periment is 20.13%, similar with that (19.9±4.2%) obtained from 8 non-lactating buffalos reported by Neglia G. et al. [6], lower than that (30.2%) reported by Huang et al. (2004). It is probably because the oocytes recovered from each donor were respectively cultured, the cultured oocytes in one drop were too less (1-6 COCs/ drop).

Table 2. Effect of OPU Replicate on Oocyte Quality

| Replicates | Grade A (%) | Grade B (%) | Grade C (%) | Grade D (%) | Only Zona (%) | Usable oocytes (%) | Oocytes Recovered |
|------------|-------------|-------------|-------------|-------------|---------------|--------------------|-------------------|
| 1st OPU    | 40          | 39 (15.12)  | 106 (41.09) | 61 (23.64)  | 24 (9.30)     | 28 (10.85)         | 145 (56.20)       | 258               |
| 2nd OPU    | 40          | 28 (15.22)  | 121 (65.76) | 22 (11.95)  | 8 (3.90)      | 8 (3.23)           | 149 (80.98)       | 187               |
| 3rd OPU    | 40          | 18 (17.41)  | 127 (63.18) | 22 (10.95)  | 6 (2.99)      | 11 (5.47)          | 145 (78.80)       | 184               |
| Tot.       | 120         | 85 (13.22)  | 354 (55.05) | 105 (16.33) | 38 (5.91)     | 47 (7.31)          | 439 (68.27)       | 643               |

Table 3. Effect of OPU Replicate on In-vitro Fertilization

| Group   | Oocytes | Cleavages | Cleavage rate (%) | Blastocysts | Blastocyst rate (%) |
|---------|---------|-----------|-------------------|-------------|---------------------|
| 1st OPU | 165     | 77        | 46.67 (77/165) aA | 31          | (18.79)             |
| 2nd OPU | 153     | 90        | 58.82 (90/153) b  | 26          | (16.99)             |
| 3rd OPU | 144     | 90        | 62.50 (90/144) bB | 36          | (25.00)             |
| Tot.    | 462     | 257       | 55.63 (257/462)   | 93          | (20.13)             |

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