

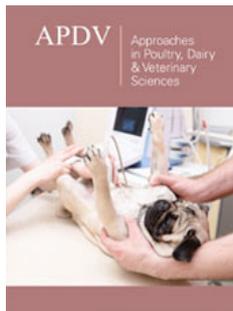
# Effect of Ovum Pick-Up Frequency on Quality of Buffalo Oocytes

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ISSN: 2576-9162



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**Submission:**  June 16, 2020

**Published:**  June 29, 2020

Volume 7 - Issue 4

**How to cite this article:** Sheng QG, Tang CM, Jian H, Hui L, Zhun TZ, Yan YC, Xiang HF, Zhuang YB, Qi YN, Shun SD, Xiang HJ. Effect of Ovum Pick-Up Frequency on Quality of Buffalo Oocytes. *Appro Poultry Dairy & Vet Sci* 7(4). APDV.000670.2020. DOI: [10.31031/APDV.2020.07.000670](https://doi.org/10.31031/APDV.2020.07.000670)

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## Abstract

The frequency of live ovum pick-up (LOPU) is one of the main factors affecting buffalo oocyte collection. This study discussed the effect of LOPU frequency on buffalo oocyte collection in purpose of improving the efficiency of water buffalo embryo *in vitro* production and laying a foundation for the establishment of a high efficient system. 92 buffalo buffaloes from Guangxi Buffalo Research Institute (including Murrah, Nili-Ravi buffalo and hybrid buffaloes), aged 4-12 years, weighing 580-700kg, were used for LOPU for 5 consecutive times at a frequency of every 3 days. The donors were not treated with hormone and the oocytes collected were divided into A, B, C and D grades. All data (the number of visual oocytes, punctured follicles, collected oocytes, available oocytes, recovery rate, availability rate, follicles, punctured follicles, recovered oocytes, etc.) was collected and counted to obtain the average number of oocytes in LOPU and the rate of high quality oocytes were evaluated. The number of admissible follicles, puncture follicle, collected oocytes were expressed by mean  $\pm$  standard error (S.E.M). The statistical software SPSS 17.0 was used to analyse the variance. The results showed that the average percentage of high quality oocytes (A and B grade) was 66.05%. When conducting continuous LOPU, the rate of high quality oocytes (grade A) from the first batch to the fifth batch were 58.95%, 64.05% and 70.28%, 69.07% and 67.86%, respectively, while the recoveries rate were 43.07%, 35.43% 4.60%, 37.42% and 37.46% respectively, both showed no significant difference between each batch ( $P > 0.05$ ). However, the rate of grade C oocytes (naked eggs and reticular cells) during 5 times were 39.99%, 24.65%, 15.95%, 23.81% and 21.17%, respectively, showed a significant difference ( $P < 0.0001$ ), and with a decreasing trend as the frequency of LOPU increases. This study laid a foundation for further improving of the efficiency of buffalo LOPU.

**Keywords:** Buffalo; LOPU frequency; Oocyte quality

## Introduction

The domestic buffalo (*Bubalus bubalis*), a multipurpose livestock species in Asia, provides milk, meat, and draught power. The low fecundity of buffalo is characterized by poor superovulation [1], low recovery of transferable embryos, and low pregnancy rate, as well as some basic physiological characteristics, such as delayed age of puberty, less pronounced signs of estrus, seasonal anoestrus, long period of post-partum anoestrus [2-4]. The Ovum pick-up (OPU) and the *in vitro* embryo production (IVEP) are currently the most competitive technologies to produce transferable embryos (TE) over the long term [5-8]. Furthermore, OPU can be performed on a wide variety of donors, such as non-cyclic animals, pregnant cows, and subjects with non-patent oviducts or genital tract infections [7,9]. It can also be carried out in animals that do not respond to multiple ovulation procedures, the last representing a high proportion in buffalo species [10]. Therefore, this procedure is a valid alternative to multiple ovulation/embryo transfer (MOET) programs [8], because of the low response to hormonal stimulation, the poor embryo recovery [1,8,11], and the impossibility to repeat continuously the MOET treatments over the long-term. The application of OPU-IVEP technology to buffalo can enhance the maternal contribution to genetic improvement [8,12], thus overcoming the reduced levels of efficiency linked to the reproductive seasonality pattern of this species. This study discussed this factor to improve the efficiency of water buffalo *in vitro* production technology system and lay a foundation for the establishment of efficient buffalo *in vitro* production technology system.

## Materials and Methods

### Reagents and media

If not otherwise stated, all chemicals and reagents were purchased from Sigma I (St. Louis, MO), with the exception of fetal bovine serum (FBS), which was purchased from Gibco BRL (Paisley, Scotland, UK).

### Animal and OPU

The study was conducted in a farm located in the veterinary staff of the Division of Animal Resources of Guangxi Buffalo Research Institute (GBRI) Chinese Academy of Agricultural Sciences (CAAS). Ovum pick-up was carried out healthy, multiparous and lactating buffalo cows (Murrah, Nili-Ravi and cross-breed buffalo) with 92 heads. The donors were under controlled nutrition, barn-housed, and restrained in a chute at the moment of the oocyte retrieval session, then prepared for follicular as described by the reporters [5,7,13] for buffaloes. HS2000 Ultrasonic imaging produced by Honda of Japan, with 7.5/5.0MHz vaginal sector scan probe (Aloka, SSD-500, Tokyo, Japan) equipped with 55cm long needle for oocyte collection, vacuum pumps (Cook IVF Co., Australia), follicular fluid collection tube, temperature devices. The number and size of follicles in each ovary was determined before puncture. Follicles of  $\geq 3$ mm in diameter were punctured and the follicle contents were drawn into the 50-ml Falcon tube using a regulated vacuum pump at 50mmHg vacuum pressure. During follicle aspiration the aspiration line was continuously rinsed with aspiration medium. OPU was conducted once every three days intervals and performed five times continuously.

### COCs processing

Aspiration medium consisted of DPBS with addition of 3% fetal bovine serum (FBS) and 100 $\mu$ g/ml, penicillin and 60mg/ml streptomycin and 10IU/ml heparin. The rinsed TCM199 medium contained 5% NBS. According to the morphological characteristics of cumulus cell complex (COCs), the oocytes were classified according to the method described by Zhang Xiufang et al. [14].

Cumulus-oocytes complexes (COCs) were classified in 4 categories.

- A. Grade A (oocytes with more than two layers of cumulus cells and homogenous cytoplasm);
- B. Grade B (oocytes with at least one layer of cumulus cells and homogenous cytoplasm);
- C. Grade C (oocytes partially denuded, but still showing homogenous cytoplasm);
- D. Grade D (degenerated oocytes with irregular shrunken cytoplasm). Grade A and B COCs were cultured *in vitro* matured medium (TCM199+ 10% FBS+ 10 $\mu$ g/mL FSH + 12 U/mL LH + 1  $\mu$ g/mL E2+ 50  $\mu$ M cysteine) under a humidified atmosphere of 5% CO<sub>2</sub> at 39°C for 22 to 24 h (Pang et al, 2009)

### Statistical analysis

The average number of oocytes obtained by OPU and the rate of high-quality oocytes were evaluated. SPSS 17.0 software was used

for statistical analysis. With different batches as fixed factors, the number of follicles was regarded as dependent variable and the number of follicles was regarded as dependent variable. Single factor analysis of variance (One-Way ANOVA) was used to test significance. The difference was significant by Duncan's method, using  $P < 0.05$  as the criterion of significance.

### Result and Discussion

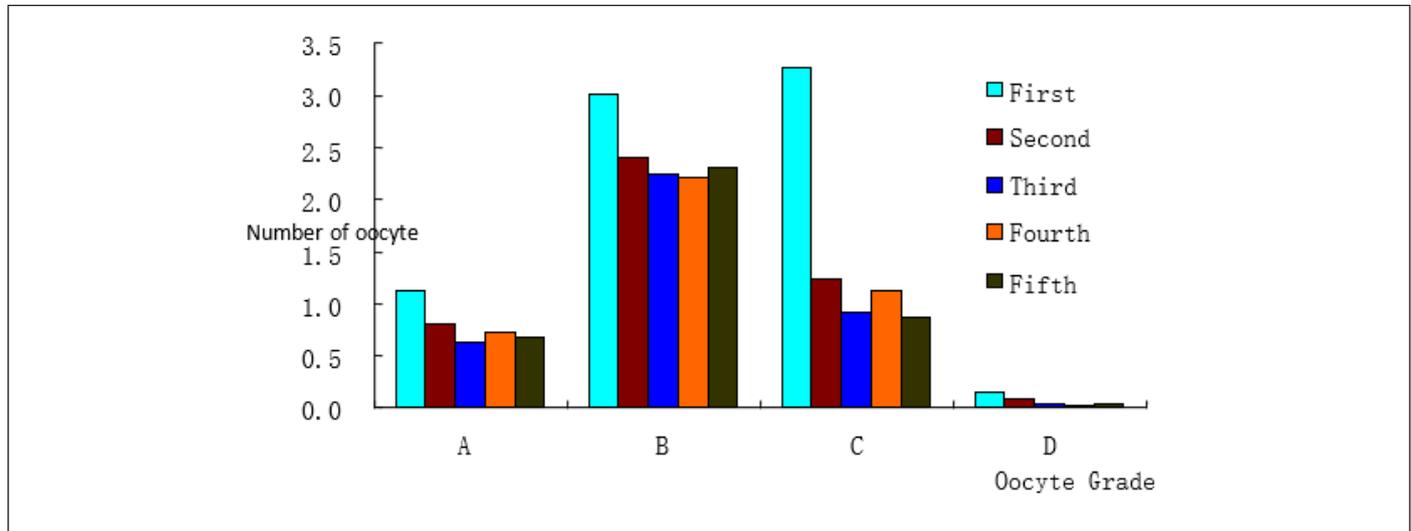
The results showed that the average percentage of high-quality oocytes (A and B grade) was 66.05%. When conducting continuous LOPU, the rate of high quality oocytes (grade A) from the first OPU to the fifth one were 58.95%, 64.05% and 70.28%, 69.07% and 67.86%, respectively, while the recoveries rate were 43.07%, 35.43% 4.60%, 37.42% and 37.46% respectively, both showed no significant difference between each batch ( $P > 0.05$ ). However, the rate of grade C oocytes (naked eggs and reticular cells) during 5 times were 39.99%, 24.65%, 15.95%, 23.81% and 21.17%, respectively, showed a significant difference ( $P < 0.0001$ ), and with a decreasing trend as the frequency of LOPU increases.

A literature [15] reported that although there were differences in the average number of punctured follicles and the number of oocytes recovered from every donor successive three times of OPU, there was no difference in the number of oocytes available to each head. In this study, the quality oocytes of grade A and B were the lowest in the first OPU, the second time was increased, the fourth time was relatively stable, but there was no significant difference among the batches of OPU. However, the changes of C grade oocytes were significantly different among batches ( $P < 0.0001$ ). With the increase of the number of OPU, the percentage of C grade oocytes (denuded oocytes and reticular cells) decreased continuously. However, there was no significant difference in the percentage of high quality oocytes and the recovery rate among the batches ( $P > 0.05$ ). The average percentage of high quality oocytes of grade A and B grade were 66.05% and was similar to the result which 70.92% [15]. Another the recovery rate of oocytes was 71.60% in Murrah buffalo which were treated with eCG [16]. Furthermore, average recovery of no hormonal treatment buffalo oocytes had the similar result (64.18%).

As for the recovery rate of OPU in buffalo, [15] reported that oocyte availability rate in the first OPU was only 56.20%, and the first oocyte availability rate in this study was 58.95%, the results were very similar. But the availability rate of the 2<sup>nd</sup> and third OPU (64.05 $\pm$ 3.63%, 70.28 $\pm$ 3.82%) was lower than that of Liang Xianwei (80.98%, 80.60%), which might be different from the variety, quantity and season of the donor, nutrition and other factors related. But the incidence of good quality oocytes (Grade A +B COCs) was also not affected by season in Mediterranean buffaloes [17]. The reason for the low availability of oocytes in the first oocyte collection may be that there are more atresia follicles at the first time than at other times. It was agree with the point of view that higher level of follicular atresia was reported [18] and, consequently, a lower number of total recoverable and viable oocytes. With the increase of the number of oocytes collected the number of the atretic follicles decreases and the quality of the oocytes increases. As a result, the proportion of available COCs recovered increased [19]. The results also showed

that the quality oocytes remained relatively stable at the fifth time. However, there was no significant difference in the recovery of oocytes. However, the changes of C-grade oocytes in different batches were significantly different ( $P < 0.0001$ ). With the increase of the number of OPU, the percentage of C-grade oocytes (denuded oo-

cytes and reticular cells) decreased continuously. The average rate of denuded oocytes recovered in this study was 25.15%, which was higher than that reported by Liang Xianwei et al. [15] (16.33%), but lower than that reported by Yadav et al [16] (39.9%) Figure 1 and Table 1.



**Figure 1:** Effect of OPU replicate on oocyte quality.

**Table 1:** Effect of OPU replicate on oocyte quality (Mean±S.E.M).

Replicate	Number	Oocyte Quality				High Quality Rate(%)	Recovery Rate(%)
		A %	B %	C %	D %		
1	92	14.56±2.30	44.39±4.77	39.99±2.65	2.41±0.82	58.95±4.77	43.07±3.52
2	92	13.60±2.43	50.45±3.83	24.65±3.02	1.36±0.55	64.05±3.63	35.43±2.72
3	92	14.76±2.61	56.13±3.95	21.17±3.20	0.94±0.50	70.28±3.82	34.60±2.62
4	92	16.93±2.83	52.14±3.91	23.81±2.88	0.56±0.38	69.07±2.38	37.42±2.75
5	92	15.01±2.67	52.85±3.93	15.95±2.55	0.76±0.7	67.86±3.93	37.46±3.02
Mean	92	14.98±1.15	51.18±1.83	25.15±1.33	1.20±0.25	66.05±1.76	37.60±1.32
P Value		0.924	0.349	<0.0001	0.148	0.243	0.285

**Conclusion**

OPU replicate effected on oocyte quality. With the increase of the number of OPU, the number of high quality oocytes (GradeA+B) increases. There was significant difference in Grade C.

**Acknowledgement**

This research project is supported by Guangxi key R & D Program (Gui Ke AB 16380040) and Guangxi Science and Technology Development Project (Gui Ke AD19259009) and State Key Laboratory for Conservation and Utilization of Subtropical Agro-bio resources ,Guangxi University (SKLCUSA-b201814).

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