

Transvaginal Ultrasound-guided Ovum Pick-up (OPU) in Cattle

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Abstract

OPU together with IVF has been taken as another challenge to the animal reproduction. While under so many years development, OPU has been considered as a mature technology. No many breakthroughs might happen to this technology. We concluded the history, present of bovine OPU, its advantages and disadvantages, factors that may influence its efficiency in this article. We hope to offer a reference for the development of bovine OPU technology in the future.

Keywords: Cattle; Ovum pick-up; Procedure; Factors

Introduction

The technique of ultrasound-guided transvaginal follicular aspiration for ovum pick-up (OPU), is a non-invasive procedure for recovering oocytes from antral follicles in live animals. It was developed in the human [1,2] to assist human infertility. When people realized its application prospect, considerable researches have been aimed at applying this technology in the bovine. In 1987, an ultrasonic-guided aspiration of bovine follicular oocytes was first proposed in Denmark [3] and in 1988; a real OPU was first established in cattle by a Dutch team [4]. Together with *in vitro* fertilization of oocytes, OPU has been taken as a most flexible and repeatable technique to produce embryos from any given live donor. Unlike MOET, OPU does not interfere with the normal reproduction and production cycles of the donor. Any female starting from 6 months of age to the third month of pregnancy and even soon after calving (2-3 weeks) could be a suitable donor. It has been shown to be a feasible and practical alternative to the conventional multiple ovulation and embryo transfer (MOET) program [5,6], and it is being more and more used for commercial applications in the world [7,8].

The first *In vitro* Produced (IVP) calf was born in 1981 [9]. Both OPU and IVF could be seen as mature technologies in the current world. The total number of transferable IVP bovine embryos worldwide was 453,471 in 2011 [10], which included OPU embryos and abattoir embryos. Although there is a large variation between donors, it is capable of producing over 50 calves per donor cow per year if the two technologies-OPU and IVF are combined. In addition, with the complementing of bovine genome sequencing and key genes for traits of economic interest becoming available in the recent years, OPU/IVP has proven invaluable in rapidly multiplying rare genes and provides the basis for more advanced technologies such as cloning [11,12] and transgenic. Brazil dominated the IVP production by performing 53,019 OPU sessions averaging 15 oocytes and 6 embryos per session. There are many embryo technology companies in Brazil, who are specializing in the production of *in vitro* embryos, embryo transfer or embryo-related technologies training to farmers or people related. This kind of companies or groups also exists in US, Canada, Italy, et al.

Different OPU Systems

The main process of OPU includes epidural anaesthesia, ovary positioning per rectum, follicle visualizing by the transvaginal transducer, oocyte aspiration by needle. There are two major OPU

systems including non stimulation and pre-stimulation procedure. As the name implies, the difference is whether the donor will be stimulated with hormone prior to OPU.

The original OPU procedure includes no hormone stimulation. It routinely performs twice a week, which allows the maximum recovery of oocytes of suitable quality for embryo production in a given time interval compared to once-a-week OPU, because no dominant follicle develops when all visible follicles are aspirated in the OPU process. While in most once-a-week collections, a dominant follicle develops at the successive collection, which causes the regression and degeneration of the subordinate follicles. In a result, oocytes collected by this scheme are relatively less in a given time interval. On a per cow per session basis, there was no difference between 'OPU 1/w' and 'OPU 2/w' protocols in terms of the average number of follicles aspirated, oocytes retrieved and blastocysts produced on Day 7. While on a weekly basis, those three indexes were significantly higher in the 'OPU 2/w' protocol than those in the 'OPU 1/w' [13]. Moreover, as it does not interfere with the normal reproduction cycles of the donor, there were no any long-term detrimental effects on the donor cow's fertility even after twice-a-week OPU for over a year [14,15] performed by experienced operators.

The main development in the OPU research is the hormone involved. The advantages of super-stimulation prior to OPU seem obvious—more follicles to puncture, more oocytes retrieved and more embryos, and less work as compared to non-super stimulated OPU. Chaubal et al. reported that the average number of follicles aspirated, oocytes retrieved and blastocysts produced on Day 7 in a FSH-treated OPU procedure were significantly higher than those in once-a-week non-stimulated OPU procedure on a per cow per session basis. While those indexes were similar to those in twice-a-week procedure when

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calculated on a per cow per week basis [13]. In order to improve the numbers of aspirated follicles, oocytes and IVF embryos, different hormone procedures have been tried and it has made some considerable gains [16,17]. In spite of all cited improvements above, the hormone-stimulated OPU has some unsolved key problems. Application of exogenous hormones disturbs donor's endocrine, especially when exogenous hormones are used to the donor for a long time, which might lead to infertility of the donor. Moreover, donor's responses to hormone stimulation are different, even the same donor shows different responses in different sessions, which leads to variation of the results. As a result, it is better to use hormones to the donor in a short period and leave a period of regulation and recovery to the endocrine system.

Factors affecting Bovine Ovum Pick Up

In order to obtain more oocytes from the donor, it is necessary to be more follicles on the bovine ovary, which is influenced by cattle breed, nutritional status and climate conditions. First, different cattle breeds present different follicle numbers on the ovaries in a follicular wave cycle. It was reported that *Bos indicus* breeds tend to have more follicular waves [18,19] and a larger population of small follicles (<5 mm) compared to *B. taurus* breeds [20]. Brazil is at the top of the list for embryos produced by OPU/IVP [9], besides other factors, there is a *Bos indicus* breed named Nelore which has a larger number of ovarian follicles with averages ranging from 18 to 25 recovered oocytes per OPU session without the use of exogenous hormones or synchronization protocols [21,22]. The effect of nutritional status on animal reproduction and follicular development is obvious [23,24]. Researchers have shown that climate temperature plays a key role on bovine follicle formation and development, oocyte quality and embryo development. Heat stress suppresses follicular dominance, resulting in a number of changes in follicular growth [25]. Zeron et al. reported that the number of follicles 3-8 mm in diameter per ovary was higher in winter (19.6) compared with summer (12.0) and 7.5 oocytes per ovary were found in winter and 5.0 oocytes per ovary in summer after aspiration of follicles [26]. Roth et al. found that bovine oocyte development was arrested in summer [27].

In addition, OPU procedure, operator's experience, individual variation (age, reproductive phase and individual response) are the important factors that affect OPU success and oocyte quality. As concluded above, there are many OPU systems including hormonal stimulation and non-stimulation, once-a-week and twice-a-week. Furthermore, there are some technical improvements which could increase oocytes aspirated. Chaubal et al. reported that removal of dominant follicle (DF) improved the blastocysts produced on day 7 [13]. Gradela et al. concluded the same results that the embryo viability rate was higher when no DF existed or DF was removed [28]. Experienced operator exerts the good vacuum pressure, needle operation and aspiration time. Under the same condition, they could obtain more available oocytes. In fact, the mechanical damage occurred during the aspiration process in the COC transport is mainly generated by the needle size and length as well as the vacuum pressure [29,30]. As to the individual variation, there are low, medium, and high responders to FSH stimulation, which show different OPU results and oocytes collected per session varied from 0-26 [31]. Rizosa et al. reported that a significantly higher number of total oocytes (4.7 versus 2.8, $P < 0.001$) and grade 1-2 oocytes recovered/animal from heifers than from cows (3.0 versus 1.8, $P < 0.05$). While there was no significant difference in the percentage of oocytes cleaving after fertilization, or in the percentage reaching the blastocyst stage between heifers and cows [32]. Moreover, the reproductive phase may influence the developmental competence of the recovered oocytes. In a Japanese OPU experiment on Black cattle,

oocyte development, the total proportions of cleavage, development into blastocysts and freezable embryos were higher for embryos obtained from pregnant cows than those obtained from non-pregnant cows [33].

Conclusion

With decades of exploration and researches on bovine OPU, OPU has been considered a mature technique. Although it is costly compared to MOET, it has distinct advantages. Its application will be invaluable in rapidly multiplying rare genes and provide basis for more advanced technologies such as cloning and transgenics.

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