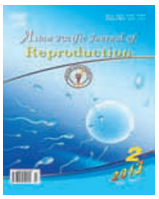
Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net



Document heading doi: 10.1016/S2305-0500(13)60139-6

Production of the first viable ovum pick-up and *in vitro* embryo produced (OPU-IVEP) buffalo calf in India

Shiv Prasad*, Beerendra Singh, Sumit Singhal, FA Khan, JK prasad, HP Gupta

Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar-263 145, India

ARTICLE INFO

Article history:
Received 1 April 2013
Received in revised form 3 May 2013
Accepted 4 May 2013
Available online 20 June 2013

Keywords:
Buffalo
Embryo transfer
Ovum pick-up
In-vitro fertilization

ABSTRACT

This report puts on record production of the first viable calf following ovum pick-up *in vitro* embryo production (OPU-IVEP) technique in buffalo (*Bubalus bubalis*) in India. A total of 313 transvaginal OPU sessions were performed in six elite Murrah buffaloes and 1 171 follicles were aspirated. From these follicles, a total of 729 immature oocytes were recovered. The recovered oocytes were graded as A (59, 8.1%), B (111, 15.2%), C (293, 40.2%) and D (262, 35.9%) following standard procedures. Maturable grade oocytes (A, B, and C; 473, 68.9%) were subjected to *in vitro* maturation in Medium 199 supplemented with 10% FBS, 0.5 μg/mL FSH, 10 IU/mL LH, 1 μg/mL estradiol-17β, 20 ng/mL EGF and 50 μM cysteamine in CO_2 incubator at 39 ℃ temperature, 5% CO_2 and high humidity. The matured oocytes (427 out of 473; 90.3%) were co-incubated with $1x10^6$ /mL buffalo sperms in modified synthetic oviductal fluid (mSOF) containing 10 ng/mL heparin for 22 hrs for *in vitro* fertilization. After fertilization the presumptive zygotes were stripped off of remaining cumulus cells and cultured in mSOF for 7 days to study embryonic development. A total of 278 (65.1%) oocytes cleaved after 24-36 hrs of fertilization. Out of the cleaved oocytes, 249 (89.6%), 216 (77.7%), 167 (60.1%), 139 (50.0%) and 73 (26.3%) developed up to 4-cell, 8-cell, 16-cell, morula, and blastocyst stages, respectively. A total of 29 embryos were transferred into 27 synchronized recipient buffaloes. Two of these buffaloes were found pregnant at 90 days. One of them aborted at 4.5 months of gestation (male fetus) and the other gave birth to a live male calf weighing 25 kg. Currently, the calf is normal and has attained the age of 4.5 years.

1. Introduction

Ovum Pick-up (OPU) technology combined with the multistep *in vitro* embryo production (IVEP) represents one of the latest assisted reproductive technologies used for faster multiplication of superior germplasm in buffalo. Considering the limited success in super ovulation in buffaloes for *in vivo* embryo production [1,2], as well as *in vitro* embryo production (IVEP) using ovaries collected from abattoir [3], the technique of ovum pick-up (OPU) for aspiration of oocytes from live animals along with *in vitro* embryo production (OPU-IVEP) seems to be a more efficient method of embryo production in buffalo. The OPU technique can be used repeatedly on live animals for collection of large number of oocytes from individual donors of all age (2 months old calves to very old cows), unknown fertility, various physiological phases of reproduction and even pregnant animals (up to 3 months), without any side effects on the donor's reproductive performance [4]. The technique was first used in buffaloes by Boni *et al* [5]. Birth of the first buffalo calf using OPU-IVEP was reported by Galli *et al*. [6] and in subsequent years, more births were reported from different parts of the world [7,8]. This paper reports birth of the first OPU-IVEP buffalo calf in India.

*Corresponding author: Shiv Prasad, Department of Veterinary Gynaecology and Obstetrics College of Veterinary and Animal Sciences G.B. Pant University of Agriculture and Technology, Pantnagar-263 145, India.
Tel: +91 9411377368
Fax: +91 5944233473
E-mail: shivp2003@yahoo.co.uk

2. Case report

A total of 313 transvaginal OPU sessions were performed in six elite Murrah buffaloes and 1 171 follicles were aspirated. From these follicles, a total of 729 immature

oocytes were recovered. The recovered oocytes were graded as A (59, 8.1%), B (111, 15.2%), C (293, 40.2%) and D (262, 35.9%) following standard procedures. Maturable grade oocytes (A, B, and C, 473; 68.9%) were subjected to *in-vitro* maturation in Medium 199 supplemented with 10% FBS, 0.5 μg/mL FSH, 10 IU/mL LH, 1 μg/mL estradiol-17β, 20 ng/mL EGF and 50 μM cysteamine in CO_2 incubator at 39 ℃ temperature, 5% CO_2 and high humidity. The matured oocytes (427 out of 473, 90.3%) were co-incubated with $1x10^6$/mL buffalo sperms in modified synthetic oviductal fluid (mSOF) containing 10 ng/mL heparin for 22 hrs for in vitro fertilization. After fertilization the presumptive zygotes were stripped off of remaining cumulus cells and cultured in mSOF for 7 days to study embryonic development. A total of 278 (65.1%) oocytes cleaved after 24-36 hrs of fertilization. Out of the cleaved oocytes, 249 (89.6%), 216 (77.7%), 167 (60.1%), 139 (50.0%) and 73 (26.3%) developed up to 4-cell, 8-cell, 16-cell, morula, and blastocyst stages, respectively. A total of 29 embryos were transferred into 27 synchronized recipient buffaloes. Two of these buffaloes were found pregnant at 90 days. One of them aborted at 4.5 months of gestation (male fetus, Figure 1) and the other gave birth to a live male calf weighing 25 kg. Currently, the calf (named "Saubhagya") is normal (Figure 2)== and has attained the age of 4.5 years.

Figure 1. Aborted fetus (4.5 months of gestation)

Figure 2. First OPU-IVEP buffalo calf with surrogate mother

3. Discussion

Results on recovery of oocytes by OPU in the present study were encouraging. Similar findings were reported by Singhal [9] and Yadav [10]. However, compared to cattle [11,12], the recovery rates were much lower, which could be attributed to the lower ovarian reserve in buffaloes [13]. Recovery rate of maturable oocytes (68.1%) was higher than 43.0% to 55.0% reported in earlier studies [4,14]. The overall maturation rate in the present study was 90.3 %, which is consistent with earlier results (86.3% [15]; 85.0% [16]; 91.1% [8]). Higher maturation rate in our study might have resulted from addition of hormones, growth factors and antioxidants. The overall cleavage rate was 65.1%, which was higher as compared to 29.8% [6] and 49.4% [4] reported earlier in oocytes collected following OPU. The higher cleavage rate could be attributed to growth factors and antioxidants used in IVM [17] and better *in-vitro* capacitation of semen in mSOF [18]. Progressive development to subsequent stages was encouraging; however, the pregnancy rate (7.4%) was comparatively lower. The parentage of the only calf born was confirmed by the genotyping of the donor, surrogate mother and calf by using micro satellite marker based parentage confirmation kit developed by National Bureau of Animal Genetic Resources (NBAGR), ICAR, Karnal. To our knowledge, "Saubhagya" is the first buffalo calf born through OPU-IVEP in India.

Conflict of interest statement

The authors declare that they have no conflict of interest.

References

[1] Misra AK. Superovulation and embryo transfer in buffalo; Progress, problems and future prospects in India, A review. *Buffalo J* 1993; **31**: 13-24.
[2] Madan ML, Das SK, Palta P. Application of reproductive technology to buffaloes. *Anim Reprod Sci* 1996; **42**: 299-306.
[3] Madan ML, Singla SK, Chauhan MS, Manik RS. *In vitro* production and transfer of embryos in buffaloes. *Theriogenology* 1994; **41**: 139-143.
[4] Boni R, Roveillo S, Zicarelli L. Repeated ovum pick up in Italian Mediterranean buffalo cows . *Theriogenology* 1996; **46**: 899-909
[5] Boni R, Di Palo R, Barbieri V, Zicarelli L. Ovum pick-up in deep anestrus buffaloes. *Proc IV World Buffalo Congress* 1994; **3**: 480-482.
[6] Galli C, Duchi R, Crotti G, Lazzari G. Embryo production by ovum pick up in water buffalo. *Theriogenology* 1998; **49**: 400.
[7] Infascelli F. First successful pregnancy from *in vitro* developed buffalo embryo. *Bubalus bubalis* 2003; **9**: 23-24.
[8] Huang Y, Zhang X, Gasparrini B, Presicce A. Oocyte recovery by ovum pick up and embryo production in Murrah and Nili-Ravi buffaloes (*Bubalus bubalis*) imported in China. *Reprod Fertil Dev* 2005; **17**: 273.
[9] Singhal S. *Effect of growth factors and antioxidants on developmental competency of buffalo oocytes collected through*

ultrasound guided trans-vaginal aspiration. M.V.Sc. thesis submitted to G.B. Pant University of Agriculture and Technology, Pantnagar, U.S.Nagar, Uttarakhand, 2007.

[10]Yadav SK. *Ultrasound guided trans-vaginal follicular aspiration of buffalo oocytes and in-vitro fertilization*. M.V.Sc. thesis submitted to G.B.Pant University of Agriculture and Technology, Pantnagar, U.S.Nagar, Uttarakhand, 2007.

[11]Kruip TAM, Boni R, Wurth YA, Roelofsen MWM, Pieterse MC. Potential use of ovum pick up for embryo production and breeding in cattle. *Theriogenology* 1994; **42**: 675-684.

[12]Broadbent PJ, Dolman DF, Watt RG, Smith AK, Franklin MF. Effect of frequency of follicle aspiration on oocyte yield and subsequent superovulatory response in cattle. *Theriogenology* 1996; **47**: 1027-1040.

[13]Danell B. *Estrus behavior, ovarian morphology and cyclical variation in the follicular system and endocrine pattern in water buffaloe heifers*. Ph.D. Dissertation, Swedish University of Agriculture Sciences, Uppasala, Sweden, 1987.

[14]Manik RS, Chauhan MS, Singla SK, Palta P. Transvaginal ultrasound guided aspiration of follicles from Indian buffaloes (*Bubalus bubalis*) with reproductive problems. *Vet Rec* 2002; **150**: 22-24.

[15]Singh G, Totey SM, Talwar GP. *In vitro* fertilization of oocytes matured *in vitro*. *Theriogenology* 1989; **31**: 255

[16]Chauhan MS, Singla SK, Palta P, Manik RS, Madan ML. *In vitro* maturation and fertilization and subsequent development of buffalo (*Bubalus bubalis*) embryos: effects of oocytes quality and type of serum. *Reprod Fertil Dev* 1998; **10**: 173-177.

[17]Singh V. *Effect of growth factors and antioxidants on in-vitro maturation and fertilization of oocytes and in-vitro development of buffalo embryo*. Ph.D thesis, G.B.Pant University of Agriculture and Technology, Pantnagar, U.S.Nagar, Uttaranchal, India, 2006.

[18]Misra AK. *Advances in embryo technologies in water buffaloes*. Proc 7th World Buffalo Congress, Manila, Philippines, 20-23 October; 2004, p. 140-156.

Manuscript contents

Authors and title page

All authors should have participated sufficiently in the work to take public responsibility for the content. Written approval signed by all authors should be presented with the manuscript.

The title page should contain, on separate lines, the title of the manuscript, the name(s) and affiliation(s) of the author(s), and the mailing address, telephone and fax numbers, and E-mail address of the corresponding author.

Manuscript

The manuscript should be submitted online through our email, later on through our website, and should be arranged in the following order: 1) Title Page, 2) Keywords, 3) Abstract, 4) Corresponding author, 5) Introduction, 6) Materials and methods, 7) Results, 8) Discussion, 9) Acknowledgments, 10) References, 11) Tables, 12) Figure Legends, and 13) Figures. Please indicate the page your manuscripts to facilitate reviewing. Except for units of measure, the use of abbreviations is discouraged. At the first appearance in the abstract and the text, abbreviations should be preceded by words for which they stand.

Tables

Tables must be concise and cited consecutively using Arabic numerals in the text (Table 1, Table 2, etc.). Each table should be typed on a separate sheet. The title of the table should clearly indicate the nature of the contents and sufficient details should be included in the footnote to facilitate interpretation without reference to the text. Use horizontal rules only.

Figures

Figures (photographs, drawings, diagrams and charts) should be clear, easily legible and cited consecutively using Arabic numerals in the text (Figure 1, Figure 2, etc.). Please supply figures 1.5 to 2 times the size at which they will be finally reproduced. For line work, submit black-ink drawings of professional quality. Micrographs or other glossy photographs must be of the highest quality. If a figure comprises more than one glossy photograph, these should be marked A, B, C, etc. Figure legends should be marked clearly with the correspond letter. Legends should contain sufficient details to permit figure interpretation without reference to the text. Scale markers should be indicated in the photographs. Color plates are also welcome. The choice of cover art illustration will be made by the Editor.

References

The Journal advocates the citation of new papers; old references are better replaced with updated ones. The authors are responsible for the correctness of references. References are numbered consecutively in the order in which they appear in the text. Citation should be labeled in superscript parenthesis and should appear in front of the period or comma at the end of the sentence/clause. All references should be cited.

References should be listed in numerical order in the Reference section. Journal titles should be abbreviated according to the list of Journals Indexed in Index Medicus or MEDLINE (www.nlm.nih.gov).

Please note that the journal requires that all references should follow the Vancouver style rules established by the International Committee of Medical Journal Editors (http://www.icmje.org/).