Birth of First In vitro Produced Calves in Bangladesh and Their Reproductive Fitness

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ABSTRACT

The goal of the current study is to produce calves by implanting recipient cows with in vitro generated embryos. For in vitro maturation, slaughterhouse-driven cumulus-oocyte-complexes (COCs) with an even cytoplasm and at least three layers of compact cumulus cells were chosen (IVM). For the purpose of in vitro fertilization, capacitated fresh spermatozoa and presumed matured COCs were co-cultured for 18 to 20 hours (IVF). Cumulus cells were removed by carefully pipetting them into TL-HEPES. The zygotes spent three days in an in vitro culture (IVC) droplet. After this initial phase of culture, the 8–32 cell embryos were moved to IVC-II media, where they remained until Day 8 of development. On day 8, these embryos were transferred into recipient cows, with two embryos transferred to each of the five recipient cows. On day 60 of embryo transfer (ET), rectal palpation was used to assess pregnancy followed by ultrasonography on day 90. Out of five recipients, one cow delivered twin calves with a pregnancy rate of 20%. The calves were named Falguni and Chaitali. The duration of gestation was 277 days. Falguni weighed 12.87 kg at birth, whereas Chaitali weighed 18.59 kg. Falguni and Chaitali both showed typical growth patterns, with an average 254.77 g and 463.15 g/day. At a regular age, both heifer calves reach puberty and give birth. This research emphasizes the possibility that ovocytes from slaughterhouse ovaries can be used to produce healthy IVP calves, which would be a major step forward in Bangladesh’s fast expansion of high-yield dairy cattle.

Keywords: Calves, Cumulus oocyte complexes (COCs), IVF, zygote.

1. INTRODUCTION

The scientific and technological advances in the field of animal reproduction resulted the development and technical implication of numbers of assisted reproductive technologies (ART). The prime focus of all of these technologies is to multiply the genetically improved germplasm across the world and increase the subsequent production capacity. This technology also allows the use of germplasm with physical deformities and subfertility conditions as well as the conservation of endangered animal species and reduce the risk of sexually transmitted diseases. In vitro embryo generation is one of the most innovative and extensively utilized assisted reproductive technologies worldwide. This method shortens generation intervals and increases selection intensity, which speeds up genetic advancement in breeding programs [1]. Increased exploitation of maternal lineage is linked to these advantageous traits [1].

With the growing popularity of in vitro embryo production technologies, the industry is growing much faster, boasting an impressive annual growth rate of 12% [2]. The International Embryo Transfer Society (IETS) reports a surplus of viable embryos produced compared to those actually transferred, resulting in a shift among livestock seedstock producers from traditional MOET to IVP [2]. However, this transition is not uniform worldwide, as developing countries like Bangladesh are still very amateur in adapting this technology.

Implementing this technology requires the establishment of a consistent and efficient system for embryo culture, storage, and transfer to recipients. Research in the field of embryo culture and transfer remains notably limited in Bangladesh. So far, progress has been achieved mainly in optimizing culture conditions and research for enhancing blastocyst development [3]–[5]. Bangladesh has
had extremely little success with in vitro embryo production in livestock species. Some progress has been made in different aspects, such as buffalo oocyte vitrification [6], in vitro zygote production using slaughterhouse-derived buffalo oocytes [7], in vitro maturation of buffalo oocytes [8], and maturation of goat oocytes [9]. However, none of these endeavors have succeeded in producing in vitro cultured calves. Given these circumstances, this research endeavors to introduce and adapt in vitro embryo production technology to enable the production of in vitro calves in Bangladesh.

2. Materials and Methods

2.1. In vitro Embryo Production

Ovaries of the slaughtered cows were collected from the local abattoir within four to five hours of the slaughter and placed in physiological saline (0.9% sodium chloride supplemented with 100 IU/mL penicillin and 0.1 g/mL streptomycin sulfate) at room temperature. In order to aspirate the cumulus-oocyte-complexes (COCs), a 10-mL disposable syringe with a 21G needle was used. Under a stereo microscope, the aspirated material was transferred to a 100-mm Petri dish filled with TL-HEPES solution, and the cumulus-oocyte-complexes (COCs) were examined. For in vitro maturation, cumulus-oocyte-complexes (COCs) with at least three layers of compact cumulus cells and an even cytoplasm were chosen. Washings of the chosen COCs (50–70 per well) were conducted twice: once in TL-HEPES and once in IVM medium (TCM199 + supplements). Fresh RCC bull semen was used to fertilize the mature COCs in vitro. Sperm were made viable by incubating them for 15 minutes in 500 μL of IVF medium (Tyrode’s lactate solution supplemented with BSA, sodium pyruvate, penicillin, and mg/mL streptomycin) that contained 20 μg/mL of heparin sodium salt. Following capacitation, the spermatozoa were diluted using IVF medium at a concentration of roughly 1 × 10^6 spermatozoa/mL. Mature COCs were placed in a 4-well dish (500–700 μL) and co-cultured with capacitated spermatozoa for 18–20 hours. Following IVF, the cumulus cells were removed using a gentle pipetting technique into TL-HEPES. Then, the presumed zygotes were put in a four-well dish with 500–700 μL of modified CR1-aa medium [10], which was supplemented with Na-pyruvate, glutamine, penicillin/streptomycin, BSA, and glutathione for three days. The assumed zygotes were subsequently cultured in a medium with the same composition until day 8 of embryonic development, with the exception that FBS was added in place of BSA (IVC II). During IVM, IVF, and IVC, the incubation conditions were 5 percent CO\textsubscript{2} in air at 38.5 °C and maximum humidity. Unless specified otherwise, all of the chemicals and reagents used in this experiment were bought from Sigma Aldrich.

2.2. Estrus Synchronization

Eight healthy and regular breeder cows (three BLRI cattle Breed-1 and two Red Chittagong) were chosen for embryo transfers (ET). Functional corpus luteum (CL) was detected through per rectum palpation and selected recipients received a single dose (2.0 ml Ovuprost) of prostaglandinF2α (PGF2α) and a single dose (2.5 mL Ovu-rellin) of gonadotrophin-releasing hormone (GnRH) on observed estrus. After receiving PGF2α, the treated individuals entered estrus in 60 to 72 hours. Teaser bull exposure, rectal examination, and careful observation of behavioral indicators were used to confirm estrus.

2.3. Embryo Transfer to Recipients

Each recipient received two embryos (late morulae to early blastocyst) depending on the morphological development stage of the blastocyst, on days 7 or 8. Embryos were inserted ipsilaterally into the uterine horn. On day 60 of the pregnancy, a rectal palpation was performed, and on day 90, an ultrasound was performed.

2.4. Statistical Analysis

Microsoft Office Excel was used to determine the mean and standard deviation.

3. Results and Discussion

3.1. Embryo Development Efficiency

A total of 220 cumulus-oocyte-complexes (COCs) were set for IVM and IVF. However, 180 presumptive zygotes were transferred into IVC-I medium for embryo development. Cleavage and blastocyst development rates were 81.11% and 36.11%, respectively (Fig. 1).

3.2. Estrus Synchronization Efficiency

Eight recipient cows were brought under hormonal treatments. After being administered PGF2α for 60 to 72 hours, each of them displayed indications of estrus (Table I). Several studies showed that the success rate of estrus synchronization varied from 50% to 80% in different estrus synchronization protocols [11], [12]. During this study, cows having active corpus luteum in their ovary were administrated with PGF2α. The PGF2α work well in the luteal phase. This fact might have contributed to bringing all the cows into the estrus during this experiment. The general management of the herd, which includes diet, health, breeding, and reproductive programs, determines the success rates of any synchronization programs [13]. Out of all the cows in estrus, five cows underwent ET since their ovaries showed developed corpus luteum. The recipient preparation rates were reported to be 62.50%.

3.3. Pregnancy Establishment

Embryos were transferred into 5 cows and one cow conceived as detected by per rectal palpation on day 60 and by ultrasonography on day 90 (Fig. 1). Overall 20% of pregnancy was achieved through embryo transfer. The conception rate following the transfer of IVF embryos ranges from 33.50% in Nelore cows [14] to 53.01% in Hanwoo cows [15] as detected on day 60 of embryo transfer. About 47% of pregnancy was established in Holstein Friesian cows after embryo transfer [16]. The lower conception rate of this study might be associated with the skill of ET technician and other unknown factors. Following the 277-day gestation period, the recipient cow delivered
two female calves (Fig. 1). The calves were named as Falguni and Chaitali. The average calving rate following transfer of fresh IVP embryos is about 30% in cattle [17]. However, depending on the recipient’s breed, condition score, and source, the average pregnancy rate ranged from less than 20% to over 50% [18]. Differences in pregnancy outcomes are associated with differences in recipient competence along with embryo competence [13]. Moreover, the transfer of embryos in the cranial part of the uterus, the lesser degree of difficulty in transfers, and the lesser time for transferring embryos increase the rate of pregnancy with ET [19]. The low pregnancy rate in this study may be associated with recipient competence and other technical issues associated with embryo handling. The recipient cows showed normal post-partum heat following the delivery of IVP calves.

3.4. Productive and Reproductive Characteristics of IVP Calves

Chaitali weighed 18.59 kg at birth, while Falguni weighed 12.87 kg. On 30, 60, and 120 days, Falguni and Chaitali weighed 23.45, 33.38, 43.44 kg, 47.40 kg, 56.00, and 74.17 kg, respectively. Body weight increases per day for up to 120 days, recorded as 254.77 gm for Falguni and 463.15 gm for Chaitali.

These calves were grown normally. The age of puberty and age at first calving of Falguni vs. Chaitali was 19.13 vs. 22.03 months and 28.21 vs. 31.20 months, respectively. The calving interval of Falguni was 18.10 months and that of Chaitali was 25.10 months, respectively Table II. Puberty age and age at first calving of RCC were 37.0 ± 2.16 and 49.67 ± 2.1 months which were higher than the present findings [20]. This performance also indicates their normal reproductive efficiency as in vivo produced cows.

4. Conclusion

*In vitro* embryo development technology can be used to produce healthy calves from oocytes derived from slaughterhouses. However, more research needs to be conducted to improve the calf production efficiency and embryo cryopreservation technique to develop a sustainable embryo production system.

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Authors’ Contributions

All the authors have contributed significantly to the planning, executing and drafting of the manuscript. All the
authors have given their consent to submit the manuscript in European Journal of Veterinary Medicine. GK Deb, MFH Miraz, MA Kabir, TN Nahar and SMJ Hossain were involved in the conceptualization, designing and execution of the experiment, writing of the original draft, and project administration. MS Ali cooperates embryo transfer in receipt cows.

CONFLICT OF INTEREST
Authors declare that they do not have any conflict of interest.

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