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Short Communication

In Vitro Embryo Production in Indian Buffalo

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ABSTRACT

The embryo production is carried out through a combination of techniques of collection of immature oocytes, in vitro maturation (IVM), fertilization (IVF) and culture (IVC). Samples collected from slaughterhouse are the cheapest and the most abundant source of primary oocytes for large scale production of embryos through in vitro maturation (IVM) and in vitro fertilization. The culture medium and selection of protein supplements and hormones for IVM play an important role in the subsequent maturation rate, and embryonic development following IVF. The in vitro fertilization procedures in buffalo and requires appropriate preparation of sperm and oocyte, as well as culture conditions that are favorable to the metabolic activity of the male and female gametes. The presumptive zygotes are then cultured in vitro up to the blastocyst stage at which these could either be transferred to synchronized recipients for producing live offspring or cryopreserved for future use.

Key words: Buffalo, Maturation, Fertilization, Embryo culture

INTRODUCTION

The buffaloes are in the order of *Artiodactyla*, the cloven-hooved mammals, genus *Bubalus* and species *bubalis*. Two main species of buffalo are found in the world: the Asiatic (water) buffalo (*Bubalus bubalis*) and the African buffalo (*Syncerus caffer*). The two buffalo types are having different habitats and chromosome numbers. There are about 170 million buffaloes in the world (Perera *et al.*, 2005). Out of this 97 percent of them are water buffaloes and are mainly found in the Asian region. Riverine buffaloes are characterized by black colour and have long curled horns (e.g. Murrah Breed) and the Swamp buffaloes are dark grey, but may also be black, black and white, or even all white, have long, gently curved horns. Riverine buffaloes (70 percent of the total world population) are reared in high numbers in South Asia, especially in India and Pakistan. The name 'swamp' has probably arisen from their preference for wallowing in stagnant water pools and mud holes (Subasinghe *et al.*, 1998). Swamp buffaloes are found mainly in southern China Sri Lanka, and the South-East Asia countries of Thailand, the Philippines, Indonesia, Vietnam, Burma (Myanmar), Laos, Cambodia and Malaysia (Chantalakhana and Falvey, 1999).

India has about 95 million buffalo's represents 56.5 percent of the world buffalo population. India is the first country in the world for rearing buffalo's production (about 134 million tons of milk). India is also the first country in Asia for scientific and technological development in buffalo nutrition, production, reproduction, biotechnologies and genetic improvement. Moreover, India has implemented national programmes known green revolution¨ for increasing crop production for animals, the "white revolution" for increasing milk productivity to satisfy human needs for animal proteins and finally the "red revolution" for increasing meat production and supporting meat industry, especially from buffalos. India possesses the best River milk breeds in Asia e.g. Murrah, Nili-Ravi, Surti and Jaffarabadi, which originated from the north-western states of India and have a high potential for milk and milk fat production in addition to use as a work animal and as a supplementary stock for meat production. The IVEP permits the preservation of genetic potential of sub-fertile or dead animals **(**Deuleuze *et al.*, 2009) by the creation of a gene bank with oocytes recovered from slaughterhouses (Seidel and Seidel, 1989**)** for the improvement of livestock productivity. The purpose of this article is to summarize the steps of in vitro embryo production in Indian buffalo.

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In Vitro embryo production

The in vitro production of embryos involves retrieval of oocytes from ovaries of slaughtered animals or live animals, in vitro maturation (IVM), fertilization (IVF) and culture (IVC) (Kumar and Anand, 2012). However, the significantly contributing factors in the success of IVEP are the quality and number of collected oocytes.

Ovaries collection and handling

Samples collected from slaughterhouse are the cheapest and the most abundant source of primary oocytes for large scale production of embryos through in vitro maturation (IVM) and in vitro fertilization (IVF) (Nandi *et al.,* 2006).

After slaughter, the left and right ovaries are excise and placed in separate conical tubes containing Washed Medium (WM) and transport to the laboratory at 35-37°C within next 2 hours after slaughter. All cystic ovaries are excluded from studies (Wang *et al*., 2007).

Oocytes collection from slaughtered animal ovaries

The in vitro production of embryos in buffalo involves retrieval of oocytes from ovaries of slaughtered animals. They are three methods used for the collection of oocytes from slaughtered animal ovaries as described by (Kumar and Anand, 2012) as follows:

1. Aspiration from surface follicles using 18-20G needle.
2. Puncturing or dissecting of prominent follicle.
3. Slicing of ovaries into small pieces.

The aspiration method is commonly employed because of the convenience associated with its application. Aspiration of oocytes is done using a needle attached to a 10-ml syringe. To avoid disruption of the surrounding cumulus cells, an 18- gauge needle is used. Possible toxicity associated with syringes containing rubber plungers and siloxane lubricants is avoided by washing and sterilizing glass syringes under the stringent conditions used for tissue culture glassware. For livestock, the use of plastic disposable syringes is acceptable.

Oocytes evaluation and regarding

Oocytes are examined under stereomicroscopy and classifying according to their compaction, number of cumulus cell layers and homogeneity of ooplasm according to (Alves *et al*., 2014), into 4 categories:

1. Grade I (GI): Oocytes with more than 4 layers of bunch of compact cumulus cells mass with evenly granulated cytoplasm.
2. Grade II (GII): Oocyte with at least 2–4 layers of compact cumulus cell mass with evenly granulated cytoplasm.
3. Grade III (GIII): Oocyte with at least one layer of compact cumulus cell mass with evenly granulated cytoplasm.
4. Grade IV (GIV): Denuded oocyte with no cumulus cells or incomplete layer of cumulus cell or expanded cells and having dark or unevenly granulated cytoplasm.

Oocyte in vitro maturation

Oocytes maturation is the most critical step towards successful in vitro embryo production. The culture medium and selection of protein supplements and hormones for IVM play an important role in the subsequent maturation rate, and embryonic development following IVF (Bavister *et al*., 1992). Several factors such as addition of FSH, LH and their combination to culture media had been considered for maximizing success (Saeki *et al.,* 1991). The most in vitro maturation medias used to maturation in vitro buffalo oocyte are tissue culture media (TCM-199), and Ham's F-10. But the most widely used TCM-199 supplemented with 10% FBS + 0.81 mM sodium pyruvate + 5% buffalo follicular fluid (buFF) + 50 µg/ml gentamycin sulfate and 5 µg/ml porcine FSH, achieving 82.3% maturation rates for 24 h incubation in a CO2 incubator (5% CO2 in air, 90-95% relative humidity) at 38.5°C (Kumar *et al*.,2007).

Oocyte in vitro fertilization

The in vitro fertilization is the most critical step of the IVEP procedures in buffalo and requires appropriate preparation of sperm and oocyte, as well as culture conditions that are favorable to the metabolic activity of the male and female gametes. The media used for IVF suggested are BO and TALP, which contain motility enhancing substance like caffeine or theophylline (Bavister, 1995).

On other hand for *in vitro* fertilization, generally TALP (Tyrode's modified medium; Parrish *et al.,* 1988) or BO (Brackett and Oliphant medium) is most widely used medium. For successful fertilization of oocytes, good sperm preparation is the essential and crucial step. Different workers tried different methods for separation of good motile sperm like swim-up (Lopata *et al*., 1976) or percoll based separation system. Sperms used for fertilization should pass through process of capacitation. Capacitation involves alterations of the sperm plasma membrane, which cause it to become unstable and to undergo vesiculation with the outer acrosomal membrane. In bovines, the capacitation occurs basically in the oviduct during the period of estrus and there is evidence that capacitation is caused by a heparin-like glycosamino-glycan in the oviductal fluid (First and Parrish, 1987). High IVF rates have been achieved by addition of heparin (Brackett and Zuelke, 1993) or its combination with penicillamine, hypotaurine and epinephrine, Ca^{++} ionophore A23187 with or without caffeine and high ionic strength media (Brackett *et al.,*1982).

In Vitro embryo culture

At the end of sperm- oocyte incubation, prior to transfer to the *in vitro* culture droplets, presumed zygotes were washed four times in embryo culture medium (mCR2aa containing 0.8% BSA) and cultured in this medium in a humidified CO2 incubator at 38.5 °C for up to 9-10 days to get the blastocyst, the embryo production rate was examined under an inverted microscope, to record the number of cleaved embryos at 8-16 cells which could either be transferred to synchronized recipients for producing live offspring or cryopreserved for future use. (Enchaparambil *et al.,* 2014).

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