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Review Article

PRESENT STATUS OF *IN-VITRO* EMBRYO PRODUCTION IN BUFFALOES

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In the last several years, there has been an increasing interest in *in-vitro* embryo production (IVEP) in buffaloes because of the low efficiency and poor adoption of AI as well as low efficiency of multiple ovulation and embryo transfer (MOET) (Madan *et al.*, 1996, Gasparrini, 2002). *In-vitro* production technologies not only helps in the production of high genetic merit animals, but also provide an excellent source of embryos for the emerging biotechniques like embryo sexing, cloning, nuclear transfer and transgenesis etc. (Gordon, 1994).

The first buffalo calf "PRATHAM" through *in-vitro* maturation, fertilization, and culture (IVMFC) of buffalo oocytes was reported in 1991 (Madan *et al.*, 1991) and cryopreserved embryo in 1993 (Kasiraj *et al.*, 1993). Since then IVMFC has been successfully used for providing buffalo morula / blastocyst (Totey *et al.*, 1992, 1996, Suzuki *et al.*, 1992, Madan *et al.*, 1994, Narula *et al.*, 1996, Chauhan *et al.*, 1997c, 1997d, 1998a-f, 1999, Nandi *et al.*, 1998) and pregnancies in buffalo (Madan *et al.*, 1994b, Gali *et al.*, 998). The efficiency in terms of transferable embryos and development up to full term has been very low (Madan *et al.*, 1996). In buffalo, a higher rate of maturation (70-94%), fertilization (60-70%) and cleavage (40-50%) with low rates of blastocyst formation (10-15 %) has been reported (Nandi *et al.*, 2002). In cattle, a comparable maturation, fertilization and cleavage rate with a higher proportion of blastocyst development varying from 30-60% has been observed (Holm *et al.*, 1999, Farin *et al.*, 2001). Even though the entire *in- vitro* system establishes in buffalo species has been developed by extrapolating information acquired in more studied species like cattle. The *in-vitro* embryo production of buffalo is considerably less efficient than that of cattle (Zicarelli *et al.*, 1996, Palta and Chauhan, 1998, Nandi *et al.*, 2002). The *in-vitro* culture system employed for performing *in-vitro* fertilized and cleaved embryos up to blastocyst stage are suboptimal and need substantial improvement (Nandi *et al.*, 2002). The present review summarises the *in-vitro* maturation, fertilization, and culture system involved in transferable production in buffalo.

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IN-VITRO MATURATION OF OOCYTES

The washed cumulus oophorus complexes (COCs) are generally cultured in 50 µl droplets (15-20 oocytes / droplet) of maturation medium in a 35 mm sterile petridish. The droplets are covered with warm non-toxic paraffin oil and placed in CO_2 incubator at 38.5^{0}C with 5% CO_2 in air and relative humidity for 24 hours.

Buffalo oocytes are mostly cultured in complex medium such as TCM-199 (Singh *et al.*, 1989, Totey *et al.* 1991, 1992, 1993, Madan *et al.*, 1994a, Dhanda *et al.*, 1996, Das *et al.*, 1996, Chauhan *et al.*, 1997a), Ham's F-10 medium (Totey *et al.*, 1993), minimum essential medium and way mouth medium (Ravindranatha *et al.*, 2001) for 24 h under standard conditions. Supplementation of IVM culture medium with fetal calf serum (FCS) (Totey *et al.*, 1996, Chauhan *et al.*, 1999), proestrus buffalo serum (PrBS) (Samad *et al.*, 1998), oestrus buffalo serum (OBS) (Singh and Majumdar 1992, Totey *et al.*, 1992), oestrus cow serum (OCS) (Samad *et al.*, 1998), steer serum (SS) (Chauhan *et al.*, 1998a, Nandi *et al.*, 2001b) or super ovulated buffalo serum (SBS) (Chauhan *et al.*, 1998a) at 10-20% oxygen tension is necessary for achieving