# Effect of Oviductal Cell Co-Culture on Cleavage and Development of Buffalo IVF Embryos

P. S. Yadav*, S. Khanna, O. K. Hooda and R. K. Sethi
Central Institute for Research on Buffaloes, Sirsa Road, Hisar, Haryana, India

**ABSTRACT** : *In vitro* fertilization can be used for salvaging superior buffalo germplasm which otherwise goes waste after the slaughter of animals. This technology has also increased our basic understanding of growth of germ cells and embryos. The requirement of growing embryos is peculiar and stage specific. In the present study the cleavage and development of buffalo embryos were studied with homologous (buffalo) and heterologous (goat) oviductal cell co-culture systems. The cleavage rate improved significantly ($p<0.01$) in both homologous and heterologous co-culture as compared to control (55.3, 46.8 and 11.4%). The morula formation using homologous and heterologous oviductal cells also increased significantly as compared to control group (43.6, 21.9 & 1.9%). There was no blastula formation in control group, but addition of oviductal cells either from homologous or heterologous species significantly increased the blastula formation (9.5, 12.5%). The cleavage rate and embryo development was slightly better (non significant) in homologous as compared to heterologous oviductal cell culture. It was concluded that the use of oviductal cell co-culture (homologous and heterologous species) have significantly improved cleavage and development of buffalo embryos *in vitro*. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 7 : 894-896*)

**Key Words** : Buffalo, IVF, Oviductal Cells, Co-Culture, Heterologous

## INTRODUCTION

A successful *in vitro* fertilization is the outcome of a series of closely linked events: oocyte maturation, sperm capacitation, fertilization, cleavage, morula and blastocyst formation. Impairment at any step due to uninvestigated factors lead to poor yield of blastocysts. The success rate of oocytes maturation and fertilization *in vitro* has been greatly improved in goat (Yadav et al., 1998), cattle (Goto et al., 1988) and buffalo (Chauhan et al., 1998). Various culture systems with varying success rate for culturing embryos include supplementation of media with steroid hormones (Fukui et al., 1982), buffalo estrus serum (Yadav et al., 1997), human serum (Walker et al., 1989). Crister et al. (1986) have shown that granulosa cell interactions with cumulus oocyte complexes during maturation *in vitro* are involved in imparting developmental competence to the maturing bovine oocytes. Co-culturing of embryos with somatic cells (Camous et al., 1984) and cumulus cells (Goto et al., 1988) have been reported as important factors for *in vitro* development of embryos in several species. Malayer et al. (1988) reported that various oviductal polypeptides, which are synthesized and secreted by oviductal cells, support the *in vitro* development of embryos. Inspite of these efforts, the percentage of oocytes reaching the blastocyst stage in a completely *in vitro* system is still low in buffaloes. Hence in this study an attempt has been made to investigate the role of homologous and heterologous oviductal cell co-culture system on cleavage and development of buffalo oocytes to morula and blastocyst.

* Corresponding Author: P. S. Yadav. Tel: +91-1662-28313, Fax: +91-1662-39604, E-mail: gopukhanna@yahoo.com, Sanjaykhanna@mailcity.com.
Received September 7, 1999; Accepted November 25, 1999

## MATERIALS AND METHODS

### Collection of oocytes

Ovaries were obtained from buffaloes killed at abattoir and were transported in laboratory in normal saline (9 g NaCl/l) at 30-37℃. These ovaries were washed twice with normal saline solution in the laboratory. The oocytes were recovered from visible surface follicles by aspiration with an 18-gauge needle fitted to a 5 ml hypodermic syringe. A small amount of aspiration medium (TCM-199 with 10% buffalo estrus serum and Phosphate Buffer Saline (PBS) with 0.4 mg/ml bovine serum albumin at 1:1 ratio) was sucked in the syringe and oocytes along with follicular fluid were sucked from follicles of 2-6 mm in diameter and then decanted into embryo searching plates (Falcon, USA). Plates were screened thoroughly and cumulus oocyte complex (COC) with one or more layers of cumulus cells were selected. These COCs were transferred to another plate and washed 3-4 times with maturation medium (TCM 199+10% buffalo estrus serum).

### Oocyte maturation

Washed oocytes (10-12 in number) were introduced into 100 $\mu$l of culture medium covered with embryo tested mineral oil (Sigma Chemicals, USA) in a 35 mm Petri dish. These Petri dishes were incubated for 24 h in a $CO_2$ incubator under 5% $CO_2$ in air and

**Table 1.** Effect of oviductal cell co-culture and development of buffalo IVF embryos

| Treatment | Trials | Oocytes used | Oocytes matured | Cleaved | | Morula | | Blastocyst | | Arrested | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Without oviductal cells | 4 | 137 | 105 (76.6)[a] | 12** | (11.4)[a] | 2** | (1.9)[a] | 0.0** | (0.0)[a] | 80* | (76.2)[a] |
| 2. Buffalo oviductal cells | 4 | 238 | 188 (78.9)[a] | 104 | (55.3)[b] | 82 | (43.6)[b] | 18 | (9.5)[b] | 46 | (24.5)[b] |
| 3. Goat oviductal cells | 4 | 210 | 173 (82.3)[a] | 81 | (46.8)[b] | 38 | (21.9)[b] | 21 | (12.1)[b] | 22 | (12.7)[b] |

Figures in parenthesis are percentages and having different superscripts in columns differ significantly; * $p<0.05$; ** $p<0.01$.

95% humidity at 38.5℃. Oocytes either with expanded cumulus cells or with first polar body with healthy appearing cytoplasm were considered matured.

**Insemination of oocytes**

Good quality semen was obtained from the germ plasm center of the Institute. To avoid variation due to semen quality, frozen semen doses of a single bull frozen on a particular day were used throughout the experiment. Frozen semen straws were thawed at 37℃ and decanted into 15 ml centrifuge tubes. The Brackett and Oliphant medium (BO medium) for sperm capacitation was prepared as per method described by Brackett and Oliphant (1975). The semen was washed with Brackett and Oliphant medium (BO) and centrifuged at 2,000 rpm for 5 minutes. This process was repeated twice. For sperm capacitation, sperm pellet was dissolved in a capacitation medium (BO medium with 20 mg BSA/ml and 10 $\mu$g heparin/ml); 100 $\mu$l of this medium with sperm concentration $1\times10^6$ sperms/ml was used to inseminate 10-12 oocytes. After 3 h coincubation of sperm and oocyte the sperm capacitation medium was replaced by maturation medium. At this stage the inseminated oocytes were divided into three groups to study the effect of oviductal cell co-culture. Group 1 (Control) without oviductal cells, Group 2 with buffalo oviductal cells, Group 3 with goat oviductal cells. In each group, the maturation medium was changed on alternate days. Zygotes were observed for cleavage 48 h after insemination under phase contrast microscope. The resulting embryos were cultured for 9 days and observed for development every 24 h.

**Collection and establishment of oviductal cell culture**

Buffalo and goat oviducts having fresh corpus luteum on their ovaries were collected from the slaughter house and were transported at 4℃ to the laboratory. Oviductal cells were prepared as described earlier by Yadav et al. (1998). The data were analysed statistically by Duncans multiple range test.

## RESULTS AND DISCUSSION

The results are presented in table 1. The numbers of oocytes used in Group 1 (without oviductal cells), Group 2 (buffalo oviductal cells), Group 3 (goat oviductal cells) were 137, 238 and 210, respectively. The numbers of oocytes matured in Group 1, Group 2 and Group 3 were 105 (76.6%), 188 (78.9%) and 173 (82.3%) respectively, which is statistically non significant. The numbers of oocytes in which cleavage was observed in Group 1, Group 2 and Group 3 were 12 (11.4%), 104 (55.3%) and 81 (46.8%) respectively, indicating a significant increase in the cleavage rate in the oocytes co-cultured either with buffalo oviductal cells or with goat oviductal cells. The percentage (based on matured oocytes) of morula formed were 1.9%, 43.6%, 21.9% in Group 1, Group 2 and Group 3, respectively. The percentage of embryos with arrested development after cleavage was significantly higher (76.2%) in Group 1 as compared to Group 2 and Group 3. The percentage of blastula formed in Group 1, Group 2 and Group 3 were 0.0, 9.5 and 12.1 per cent respectively.

The results of this study indicated that co-culture of embryos either with homologous or heterologous oviductal epithelial cells has marked effect on the *in vitro* development of buffalo embryos. Similar observations were also reported in buffalo (Madan et al., 1994), goat (Yadav et al., 1998), and cattle (Watson et al., 1994). This significant increase in development of embryos *in vitro* may be attributed to the synthesis and secretions of some oviductal polypeptides which may be a beneficial factor during co-culture (Malayer et al., 1988). The availability of fresh oviducts is one of the limiting factors for a co-culture system (Xu et al., 1992). The use of heterologous co-culture system may be helpful in IVF embryo production. In this study the cleavage rate and morula formation with homologous oviductal cell co-culture (Group 2) was slightly higher (statistically non significant) as compared to heterologous oviductal cells (Group 3). This is supported by Yadav et al. (1998) who reported that cleavage and morula formation in goats using goat and buffalo oviductal cells were 57.6% vs 59.2%, and 23.6% vs 26.6% respectively. The use of heterologous oviductal cells indicated that oviductal cells from other species can also be used successfully for a co-culture system and non availability of homologous oviductal cells cannot be a limiting factor for *in vitro* development of embryos. It was concluded that both buffalo and goat

oviductal cell co-culture have significantly improved the cleavage and development of buffalo embryos.

## REFERENCES


Brackett, B. G. and G. Oliphant. 1975. Capacitation of rabbit spermatozoa *in vitro*. Biol. Reprod. 12:262-274.

Camous, S., Y. Heyman, W. Meriou and Y. Menezo. 1984. Cleavage beyond the block stage survival after transfer of early bovine embryos cultured with trophoblastic vesicles. J. Reprod. Fert. 72:479-485.

Chauhan, M. S., S. K. Singla, P. Palta, R. S. Manik and O. S. Tomar. 1998. Development of *in vitro* produced buffalo (*Bubalus bubalis*) embryos in relation to time Asian-Aust. J. Anim. Sci. 11:398-403.

Crister, E. S., M. L. Leibfried-Rutledge, W. H. Eyestone, D. L. Norley and N. L. First. 1986. Acquisition of developmental competence during maturation *in vitro*. Theriogenology. 25:150(Abstr.).

Fukui, Y., M. Fukushima, Y. Terawaki and H. Ono. 1982. Effect of gonadotropins, steroids and culture media on bovine oocyte maturation *in vitro*. Theriogenology. 18:161-175.

Goto, K., Y. Kajihara, S. Kosaka, M. Koba, Y. Nakanishi and K. Ogawa. 1988. Pregnancies after co-culture of cumulus cells with bovine embryos derived from *in vitro* fertilization of *in vitro* matured oocytes. J. Reprod. Fert. 83:753-758.

Madan, M. L., M. S. Chauhan, S. K. Singla, R. S. Manik. 1994. Pregnancies established from water buffalo (*Bubalus bubalis*) blastocysts derived from *in vitro* matured, *in vitro* fertilized oocytes and co-cultured with cumulus and oviductal cells. Theriogenology. 42:591-600.

Malayer, J. R., P. J. Hansen and W. C. Buhi. 1988. Secretion of proteins by cultured bovine oviducts collected from estrus through early diestrus. J. Exp. Zool. 248:345-353.

Walker, S. K., R. J. Lampe, R. F. Seamark. 1989. Culture of sheep zygotes in synthetic oviductal fluid medium with different concentration of sodium bicarbonate and hepes. Theriogenology. 32:797-804.

Watson, A. J., P. H. Watson, D. Warnes, S. K. Walker, D. T. Armstrong and R. F. Seamark. 1994. Preimplantation development of *in vitro* matured, *in vitro* fertilized ovine zygotes. Comparison between oviductal epithelial cell monolayer and culture under low oxygen atmosphere. Biol. Reprod. 50:715-724.

Xu, K. P., B. R. Yadav, R. W. Rorie, L. Plante, K. J. Betteridge, W. A. King. 1992. Development and viablity of bovine embryos derived from oocytes matured and fertilized *in vitro* co-cultured with bovine oviductal epithelial cells. J. Reprod. Fert. 94:33-43.

Yadav, P. S., Anil Saini, V. S. Solanki, O. K. Hooda and S. K. Jindal. 1997. Comparision of follicular fluid and estrus serum on *in vitro* maturation fertilization and development of buffalo embryos. International J. Anim. Sci. 12:193-196.

Yadav, P. S., Anil Saini, A. Kumar, G. C. Jain. 1998. Effect of oviductal cell co-culture on cleavage and development of goat IVF embryos. Anim. Reprod. Sci. 51:301-306.