Effect of culture media and serum supplementation on the development of in vitro fertilized buffalo embryos

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In vitro production of buffalo embryos

Practical applications:

- ✓ Faster multiplication of superior germplasm
- ✓ Production of transgenic animals
- ✓ Production of cloned animals
- ✓ Preservation of germplasm
- ✓ Generation of embryonic stem cells

Limitations:

- Yield of blastocysts is very low i.e, 6-10% of the oocytes taken
- Complicated culture procedures
- Poor availability of usable quality and total oocytes

Objectives

- To compare the development of buffalo embryos in simple and complex culture media.
- To determine the effects of serum supplementation on the development of buffalo embryos.

Collection & grading of oocytes

Buffalo ovaries from Delhi slaughterhouse

Transport to lab in saline + antibiotics at 32 to 37°C

Aspiration of follicles (2-8 mm) In aspiration medium (TCM-199 + 10% FBS)

Grade A: Compact cumulus oocyte complexes (COCs) with an unexpanded cumulus mass having 3 - 4 layers of cumulus cells, and with homogenous evenly granular ooplasm

Grade B: COCs with 2-3 layers of cumulus cells and a homogenous evenly granular ooplasm

Grade C: Oocytes partially or wholly denuded or with expanded or scattered cumulus cells or with an irregular and dark ooplasm

In vitro maturation, fertilization & culture

Oocytes of Grades A +B

↓ Washed with maturation medium (TCM-199 + 10%FBS + 5µg/ml FSH-P

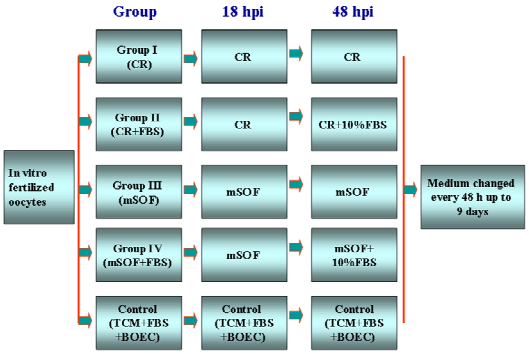
↓ Placed in 50-100 µl droplets of maturation medium in 35 mm Petri dish in groups of 10-15

↓ Cultured for 24 h in a CO_2 incubator (5% CO_2 in air, 90-95% humidity) at 38.5°C

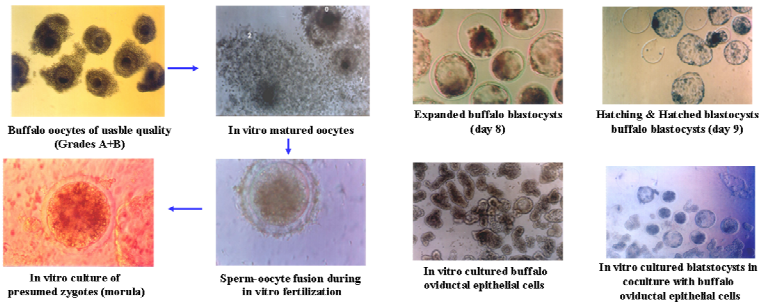
↓ 0 hr: Incubated with 1-2 million spermatozoa/ml in BO medium

↓ 18 hr: Divided in 5 groups; washed with and cultured in respective media

Experimental design



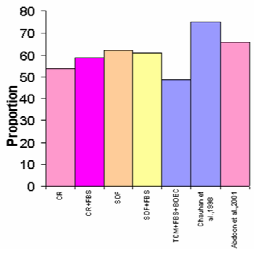
Results & Discussion



Buffalo oocytes subjected to IVM, IVF and the cleavage rates in different trials

Trial No.	No. of oocytes of Grades A + B matured in vitro	No. of oocytes fertilized in vitro	No. of cleaved embryos at 48 hpi	Cleavage rate (%)
1	140	126	76	60.3
2	190	153	74	48.3
3	182	125	64	51.2
4	185	170	72	42.3
Total	697	574	286	50.5

Proportion of cleaved embryos developed to morulae + blastocysts on day 8 post insemination



Proportion of cleaved buffalo embryos, developed to the morula and blastocyst stages, after IVC in different media

Medium	No. of cleaved embryos (n)	Day 8 M n (%)	Day 8 B n (%)	Day 8 M + B* n (%)	Day 9 M n (%)	Day 9 B n (%)	Day 9 M + B** n (%)
mCR2aa	59	12 (20.3)	20 (33.9)	32[a] (54.2)	15 (25.4)	19[a] (32.2)	34[a] (57.6)
mCR2aa + FBS	58	16 (27.6)	18 (31.0)	34[a] (58.6)	8 (13.8)	28[b] (48.3)	36[a] (62.1)
SOF	69	16 (23.2)	27 (39.1)	43[b] (62.3)	13 (18.8)	29[b] (42.0)	42[b] (60.9)
SOF + FBS	65	15 (23.1)	25 (38.5)	40[b] (61.5)	14 (21.5)	27[b] (41.5)	41[b] (63.1)
TCM-199 + FBS + co culture with BOEC (Control	35	6 (17.1)	11 (31.4)	17[c] (48.6)	7 (20.0)	5[c] (14.3)	12[c] (34.3)

M; Morulae + compact morulae, Blastocysts; blastocysts + expanded blastocysts + hatching/hatched blastocysts. Values in parenthesis are percentage of cleaved embryos taken. Values with different superscripts differ significantly. *(P<0.05), **(P<0.01).

Conclusion

- ✓ Simple media like mCR2aa or mSOFaa are capable of supporting the development of buffalo embryo.
- ✓ Yields of blastocysts were same even in the serum starved media.
- ✓ MSOFaa appears to be the most effective medium for supporting the development of buffalo embryos.

References:

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