Mansoura Veterinary Medical Journal

# IMPROVEMENT OF BUFFALO EMBRYO DEVELOPMENT IN VITRO; EFFECT OF DIFFERENT CULTURE MEDIA

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## ABSTRACT

*The aim of the present study to compare 4 different types of media, SOF, TCM-119, CR1aa and KSOM, for in vitro culture of buffalo embryo. This study was under taken in the Department of Artificial insemination and embryo transfer, Animal Reproduction Research Institute (ARRI), Al-Haram, Giza .The selected cumulus oocyte complexes(COCs) with a compact cumulus cells and evenly granulated ooplasm were in vitro matured at 38.5°C in atmosphere of 5% $CO_2$ with high humidity .After 24h of culture, oocyte were used for in vitro fertilization . Following 18h of insemination, oocyte were randomly assigned to investigate the effect of different types of media from the zygote to day 7 post-insemination . there were no significant difference in cleavage rate among 4 media However, the morula and blastocyst stage increased significantly($P<0.05$) in using SOF(26.03, 17.88% respectively) and also by using CR1aa (25.37, 16.42% respectively) compared with TCM(11.86, 5.08% respectively) and KSOM(9.62, 3.85 %respectively).There is no significant difference between SOF and CR1aa.In conclusion, the current data reveled that SOF is the best media used in embryo development in buffalo.*

***Key words**: SOF. In vitro culture media. Buffalo*

## INTRODUCTION

*In vitro* embryo production(IVP) systems in buffaloes are comparatively more sub-optimal than in cattle and require substantial improvements ( **Gasparrini, 2002 and Nandi *et. al.*, 2002)** . In buffaloes, even though a similar maturation rate with cattle (87 versus 94%) but significantly lower cleavage rate (65 versus 84%) was observed in comparison to cattle **(Gasparrini 2002 and Neglia *et al.* 2003).** Approximately 80% of buffalo oocytes fail to develop to blastocyst, also when this blastocyst developed the high percentage are incapable of developing to term **(Nandi *et. al.*, 2002).**

The major cause of the fall-off in development of embryos occur during the last part of IVP ( between the formation of zygot and blastocyst) this made the period of post fertilization is very critical period. The blastocyst quality depend on this period but the oocyte quality is responsible for blastocyst yield**(Lonergan et al.,2003**) .

There are several environmental factors which impact IVF of mammalian oocytes, such

as temperature, type of medium, pH, gas phase and other factors. Mammalian Cells can only exist outside their natural in vivo surroundings, only if the *in vitro* environment mimics that of living body, in this respect culture media must be provide with the chemical and physiological elements which cells need to survive *in vitro* **(Harlow and Quinn 1982).**

In the present study, we compared 4 different type of media to select optimal culture media used in buffalo.

## MATERIAL AND METHODS

The chemicals used in this study were purchased from Sigma Chemical Co (St. Louis, MO, USA) .

**In vitro oocyte maturation** : Ovaries from apparently normal reproductive organ of heifers or adult buffaloes of unknown age and breeding history slaughtered in EL-sharkawy abattoir were collected within 30 minutes after slaughter and evisceration of animals. The ovaries were kept in a thermos flask containing warm normal saline with 50ug/ml gentamycine. Then transported to the lab within 2-3 h from slaughter. Immature cumulus-oocyte complexes(COCs) were aspirated from medium sized(2-8mm) follicles with 18 gause needle connected to a 10ml sterile disposable syringe . Oocytes with more than two layers of compact cumulus cells and homogenous granular ooplasm were selected for in vitro maturation, according to **Totey et al(1992)** . The COCs were rapidly washed twice in modified Dulbecco' s phosphate-buffered saline, before transferring to the maturation media. The basic media for oocyte maturation is TCM-199 supplemented with 10% heat inactivated fetal calf serum, 10 µg/ml Luteinizing hormone, 5 µg/ml follicle stimulating hormone and 1 µg/ml estradiol-17β. The PH of the media was adjusted to 7.4 and osmolarity 295-310 mosm. The oocytes( 10- 15) transferred to a 100µl drop of maturation medium under mineral oil and incubated for 20-24 hour at 38.5 ◦C in 5% of CO2 in air with maximum humidity.

**Assessment of maturation rate:** This occur under steromicroscope by expansion of cumulus cell around oocyte .oocyte with full or moderate cumulus expansion and also the oocyte with slight or no cumulus cell expansion but have the first polar body extruded in preivitilline space may considered matured **(Nandi et al.,2002).**

**Sperm preparation and IVF**:Mature oocytes were partially denuded from the surrounding cumulus cell to allow easy penetration of the sperm by repeat gentle pipetting and washed three times in TALP then added to the fertilization drops 10 oocyte in each drop.Spermatozoa were capacitated in vitro using TALP medium .Three straws of frozen semen with known sperm concentration($2\times10^6$ sperm cell/ml) are thawed for one minute in 37°C water bath. immediately after thawing, the most motile spermatozoa were separated by swim up technique in sperm –TALP medium containing 6mg/ml BSA, for one hour**(Parrish et al.,1986)** then the uppermost layer of the medium containing the most motile spermatozoa was collected by using aplastic Pasteur pipette put in small test tube and 3 ml of SP-TALP medium. the freezing media is washed out by centrifugation at 2000 round per minute for 5 minutes to form sperm pellet, the supernatant is discarded and one ml of TALP is added and mixed with pellet then centrifuged and supernatant is discarded again to completely wash out the freezing media. one ml TALP medium containing 10mg /ml heparin for in vitro capacitation of sperm is added and mixed with sperm pellet and left in the 38.5 ◦C in 5% of CO2 maximum humidity

incubator for 10 minutes then checked for motility. If motility is accepted, 20 µl of semen is co incubated with each fertilization drop for 18 hour

**Embryo developmental culture** :The oocytes were freed from loosely bound spermatozoa and remaining attached cumulus cells by gentle pipetting . Denuded zygote were washed three times (each drop separately) with culture medium and . All the culture media performed in an atmosphere of $CO_2$ in air with maximum humidity at 39°C. The culture media medium was replaced every 48 h with afresh medium until day 7pi to prevent toxic accumulation of ammonium as a result of amino acid degradation and oocytes that had not cleaved must be removed from the culture media, leaving only those that undergo cleavage. The percentage of cleaved oocytes was recorded 48h post-insemination and those developed to morula and blastocyst stage were recorded at 5-7day post-insemination. During observation period ,gentle shaking of the culture dish was done to allow a uniform environment among fertilized oocyte.

**Assessment of fertilization and embryonic development:**At 18 h following insemination, the presumptive zygotes were denuded completely from surrounding cumulus cells and mounted on slides with coverslips and then fixed with acetic acid/ethanol (1:3) solution for at least 24 h. The presumptive zygotes were stained with 1% orcein dissolved in *45%* acetic acid solution and examined for evidence of fertilization penetration of the sperm was identified by observing decondensed sperm heads or male pronuclei with their accompanying sperm tails in the cytoplasm. Oocytes with two pronuclei and a clear second polar body were considered normally fertilized. The percentage of cleaved oocytes was assessed at 48 h after insemination. On day 5 and day 7 following insemination, the embryos were observed under a microscope to compare the percentages of embryonic development to morula and blastocyst at each group respectively.

**Experimental design**

**Experiment 1 : Effect of different culture media on in vitro embryo development in buffalo:** fertlilized oocytes were divided into four culture groups.

In the first group, the fertilized oocytes were cultured in TCM-199 media.

In second group, the fertilized oocytes were cultured in synthetic oviductal fluid media (SOF) media.

In third group, the fertilized oocytes were cultured in CR1aa media.

In fourth group, the fertilized oocytes were cultured in potasium complex optimized media KSOM.

**Statistical analysis:**

The experiment was replicated at least 3 times .The developmental competences were assessed using Chi-square at ($p<0.01$ and 0.05, respectively).

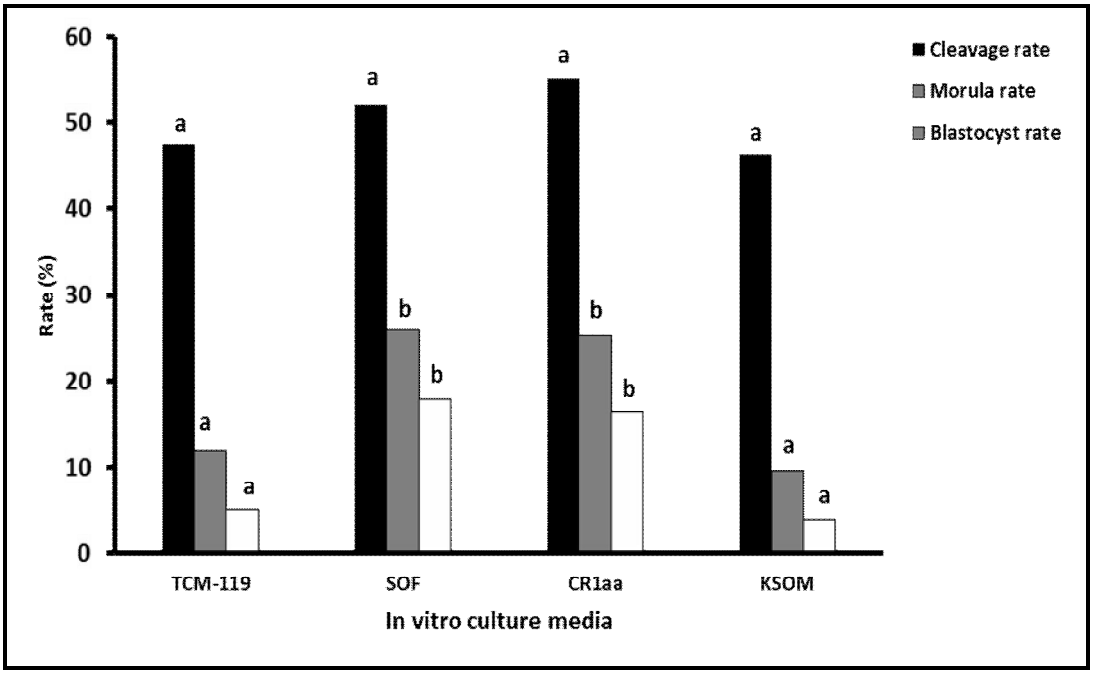
## RESULTS

In the present study there is no significant difference among different types of culture media used in relation to cleavage rate TCM, SOF, CR1aa, KSOM (47.45, 52.05, 55.22, 46.15% respectively). However the morula and blastocyst stage increased significantly($P<0.05$) in using SOF(26.03, 17.88% respectively) and also by using CR1aa (25.37, 16.42% respectively) compared with TCM(11.86, 5.08% respectively) and KSOM(9.62, 3.85 %respectively).There is no significant difference between SOF and CR1aa( **Table** 1) **(Fig 1)**.

**Table 1:** Effect of different culture media on buffalo embryo development in vitro:

| Treatment | No. oocytes | Cleavage rate | Morula stage | Blastocyst stage |
|---|---|---|---|---|
| **TCM-199** | 59 | 28 (47.45) [a] | 7 (11.86) [a] | 3 (5.08) [a] |
| **SOF** | 73 | 38 (52.05) | 19 (26.03)b | 13 (17.88) [b] |
| **CR1aa** | 67 | 34 (55.22) [a] | 17 (25.37) [b] | 11 (16.42) [b] |
| **KSOM** | 52 | 24 (46.15) [a] | 5 (9.62) [a] | 2 (3.85) [a] |

Values with different superscript letters in the same columns are significantly different at least (P<0.05).



## DISCUSSION

Culture media components and culture conditions can mark and even modify the *in vitro* development of mammalian embryo **(Hardy et al.,2000)**. Data present in the current study revealed that SOF is the best media used in embryo development in buffaloes at morula stage and blastocyst stage compared with TCM-199 and KSOM However, there is no significance difference (P<0.5) between SOF and CR1aa. These findings agree with previous study in cattle **Fukui et al(1991)** who found that SOF was superior for both cleavage and development to blastocyst stage than TCM-199.Also **Merton and Mullaart(1999)** stated that the percentage of transferable cattle embryos by using semi-defined culture media(SOFaa+BSA)than (TCM-199+FCS+co-culture) in the same point of view **Wrenzychi et al(2001**) and **Yaseen et al(2001)** found that embryos of cattle produced by SOF culture media have greater similarities to in vivo produced embryos than those produced by TCM-199 culture media.

Moreover,the data present in current study agree with **Boni et al( 1999)** and **Kumar et al (2007**) who evaluate the possibility of culturing buffalo embryos in defined media in absence of serum and co-culture and found that SOF is the best media used for supporting embryo development in buffalo by increase morula and blastocyst yields than TCM-199 with serum and co-culture.

This findings could be explained by the fact that higher rate of development in simple media as SOF,CR1aa than TCM-199 is due to high concentration of glucose in TCM-199(5.5m M)(**Kumar et al.,2007).**Glucose has inhibitory effect on embryos development in cattle, those embryos require pyruvate and / or lactate through embryo development **(Bavister 1995**).when glucose concentration above 3m M in SOF this lead to poor in development in embryos(**Mastsuyama et al.,1993).**

The result of the current study disagree with **Nandi et al(2002**) who found that ( TCM-199 +steer serum +buffalo oviductal epithelial cells) lead to higher blastocyst yield in culture media in buffalo then m SOF. This may be attributed by when used TCM-199 with co- culture system this somatic cell decrease glucose concentration in it and increase pyruvate and lactate **(Edwards et al.,1997**).

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