IN VITRO STUDIES ON MATURATION, FERTILIZATION AND DEVELOPMENT OF EGYPTIAN BUFFALO OOCYTES

By

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B. Sc. Agric. Sc. (Animal Production), Ain Shams University, 1997

M. Sc. Agric. Sc. (Animal Physiology), Ain Shams University, 2003

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ABSTRACT

Assem Adou Metwally Ramadan: In Vitro Studies on Maturation, Fertilization and Development of Egyptian Buffalo Oocytes. Unpublished Ph. D. Thesis, Department of Animal Production, Faculty of Agriculture, Ain Shams University, 2010.

The present study was designed to examine the Influence of different media supplements of hormones and antioxidants on *in vitro* maturation (IVM), suitable media for *in vitro* fertilization (IVF) and effect of granulosa cells on cleavage and embryo development of buffalo oocytes.

* The follicular oocytes were collected from ovarian follicles (2-8 mm in diameter) by aspiration method using either M-PBS or Hepes TALP and examined under stereomicroscope for evaluation and selection of suitable oocytes. Oocytes were classified under stereomicroscope according to the number of cumulus cell layers and morphology of ooplasm into excellent, good or fair. Excellent and good quality oocytes were selected for complete of maturation *in vitro* then immature oocytes were cultured for maturation in TCM-199 enriched with either 10% fetal calf serum (FCS) or 0.3 % bovine serum albumin (BSA). Matured oocytes were classified into five groups; group (1) TCM-199 served as a control; group (2) Hams F-10; group (3) TCM-199 supplemented with gonadotropins (PMSG), LH and Estradiol; group (4) in which TCM-199 was supplemented with β-mercapto ethanol and group (5) TCM-199 supplemented with both β-mercapto ethanol and the same hormones mentioned above. The maturation rate was assessed by the degree of cumulus cell mass expansion or by the presence of first polar body and reaching metaphase II stage oocytes.

The results indicated that :

* Average recovery rate in this study was 2.56 Oocyte / ovary with 72.5 % of excellent and good COCs.
* There was no significant difference between BSA and FCS as a source of protein in the maturation media.
* Moreover, TALP HEPES resulted in significantly (p<o.o5) higher maturation rate (81.25) than M-PBS (74.25).
* There was no significant difference between TCM-199 and Hams F-10 as maturation medium for maturation rate *in vitro*.
* The addition of PMSG, LH and Estradiol to the IVM medium and / or β-mercapto ethanol progressively (p<o.o5) enhanced the developmental competence of buffalo oocytes as compared to control medium.
* TALP medium supported significantly (p<o.o5) fertilization and cleavage rates (73.75% - 60.5%) than BO medium (67% - 51.25%) respectively.
* The cleavage rate of embryos cultured with granulosa cells (60.67%) was siginificantly higher (p<o.o5) than the control (55.15%).
* High percentage of the embryos development were blocked at the 8 and 16 cell stage.

In conclusion, excellent quality oocytes cultured for IVM in TCM-199 medium supplemented with hormones and antioxidant then fertilized with capacitated buffalo spermatozoa in TALP medium and cultured with granulosa cells progressively enhanced developmental competence of buffalo oocyte.

Key Words : Buffalo, IVM, IVF, IVC, proteins, hormones, antioxidants, granulosa cells, types of media.

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LIST OF ABBREVIATIONS

AR	Acrosome Reaction
BCS	Bovine Calf Serum
βME	Beta Mercapto Ethanol
BO	Brackett and Oliphant medium
BSA	Bovine Serum Albumin
cAMP	Cyclic Adenosine monophosphate
COCs	Cumulus Oocytes Complexes
DO	Denuded Oocytes
E2	Estradiol 17-β
ECS	Estrous Cow Serum
FBS	Fetal Bovine Serum
FCS	Fetal Calf Serum
GAG	Glycosaminoglycans
GSH	Glutathione
HEPES	Hydroxy Ethyl Piperazine Ethanosulphonic acid
IVC	*In Vitro* Culuture
IVF	*In Vitro* Fertilization
IVM	*In Vitro* Maturation
LH	Luteinizing Hormone
M II	Metaphase II
MPBS	Modified Phosphate Buffer Saline
PFS	Pre Antral Follicles
PMSG	Pregnant Mare Serum Gonadotrophins
POCs	Partially denuded Oocytes complexes
ROS	Reactive Oxygen Species
TALP	Tyroid Albumin Lactate Pyrovate medium
TCM-199	Tissue Culture Medium-199

1. INTRODUCTION

Several international organizations have emphasized the potentiality of the buffalo in the economy of a number of developing countries, due to its ability to produce and reproduce under the harsh environmental conditions compared to the dairy cattle. Water buffalo contributes currently by 70% of milk and 40% of meat production **(FAO, 2005)** in Egypt. This gives the buffaloes a great attention for spreading the superior genetic material.

The *in vitro* maturation, fertilization and culture (IVMFC) procedures have been successfully used for routine production of embryos from slaughterhouse ovaries in buffaloes **(Chauhan *et al*., 1998)**. The practical application of these techniques is, however, severely hampered by very poor recovery of total oocytes and IVMF (**Madan *et al*., 1994)**. Conditions during IVMFC are believed to play a role in the acquisition of developmental competence of embryos **(First and Parrish, 1987; Brackett *et al*., 1989).**

Quality of oocytes is one of the important factors affecting the successful rate of these techniques. Presence of an intact complement of cumulus cells surrounding the oocyte and a homogenous appearing ooplasm have been the best indicators of an immature oocyte`s ability to undergo maturation and embryonic development **(Madison *et al*., 1992**).

Several trials were conducted to process appropriate media for IVM and IVF through adding some hormonal supplements **(Beker *et al*., 2002; Mingoti *et al*., 2002).**

In vitro culture results in higher oxygen concentrations than in vivo environments, leading to an increased level of reactive oxygen species (ROS) that cause lipid peroxidation of cellular membranes, So supplementation with antioxidants – such as beta-

mercaptoethanol (bME) during IVF procedures improve intracellular glutathione content in oocytes in several species **(Comizzoli *et al.,* 2000; Mizushima and Fukui, 2001; De Matos *et al.,* 2002; Songsasen and Apimeteetumrong, 2002; Rodriguez-Gonzalez *et al.,* 2003)** and has a beneficial effect in maintaining the function of gametes, the incidence of normal fertilization and, consequently, the quality of IVF embryos **(Hiroaki, 2005).**

In Egypt, a few studies **(Abbas, 1998; Omaima *et al*., 1999; Abdoon *et al*., 2001)** have been carried out to improve the efficiency of buffaloes.

Therefore, the present study aimed to increase the developmental competence of buffalo's oocytes by studying the effect of protein additives (fetal calf serum and Bovine serum albumin), type of IVM media (TCM-199 and Hams-F10), and its hormonal supplements (PMSG, LH and Estradiol) and/or antioxidant (bME), type of IVF media and the influence of granulosa cells in the IVC media on buffalos embryos development.

2. REVIEW OF LITERATURE

The *In vitro* maturation, fertilization and culture (IVMFC) procedures have been successfully used for routine production of embryos from slaughter house ovaries in buffaloes **(Chauhan *et al*., 1998)**. The practical application of these techniques is, however, severely hampered by very poor recovery of total oocytes and IVMF (**Madan *et al*., 1994)**. Conditions during IVMFC are believed to play a role in the acquisition of developmental competence of embryos **(First and Parrish, 1987; Brackett *et al*., 1989).**

2.1. Collection of ovaries

Large number of good quality primary oocytes suitable for culturing are nessesary for successsful production of buffalo embyos **(Totey *et al*., 1992**). Such oocytes may be obtained from the slaughter houses **(Fukui *et al*., 1990**) in a saline at 30-35°C.

Abattoir derived ovaries provide a cheap and abundant source of oocytes, **Lee and Fukui (1995)** collected cow ovaries in a thermos flask containing physiological saline (0.9% Nacl) at a temperature ranged from 30-35°C. They transported them within one hr to the laboratory.

Martinez *et al*. (1998) and Sakaguchi *et al*. (2002) obtained bovine ovaries from abattoir and transported them to the laboratory in 0.9% Nacl within 3-6 h at a temperature of about 30°C.

Ali *et al*. (2004) used 0.9% Nacl solution containing 10.000 iu/l penicillin, 100 mg/l streptomycin, and 250 mg/l amphotericin B as a medium for preserving ovaries during their transport to the laboratory.

Nowshari (2004) collected camel ovaries within 15 min after slaughter and placed them into warm 0.9% Nacl at 37 °C for transportation to the laboratory.