EFFECTS OF YEAR SEASON AND MATURATION MEDIUM ON DEVELOPMENT OF BUFFALO OOCYTES

By

EMAD ABDEL-AZIZ MOHAMED ABD-ALLA

B.Sc. (Chemistry), Ain Shams University, 1995

A thesis submitted in partial fulfillment

of

the requirements for the degree of

MASTER OF SCIENCE

in

Agricultural Science

(Animal Physiology)

Animal Production Department

Faculty of Agriculture

Ain Shams University

2011

Approval Sheet

EFFECT OF YEAR SEASON AND ATURATION MEDIUM ON DEVELOPMENT OF BUFFALO OOCYTES

By

EMAD ABDEL-AZIZ MOHAMED ABD-ALLA

B.Sc. (Chemistry), Ain Shams University, 1995

This thesis for the M.Sc. degree has been approved by:

Dr. Abdel-Khalek El-Sayed Abdel-Khalek ……..……..
Prof. of Animal Physiology, Faculty of Agriculture, Mansoura University

Dr. Mohamed Mohamed Sabra.....
Prof. of Animal Physiology, Faculty of Agriculture, Ain Shams University

Dr. Ahmed Mohamed Saied Abdel- Aziz ...……
Prof. Emeritus of Animal Physiology. Faculty of Agriculture, Ain Shams University

Date of Examination 9 / 11 / 2010

EFFECTS OF YEAR SEASON AND MATURATION MEDIUM ON DEVELOPMENT OF BUFFALO OOCYTES

By

EMAD ABDEL-AZIZ MOHAMED ABD-ALLA

B.Sc. (Chemistry) Ain Shams University, 1995

Under the supervision of:

Dr. Esam El-Din Tharwat

Prof. of Animal Physiology, Faculty of Agriculture, Ain Shams University. (Principal Supervisor)

Dr. Ahamed mohamed Saeed Abdel- Aziz

Prof. Emeritus of Animal Physiology, Faculty of Agriculture, Ain Shams University

Dr. Fikry El-SAyed El-Keraby

Head Research Emeritus of Animal Physiology, Department of Biotechnology, Animal Production Research Institute, Agricultural Research Center

LIST OF PLATES

ABBREVIATIONS

Abbreviation	Description
IVM	In vitro maturation
IVF	In vitro fertilization
IVC	In vitro culture
EAA	Essential amino acid
NEAA	Non essential amino acid
DO	Denuded oocyte
ZP	Zona pellucida
GA	Oocyte grade A
GB	Oocyte gr
GC	Oocyte grade C
GD	Oocyte grade D
GE	Oocyte grade E
GV	Germinal vesicle
GVBD	Germinal vesicle break down
M I	Metaphase I
M II	Metaphase II
TALP	Tyroide albumin lactate pyruvate medium
H-TALP	Hepes- TALP
IVF-TALp	In vitro fertilization- TALP
BGM-3	Bovine Gamete Media-3
FF	Follicular fluid
FCS	Foetal calf serum
EBS	Estrus buffalo serum
OMI	Oocyte maturation inhibitor factor
CEO_s	Cumulus enclosed oocytes
HEPES	4-(2-hydroxyethyl)-piprazine ethane sulfonic acid

ACKNOWLEDGEMENTS

First and foremost, all praise to Allah; the magnificent, the most merciful, and the most gracious, without whose blessing and guidance this work would never has been started nor completed.

I would like to express my deepest and sincere gratitude and appreciation to Prof. Dr. **Esam El-din Tharwat**, Professor of Animal Physiology, Department of Animal Production, Faculty of Agriculture, Ain Shams University for his guidance, supervision, encouragement, reading the manuscript, and invaluable criticism and comments.

I would like to express my deepest. Sincere gratitude and appreciation to Prof. **Dr. Fikry. El-Keraby**, Emeritus Head of Research, Animal Production Research Institute, Ministry of Agriculture, for his kind supervision, encouragement and great help in thesis revision.

My deepest gratitude and appreciation are sincerely directed to **Dr. Ahamed said,** Emeritus Professor of Animal Physiology, Department of Animal Production, Faculty of Agriculture, Am Shams University, for his encouragement.

I would like to express my sincere appreciation and deep gratitude to Dr. **Sherif Shamiah**, Researcher, Department of Biotechnology Research, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture for his guidance, continuous help and encouragement.

I wish to express my deep thanks to **Dr. Arafa Halawa,** Researcher of Animal breeding, Department of Cattle Breeding Research, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, for his help in statistical analysis.

I also would like to thank my colleagues and the staff members at Sakha Animal Production Research Station and Sakha International Livestock Management Training Center for their continuous encouragement and support.

No words can express my great gratitude to all members of my family, especially my father, my sisters and my wife for her continuous encouragement. Smiling face of my kids, Rawdah, Nada, and Omar helped ease many of my difficult times.

Finally, I remember my mother the deceased and I am asking the Almighty Allah to mercy her soul and to make the place of her soul in the heaven.

1. INTRODUCTION

The Egyptian buffalo population is counted to be approximately 3.6 million **(FAO, 2004).** Buffaloes play a great role in livestock production and the economy in Egypt by producing 2.050.610 metric tons of milk/year and 306 ton of meat/year **(FAO, 2001).**

Buffalo reproductive performance suffer from a number of genetic problems that include silent ovulation, delayed maturity, breeding seasonality, prolonged generation interval, low conception rate, low population of primordial follicles, and inactivated ovaries. These problems cause low efficiency of reproductive performance. These limitations are emerged during summer season when food availability and quality are reduced and fertility significantly decreased. **(De Rensis et al. 2008).** In the last decade, Assisted Reproductive Technologies (ART) such as *in vitro* fertilization (IVF), *in vitro* maturation (IVM), artificial insemination (AI), multiple ovulation and embryo transfer (MOET), have been applied to improve reproductive efficiency, and genetic potential in this species **(Shi, 2007).** In cattle *in vitro* maturation and *in vitro* fertilization (IVM/IVF) techniques have been developed to introduce another way to producing embryos. A great deal studies are executed to estimate different parameters responsible for oocyte maturation, fertilization and subsequently embryonic development. There are some fundamental factors affecting on *in vitro* production of embryos such as inadequacy of culture media, media supplementations, and seasons of the year, **(Samad et al., 1999**). The major problems recorded by investigators in buffaloes the poor development of embryos cultured *in vitro*. **Kadoom (1995)** found that most of the cleared oocytes were blocked at 2-cell stages.

The current study was designed to investigate:

1- Effect of presence of CL on the ovary and year season (spring, summer, autumn and winter) on follicular population, recovery rate and quality of buffalo oocytes.

2- Effect of presence of CL on the ovary, year season and addition of Estrus Buffalo Serum (EBS), Buffalo Follicular Fluid (BFF) and hormones (Pregnant Mare Serum Gonadotrophins, PMSG , LH and Estradiol β17) to tissue culture medium as maturation medium on *in vitro* maturation, fertilization and development rates of buffalo oocytes.

2. REVIEW OF LITERATURE

2.1. Buffalo Follicular Population

Aboul-Ela (2000) stated that the major constraint in the ovary appears to be low follicular population and high rate of atresia among growing follicles in Egyptian buffaloes.

Very few studies were carried out to determine follicular population in buffaloes. **Danell (1987)** was the first one, who studied buffalo follicular population and reported that the total number of primordial follicles per ovaries / head was estimated as 10,000 in mature Surti buffalo heifers. **Smith (1990)** studied follicular dynamics in Philippine water buffaloes and reported that the number of primordial follicles in 2 years old buffalo heifers to be 47,200 which decreased to 6000 in 7-8 years old buffaloes. In Nili-Ravi buffaloes the number of primordial estimated follicles per pair of ovaries varied between 12,000 **(Danell, 1987)** and 8,000 **(Samad and Nasseri, 1979)**.

In Egyptian buffaloes, **Kadoom (1995)** and **Shamiah (1997**) estimated the total number of visible follicles, which appear on ovarian surface as 7.8 and 11.9**.** The results recorded by **Shamiah (1997)** indicated that the numbers of follicles of different size in buffalo ovaries were about 50% of those in cattle.

2.2. Biometry of ovaries of Egyptian buffaloes

Fadle *et al.* (1974) and **El-Wishy *et al.* (1971)** demonstrated that maximum size and weight were observed when complete developed corpus luteum present on the ovary and the minimum and maximum average weight was 2.9 and 6.1 gm in buffaloes and 3.9 and 9.9 gm in cattle,respectively.

2.3. Factors affecting oocyte Recovery Rate

2.3.1. Method of oocyte recovery

Rate of oocytes recovery varies according to method of oocytes collection. There are different methods that facilitate the collection of several good oocytes per ovary. These methods are Dissecting, Aspiration, Slicing and Follicle Puncture.

Using three methods for oocytes collection from buffalo ovaries, **Das *et al.* (1996)** found that number of oocytes per ovary (follicles with 2-6 mm in diameter) recovered by slicing was significantly ($P<0.01$) higher (5.7/ovary) than that achieved by follicles puncture (2.6 / ovary) and aspiration (1.7/ovary).

The disadvantage of aspiration method was oocytes recovered represented 30-60% of the punctured follicles collection; in contrast the advantage of follicle aspiration is in term of speed operation **(Gordon 2003).**

The oocytes yield increased by using slicing method and there is a higher proportion of poor oocytes due to heterogeneous population of oocytes retrieved from all follicles which distributed through the stroma of ovaries **(Gasparrini, 2001).**

Kumar *et al.* (1997) in a comparison among techniques used for oocyte recovery by an aspiration, slicing and follicle puncture they reported that the rate of oocyte recovery was greater ($p<0.05$) using slicing method. **Katska (1984)** stated that dissection method gave higher oocyte recovery rates in cattle. **Choi *et al.* (1993)** reported that slicing of equine ovaries is a useful tool for increasing the number of oocytes available for *in vitro* maturation (IVM). Compact cumulus oocytes complexes recovered by aspiration and by additional slicing have the same ability to reach metaphase II *in vitro*. **Gupta and Sarma (2001)** stated that there was no effect among slicing, aspiration and combined methods on the recovery of buffalo oocytes.

Datta and Goswami (1998) found that total number of oocytes recovered per buffalo ovary was significantly ($P<0.01$) lower

using aspiration method than slicing and dissection methods, but processing of aspiration required less time than those of slicing and dissection methods.

Raza *et al.* (2001) concluded superiority of slicing method over that of aspiration method for ovary oocyte collection or yield. The low recovery rate of immature oocytes from slaughterhouse ovary is a major factor in the *in vitro* embryo production (IVEP) in buffaloes **(Gasparrini, 2001** and **Chohan and Hunter 2003).**

Palta and Chauhan (1998) summarized the reasons responsible for poor oocytes yields from buffalo ovaries as follow:

1- Low number of primordial follicles in buffalo, being from 10.000 to 19.000 **(Samad and Nasseri, 1979 and Danell, 1987).**
2- Low population of antral follicles at all stages of heat cycle in buffalo **(Le Van Ty *et al.,* 1989 and Manik *et al.*, 1998)**
3- High incidence of deep atresia, being reported to be 82% **(Ocampo *et al.*, 1994)** or 92% by **(Palta *et al.,* 1998)** in ovarian follicles from an abattoir ovary.
4- Slaughter subfertile and unreproductive state buffaloes **(Selvaraj *et al.,* 1992).**
5- Presence of corpus luteum reduces the recovery of total and acceptable quality oocytes **(Das *et al.*, 1996** and **Kumar *et al.*, 1997).** By aspiration of 2-6 mm follicles the average of total oocytes recovery per ovary recorded to vary from 0.7 **(Totey *et al.*, 1992),** to 1.7 **(Das *et al.,* 1996)** or to 2.4 **(Kumar *et al.*, 1997).**

Raza *et al.* (2001) and **Jainudeen *et al.* (1993)** reported recovery rates of 3.85 and 4.1 oocyte / ovary, respectively.

The overall yield of oocytes per ovary by aspiration method was 1.37 and the acceptable quality oocytes was 1.0 **(Gupta and Sarma 2001).** However, **Chohan and Hunter (2003)** reported that

the recovery rate of buffalo oocytes per ovary was 1.49 and acceptable quality oocytes per ovary were 1.06 aspirated from 2-6 mm follicles.

Regarding the aspiration method achieved to collect buffalo oocyte, **Suzuki *et al.* (1992)** obtained 536 oocytes from 518 follicles of 2-4 mm diameter. However, **Ganguli *et al.* (1998)** gave recovery rate of 79.68 % in buffalo oocytes.

2.3.2. Presence or Absence of Corpus Luteum (CL) on Ovaries

The effect of size of the ovary on oocyte yield was not significant while the absence of CL on ovaries had highly significant effect on the oocyte harvest. **Huma *et al.* (2008)** showed that significantly greater population of oocytes per ovary was recovered from ovaries without CL than that from ovaries with CL. On the contrary, **Varisanga *et al.* (1998)** concluded that ovaries with CL showed improvement in the recovery rate of oocytes compared with non CL bearing ovaries. According to **Nandi *et al.* (2000),** the oocytes recovery rate decreased when ovaries bear CL because lutein cells occupy most of the ovary, so follicular development is restricted.

Abbas *et al.* (2002) found significant ($P<0.01$) variation in the distribution of oocytes at different stages of estrous cycle according to presence or absence of CL, where comparison was carried out and stated that good quality oocytes including the compact oocytes (38.9 vs. 42.1%) partial compact oocytes (16.7 vs. 5.0%) denuded oocytes (22.2 vs. 26.3 %) degenerated oocytes (22.2 vs. 10.5%), respectively. The yield of ovaries bearing CL had significantly lower good quality and total oocytes (2.63 and 3.76 per/ ovary) than the ovary without CL (4.48 and 5.88 per ovary). **Hafez and Hafez (2000)** reported that the lower yield of oocytes from ovaries bearing CL due to the fact that CL reduces the growth of follicles and increase the atresia of follicles

In bovine, **Hazeleger *et al.* (1995)** reported that number of bovine cumulus oocytes complex per cow ranged between 34 to 93. Presence of CL or large follicles had no effect on the number of cumulus oocytes complex. However in buffalo, oocytes yield from corpus luteum (CL)-bearing ovaries decreased significantly ($P<0.01$) as compared to non – CL – bearing ovaries **(Das *et al.*, 1996).**

2.3.3. Estrous Cycle

Takagi *et al.* (1992) and **Boedino *et al.* (1995)** and **Arlotto *et al.* (1996)** stated that there was no impact of different stages of estrous cycle on maturation, fertilization and embryonic cleavage.

Machatkova *et al.* (2004) showed that the mean number of embryos per donor and the development rate of oocytes into blastocyst was higher ($P\geq0.012$) during the growth phase than in the dominant phase (8.0 vs. 3.8) and (30.3 vs. 4.9 %), respectively. **Hendriksen *et al.* (2004)** indicated that the dominant bovine follicle reduces the developmental competence of oocytes from subordinate follicles at a relatively late stage of dominance.

2.4. *In Vitro* Maturation (IVM)

Oocyte maturation is the first and most critical step towards successful *in vitro* embryo production. An interesting feature of the mammalian oocyte was that resumption of meiosis before ovulation after long time of meiotic arrest **(Gasparrini 2001). Pincus** and **Enzmann (1935)** and **Edwards (1965)** reported that *in vivo* bovine oocyte resume meiosis after the preovulatory LH peak, while resumption of meiosis occurs spontaneously when cumulus oocytes complexes (COCs) were removed from their follicles and cultured *in vitro* under proper condition.

Eppig (1991) defined oocyte maturation as the reinitiating and completion of the first meiotic division to metaphase II and the

associated cytoplasmic process which necessary for fertilization and subsequently embryo development.

Salustri, *et al.* (1989) reported that the developmental competence of bovine oocytes matured in vitro was significantly lower than those matured in vivo. **Loos *et al.* (1991)** reported that *in vitro* maturation of oocytes represents the most challenging step due to events during IVM have been demonstrated to effect not only on the process of fertilization but also on the subsequent steps of early cleavage, blastocyst formation and successful implanting.

Maturation of the oocyte can be divided into an inductive phase and synthetic phase; the inductive phase lasts 6-8 h culminating in germinal vesicle breakdown (GVBD) and in which a reprogramming of oocyte by somatic elements was carried out in the follicle while in synthetic phase which had duration of 18 h and it is believed that in which the cumulus cell act in crucial supportive role **(Gordon 2003). Cui –Yali** and **Sang-Runzi (1999)** stated that the maturation rate of oocytes recovered at follicular phase or luteal phase of the ovary was not significantly different (63.9 and 58.7%, respectively).

2.4.1. Criteria of Evaluating Oocyte Maturation

2.4.1.1 Expansion of cumulus – oocytes complexes (COCs)

Cumulus expansion was routinely employed in buffalo IVM for evaluating oocyte maturation **(Palta and Chauhan 1998; Nandi *et al.* 2002 and Chauhan *et al.* 1997b)** reported that the maturation of cumulus oocytes complexes can be evaluated by assessing the degree of cumulus expansion by a classification scheme.

Alternatively the maturation process was evaluated by stripping off the cumulus mass followed by staining the oocytes with Giemsa stain (**Das *et al.*, 1992** and **Chauhan *et al.*, 1996)** or aceto-orcien **(Totey *et al.* 1992** and **Madan et al. 1994 b).**