Aneuploidy in In-vitro Matured Buffalo Oocytes with or without Cumulus Cells

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Abstract: Proper oocytes selection in the laboratory is crucial for successful embryo production in vitro. 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 ability to undergo maturation and embryonic development. The present study was undertaken to investigate the effect of cumulus cells on the in vitro maturation and aneuploidy rate of buffalo oocytes. Ovaries were collected from slaughtered buffaloes. The follicular contents were collected from 2 to 5 mm diameter ovarian follicles by aspiration. Oocytes with homogenous cytoplasm (450) were selected for in vitro maturation. Oocytes with or without cumulus cells were cultured for 24-26 h in TCM-199 supplemented with 10 % fetal calf serum and 50 µg/ml gentamycin sulfate. Matured Oocytes were fixed and stained for nuclear evaluation. The meiotic stages and rate of aneuploidy were determined. Maturation rate represented by the percentage of oocytes reaching Telophase I and Metaphase II stages was higher ($p < 0.005$) in oocytes with cumulus cells than without cumulus cells. Aneuploidy percentage represented by hypoploids and hyperploids MII were nearly identical for both types of oocytes (overall average 4.6 ± 0.8 % for oocytes with cumulus and 5.6 ± 1.1 % for denuded oocytes). The total abnormalities represented by aneuploid and diploid MII also were similar for oocytes with and without cumulus. In conclusion, the maturation rate was improved by the presence of cumulus cells, but aneuploidy percentage was not affected. [Nature and Science 2010;8(9):46-51]. (ISSN: 1545-0740).

Key words: Buffalo oocytes, cumulus cells, meiotic maturation, aneuploidy.

1. Introduction

It has been shown that the presence of cumulus cells was necessary for cytoplasmic and\or nuclear maturation of cattle (Liu and Foote, 1995; Zhang et al., 1995) and buffalo (Das et al., 1997) oocytes. Cumulus cells benefit oocytes development either by secreting soluble factors, which induced developmental competence, or by removing an embryo development-suppressive component from the medium (Hashimoto et al., 1998). Cumulus cells supported IVM of oocytes to the MII stage and were involved in the cytoplasmic maturation needed for optimal developmental competence, such as male pronucleus formation and development to the blastocyst stage. Cumulus cells might be a good indicator for an oocytes ability to undergo meiosis I *in vitro* and that the developmental problems of denuded oocytes were due to deficient cytoplasmic maturation (Leibfried-Rutledge et al., 1987).

Oocytes are most remarkable cells. They are the only cells, which can form a new individual after fertilization. During maturation, oocytes undergo changes in nuclear status that involve exit from diplotene stage of the first meiotic prophase, known as germinal vesicle stage (GV) and progression to the metaphase II stage with extrusion of the first polar body (Edwards, 1962). The observation of the oocytes chromosomes at this stage is more reliable mean for defining the *in vitro* maturation progress **(**Mahmoud et al. 2003 and Mahmoud and Eashra 2004). Aneuploidy is a common phenomenon observed in mammalian gametes (plachot, 2001 and 2003). It is well documented, especially in humans, in which the rate of aneuploid germ cells is quite high in oocytes (4-57.7%, estimated overall frequency 13% ; Zenzes and Casper, 1992) and in spermatozoa (up to 10%; Martin, 1984). The overall, non-disjunction rate in oocytes of domestic animals ranges between 2% and 7%, in a relatively low number of analyzed cells (King 1990; Lechniak and Switonski, 1998). Aneuploid oocytes- when fertilized-give rise to aneuploid embryos, which usually die during early pregnancy. The frequency of aneuploid embryos in domestic animals does not exceed 2 % (King, l990). Aneuploid germ cell may be attributed to the chromosome non-disjunction at the first or second meiotic division during gametogenesis or anaphase lag. However, there are also reports of aneuploid bull primary spermatocytes that arose because of a non-

disjunction process in mitotic cleavage of spermatogonia (Switonski et al., 1991).

Oocyte meiosis is very sensitive to endogenous and exogenous factors that could result in oocytes with chromosomal abnormalities. Aneuploidy may be affected by factors that appear in the *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) system. It has been shown that media used for *in-vitro* oocyte maturation may cause maturation delay and aneuploidy (A'arabi et al., 1997). Moreover, patient-related factors as infertility history, female age and stimulation regimens contribute to aneuploidy (Zhivkova et al., 2007). However, the effect of cumulus cells on the rate of aneuploidy need to be studied in buffalo. So, the present study aimed to investigate the effect of cumulus cells on the maturation and aneuploidy rate of buffalo oocytes.

2. Materials and methods

2.1. Oocyte recovery and selection

Ovaries were collected from buffaloes within 2 h of slaughter. Ovaries were transported to the laboratory in physiological saline (0.9%, w/v, NaCl) with 100 µg/ml streptomycin and 100 IU/ml penicillin maintained at 30°C. Ovaries were washed three times in phosphate-buffered saline (PBS). Oocytes were aspirated from 2- to 5-mm follicles with a 20-gauge needle attached to a 5-ml syringe containing PBS with 3% bovine serum albumin (BSA), fraction V, 100 µg/ml streptomycin, and 100 IU/ml penicillin. Oocytes were collected using a stereomicroscope (20X), then washed 2-3 times in maturation medium (TCM-199, 10% fetal calf serum and 50 µg/ml gentamycin sulfate) and evaluated morphologically. Oocytes with or without intact complement of cumulus cells and a homogenous appearing ooplasm were selected for maturation in vitro.

2.2. Oocyte maturation

Oocytes were washed 3 times in TCM-199 with Earl's salts and 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) supplemented with 10% fetal calf serum (heat treated at 56°C for 30 min.) and 50 µg/ml gentamycin sulfate (Mahmoud, 2001). Oocytes were cultured in 4-well plastic Petri-dishes containing 100 µl of culture medium (the same as washing medium), prepared 24 hrs before culturing of oocytes. Each drop of media contains about 10-15 oocytes/100 µl of medium covered with a layer of mineral oil to prevent evaporation and also to prevent contamination with microorganisms. These culture dishes were incubated for 24-26 hrs at 38.5°C in 5% CO_2 in air and 95% humidity.

2.3. Chromosomes preparation from oocytes

At the end of the culture period, slides of chromosomes were prepared according to the procedure described by Tarkowski (1966). Briefly, cumulus cells were removed mechanically by gentle pipetting. Each oocyte was transferred to 1% hypotonic sodium citrate solution for 10 min and then placed on a microscope slide with a minimal amount of hypotonic solution. Three drops of fixative (methanol: acetic acid, 3:1) were dropped onto the oocytes. Subsequently, the fixed material was stained with 1% orcein. The state of nuclear maturation was determined as described earlier by Mahmoud (2004). Oocytes that reached telophase I or metaphase II stages were considered matured. Meiotic stages and abnormalities in metaphase II were recorded according to Lechniak, et al. (1996). The Oocytes with less than 25 chromosomes were scored as hypohaploid. The Oocytes with more than 25 chromosomes were scored as hyperhaploid. The Oocytes with 50 chromosomes were scored as diploid oocytes. The rates of chromosomal abnormalities were calculated by summing the number of aneuploid (hypohaploid + hyperhaploid) and diploid oocytes (Train and pellicer, 1990).

2.4. Statistical analysis

Data were subjected to statistical analysis according to Snedecor and Cochran (1982).

3. Results

Table (1) demonstrated the meiotic stages of immature buffalo oocytes after *in vitro* maturation for 24 – 26 h. Two grades of collected oocytes with and without cumulus were studied. Meiotic stages as germinal vesicle break down (GVBD), Metaphase I (MI), Anaphase I (AI), Telophase I (TI) and Metaphase II (MII) are recorded (Figure, 1).

Maturation rate represented by the percentage of oocytes reaching Telophase I and Metaphase II stages was higher ($p < 0.005$) in oocytes with cumulus cells than without cumulus cells (Table 2). The results of aneuploidy percentage represented by hypoploids (Figure, 2A) and hyperploids (Figure, 2B) MII were nearly identical for both types of oocytes (overall average 4.6 ± 0.8 % for oocytes with cumulus and 5.6 ± 1.1 % for denuded oocytes (without cumulus). The total abnormalities represented by aneuploid and diploid MII (Figure 2C) also were similar for oocytes with and without cumulus.

Table 1: Cytogenetic evaluation of cumulus and denuded immature buffalo oocytes after maturation in vitro.

Oocytes	No. of oocytes	Total No. of meta-phases	Undefined metaphases	GVBD	MI	AI	TI	Metaphase II (MII)			
								Haploid (normal)	Diploid	Hypo-haploidy	Hyper-haploidy
With cumulus	220	178	14	6	4	4	4	136	2	6	2
Without cumulus	230	180	24	20	8	10	4	102	2	8	2
Total	450	358	38	26	12	14	8	238	4	14	4

GVBD: Germinal vesicle break down , MI: Metaphase I, AI: Anaphase I, TI: Telophase I

Table 2: Maturation rates and chromosomal abnormalities percentages of cumulus and denuded immature buffalo oocytes after maturation in vitro (% ± S.E).

Oocytes	No. of examined oocytes	Total No. of metaphases	Matured oocytes (TI + MII)		Aneuploidy (hypo + hyperploidy)		Total abnormalities (Aneuploidy + Diploid)	
			NO	%	NO	%	NO	%
With cumulus	220	178	140	78.9 ± 0.9*	8	4.6±0.8	10	5.5 ± 1.6
Without cumulus	230	180	106	58.9 ±2.9	10	5.6 ± 1.1	12	6.7 ± 1.9

* P< 0.05 (t- test).

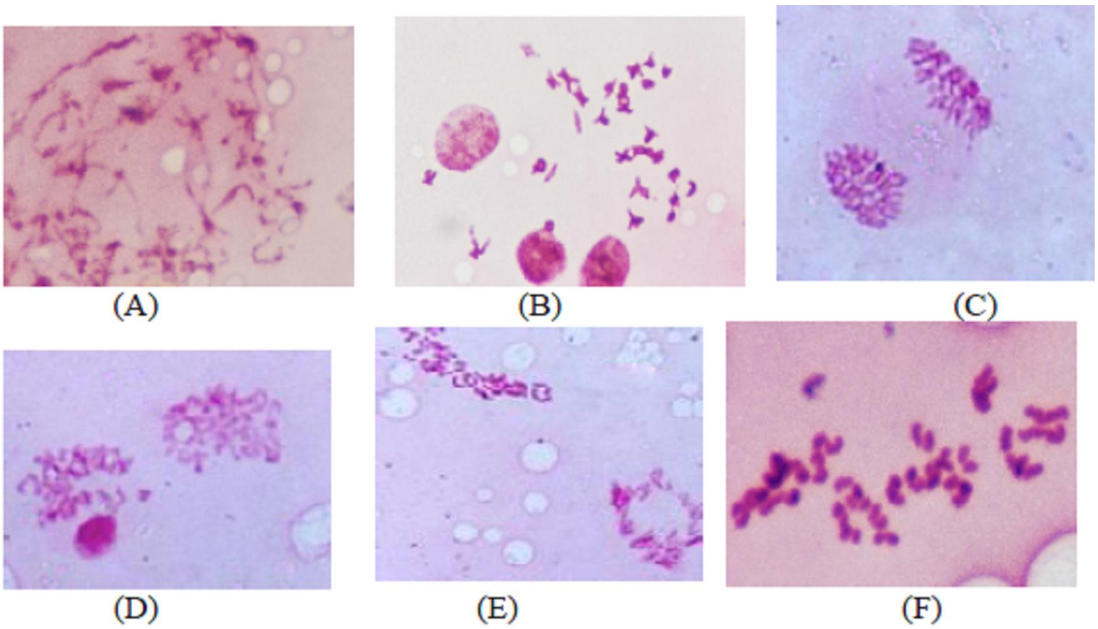

Figure 1: Buffalo oocytes at germinal vesicle break down with a condensation of chromatin (A), Metaphase I stage (B), anaphase I stage showing complete homologous segregation of chromosomes (C), Telophase I stage showing two groups of equally spread homologous chromosomes (D), Metaphase II Note the normal haploid number and the first polar body chromosomes have undergone degeneration (E). Metaphase II stage (F).

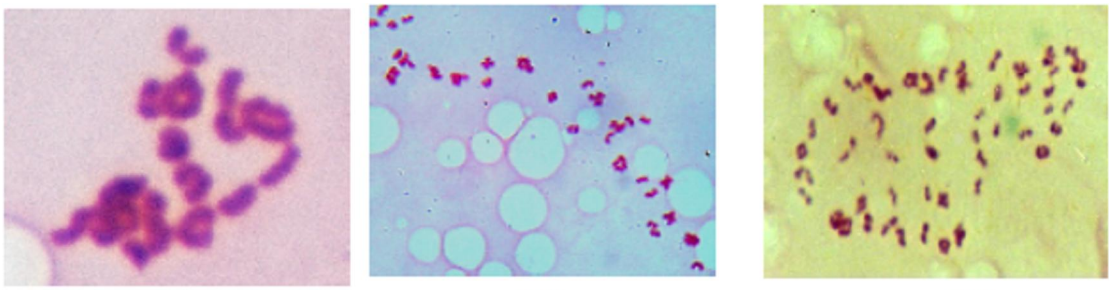

Figure 2: Buffalo oocytes chromosomes showing hypohaploidy (A), hyperhaploidy (B), diploid number (C).

4. Discussion

In our study the immature oocytes were cultured for 24-26 h *in vitro* for the evaluation of nuclear maturation. In buffalo, Yadav et al (1997); Datta and Goswami, 1999) reported that MII stage reached a peak value at 24 h of *in- vitro* culture. In the present investigation, the maturation rate represented by the percentage of the oocytes reaching Telophase I and Metaphase II stages was higher in oocytes with cumulus cells than without cumulus cells. These findings are in agreement with Warriach and Chohan (2004) who suggest that buffalo oocytes with intact layers of cumulus cells show better IVM rates than oocytes without cumulus cells and the co-culture of poor quality oocytes with cumulus cells improve their meiotic competence. Also, Abd El-Kader (2005) indicated that nuclear maturation percentage in in-vitro matured buffalo oocytes was significantly higher ($P< 0.05$) in excellent and good quality oocytes when compared with denuded oocytes. The oocyte - granulosa cell gap junction is required for the coordination of nuclear and cytoplasmic meiotic competence, and are vital for oocyte maturation and subsequent embryo development (Carabatsos et al., 2000; Magnusson et al., 2008). Moreover, Physical contact between oocytes and cumulus cells has been considered necessary for the transfer of nutrients and factors essential for oocytes development **(**Albertini et al., 2001). In the present study, two types of oocytes with homogenous cytoplasm were selected. In this respect, Leibfried and first (1979) and De loos et al. (1989) cited that oocytes with expanded, clumped cumulus cells complex and irregular ooplasm exhibit decreased capacity to mature in vitro.

The frequency of aneuploidy noticed in the present study (4.6 % for oocytes with cumulus cells and 5.6% for denuded oocytes) falls into the range of results published for various mammalian species: cattle, 5.8% (Yadav et al., 1991) and 7.1 % (Lechniak and Switonski, 1998); horse, 5.5% (King et al., 1990) and mouse, 2.7% (A'arabi et al. 1997). It has been suggested that the origin of most aneuploidies in humans is non-disjunction during first meiotic division, and FISH analysis of the first polar body chromosomes may be a sufficient test for aneuploidy (Munne et al., 1995). In the report of A'arabi et al. (1997), hyperhaploid complements were more often found in polar bodies than in oocytes. The rate of aneuploidy was calculated by summing the number of hypohaploid and hyperhaploid oocytes. Theoretically, the process of non-disjunction should produce an equal number of hypo- and hyper haploid gametes. However in many cytogenetic studies a significant excess of hypohaploid complements is reported. Thus, hypohaploid chromosome sets might be a technical artifact and hyperhaploid spreads are considered as evidence for non disjunction. In the study of Martin (l984), the frequencies of hyperhaploid and hypohaploid human spermatozoa were almost equal, whereas hamster eggs contained more hypo- than hyperhaploid complements. Chromosomal non-disjunction in female meiosis gives rise to reduced fertility and trisomy in humans (Shen et al., 2008).

In conclusion, the maturation rate was improved and aneuploidy percentage may not be affected by the presence of cumulus cells. This study opens the door to utilize all the available oocytes with homogenous cytoplasm with or without cumulus cells.

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References

[1] A`arabi SY, Roussel JD, Chandler JE. Chromosomal analysis of mammalian oocytes matured in vitro with various culture systems. J Theriogenology 1997; 48: 1173-1183.

[2] Abd El-Kader HAM. In vitro maturation, fertilization and culture to blastocyst of buffalo

oocytes in different culture media. PH. D. Thesis, Faculty of Science, Cairo University, 2005.
[3] Albertini DF, Combelles CM, Benecchi E, Carabatsos MJ**.** Cellular basis for paracrine regulation of ovarian follicle development. J Reproduction 2001; 121: 647-653.
[4] Carabatsos M J, Sellitto C, Goodenough DA, Albertini D F. Oocyte-granulosa cell heterologous gap junctions are required for the coordination of nuclear and cytoplasmic meiotic competence. J Dev Biol 2000; 226: 167–179.
[5] Das S K, Chauhan M S, Palta P, Tomer O S. Influence of cumulus cells on in vitro maturation of denuded buffalo oocytes. J Vet Rec 1997; 141:522-523.
[6] Datta T K, Goswami S L. Time dynamics and chronology of meiotic progression of buffalo (Bubalus bubalis) oocytes during in vitro maturation. Buffalo J 1999; 1: 53-60.
[7] De Loose FAM, Van Vliet C, Van Maurik P, Kruip T AM**.** Morphology of immature bovine oocytes. Gamete Res 1989; 24: 197 – 204.
[8] Edwards R G. Meiosis in ovarian oocytes of adult mammals. J Nature 1962; 196: 446- 450.
[9] Hashimoto S, Saeki K, Nagao Y, Minami N, Yamade M, Utsumi K. Effects of cumulus cell density during in vitro maturation of the developmental competence of bovine oocytes. J Theriogenology 1998; 49: 1451-1463.
[10] King W A. Chromosome abnormalities and pregnancy failure in domestic animals. J Adv Vet Sci Comp Med 1990; 23: 229-250.
[11] Lechniak D, Switonski M, Sosnowski J. The incidence of diploid bovine oocytes matured in vitro. J Theriogenology 1996; 46: 267- 277.
[12] Lechniak D, Switonski M.. Aneuploidy in bovine oocytes matured in vitro. J Chromosome Research 1998; 6: 504- 506.
[13] Leibfried L, First N L. Characterization of bovine follicular oocytes and their ability to mature in vitro. J Anim Sci 1979; 48: 76 – 86.
[14] Leibfried-Rutledge M L, Crister E S, Eystone W H, Northey D L, First N L. Developmental potential of bovine oocytes matured in vitro or in vivo. J Biol Reprod 1987; 36: 376-383.
[15] Liu Z, Foote R H. Effects of amino acids on the development of in vitro matured, in vitro fertilized bovine embryos in a simple protein free medium. J Hum Reprod, 1995**;** 10: 2985-2991.
[16] Magnusson V, Feitosa W B, Goissis M D, Yamada C, Tavares L M T, Ortiz M E, Assumpcao D'A´vila, Visintin J A. Bovine oocyte vitrification: Effect of ethylene glycol concentrations and meiotic stages. J Anim Reprod Sci 2008; 106: 265–273.
[17] Mahmoud KGhM. Cytogenetic studies on in - vitro fertilization in buffaloes. Ph.D. Thesis (Theriogenology), Faculty of Vet Med Cairo University Egypt, 2001.
[18] Mahmoud KGhM. Meiotic stages and incidence of diploid oocytes in Egyptian cattle and buffaloes. Assiut Vet Med J 2004; 50: 336-347.
[19] Mahmoud K Gh M, Eashra N T. Meiotic chromosomes of cattle oocytes in relation to seasonal variation. Egypt J Vet Sci 2004; 38: 1-9.
[20] Mahmoud K Gh M, El-shahat K H, El-Natat W S. Chromosome configuration during in vitro maturation of Dromedary camel oocytes. Vet Med J 2003; 51: 411 – 420.
[21] Martin R H. Comparison of chromosomal abnormalities in hamster egg and human sperm pronuclei. J Biol Reprod 1984; 31: 819- 825.
[22] Munne S, Dailey T, Sultan K M, Grifo J, Cohen J. The use of first polar bodies for preimplantation diagnosis of aneuploid. J Hum Reprod 1995; 10: 1014-1020.
[23] Plachot M. Chromosomal abnormalities in oocytes. J Molecular and Cellular Endocrinology 2001; 183: 59- 63.
[24] Plachot M . Genetic Analysis of the Oocyte - A Review. Placenta 2003; 24: 66 – 69.
[25] Shen Y, Betzendahl I, Tinneberg H, Eichenlaub-Ritter U. Enhanced polarizing microscopy as a new tool in aneuploidy research in oocytes. Mutation Research 2008; 651: 131–140.
[26] Snedecor G W, Cochran W G. Statistical Methods, 7th ed., Iowa Univ. Press U.S.A, 1982.
[27] Switonski M, Stranzinger G, Basrur P K, Kenney R M**.** Chromosome-pairing in trisomic spermatocytes of males with normal or altered karyotypes. J Genet Sel Evol 1991 **;**23 (Suppl.) 206s-211s.
[28] Tarkowski A K. An air-drying method for chromosome preparations from mouse eggs. Cytogenetics 1966; 5: 394-400.
[29] Train J, Pellicer A. Ovarian response to gonadotopines: a cytogenetic analysis of unfertilized human oocytes. J Fertil Steril 1990; 54: 665-670.
[30] Warriach HM, Chohan K R. Thickness of cumulus cell layer is a significant factor in meiotic competence of buffalo oocytes. J Vet Sci 2004; 5: 247-251.
[31] Yadav BR, Katiyar PK, Chauhan MS, Madan, M L**.** Chromosome configuration during in vitro maturation of goat sheep and buffalo oocytes. J. Theriogenology, 1997; 47: 943-951.

[32] Yadav BR, King WA, Xu K P, Pollard J, W Plante L. Chromosome analysis of bovine oocytes cultured in vitro. J Genet Sel Evol 1991; 23: 191- 196.
[33] Zenzes M T and Casper R F. Cytogenetics of human oocytes, Zygotes, and embryos after in vitro fertilization. J Hum Genet 1992; 88: 367-375.
[34] Zhang L, Jiang S, Wozinak PJ, Yang X, Godke R A. Cumulus cell function during bovine oocyte maturation, fertilization and embryo development in vitro. J Mol Repord Dev 1995; 40: 338-344.
[35] Zhivkova RS, Delimitreva SM, Toncheva DI, Vatev IT. Analysis of human unfertilized oocytes and pronuclear zygotes-Correlation between chromosome/chromatin status and patient-related factors. European Journal of Obstetrics and Gynecology and Reproductive Biology 2007; 130: 73–83.

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