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MEIOTIC STAGES AND INCIDENCE OF DIPLOID OOCYTES IN EGYPTIAN CATTLE AND BUFFALOES

(With One Table and 2 Figures)

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(Received at 20/5/2004)

الاطوار الميوزية ونسبة البويضات ثنائية العدد الكروموسومى فى الابقار والجاموس المصرى

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يقـوم هذا البحث بتحليل وراثى سيتولوجى لبويضات الأبقار والجاموس المصرى الناضجة معمليا، وذلك بتحديد الأطوار الميوزية المختلفة لمعرفة معدل نضج البويضات وتسجيل نسب البويضـات الشـاذة المحتوية على عدد ثنائى من الكروموسومات. تم تجميع البويضات بعد الذبـح مباشرة من مبايض الأبقار والجاموس ونضجها معمليا فى اوساط انمائية فى حضانة غاز ثانى أكسيد الكربون عند درجة حرارة ٣٩ درجة مئوية لمدة فترتين من الزراعة ٢١-٢٤ سـاعة، ٢٥-٢٨ سـاعة. أوضح التحليل الكروموسومى أن معدل نضج البويضات فى الأبقار يزداد معنوي (٠,٠٥) عن الجاموس عند فترة الزراعة ٢١-٢٤ ساعة ولكن لم تكن هـناك اختلافات معنوية عند فترة الزراعة ٢٥-٢٨ ساعة. وجد ان بويضات الابقار تنضج مـبكرا عـن الجاموس و ذلك بوصول جميع البويضات الى مرحلة الطور الاستوائى الثانى والطور النهائى الاول فى الفترة ٢١-٢٤ ساعة. بينما توجد فى الجاموس نسبة غير ناضجة فى مرحلة الطور الاستوائى الاول والطور الانفصالى الاول عند فترة ٢٥-٢٨ ساعة. وجد ان نسـبة البويضات الشاذة التى تحتوى على عدد ثنائى من المجموعة الكروموسومية تزداد معنويا (٠,٠٥) فى الابقار عند فترة ٢٥-٢٨ ساعة عن ٢١-٢٤ ساعة. ولكن فى الجاموس لم تكن هناك اى اختلافات معنوية فى نسبة البويضات الشاذة بين الفترتين.

SUMMARY

The present work describes a cytogenetic study of in vitro matured oocytes from cattle and buffalo to analyze the meiotic stages,to determine the incidence of diploid metaphase II and to evaluate the in vitro maturation progress. Oocytes were collected by aspiration from ovaries of slaughtered cattle and buffalo.Recovered oocytes with at least two layers of compact cumulus cells and homogenous cytoplasm were

selected. A total of 721 cattle and 989 buffalo oocytes were matured in droplets of TCM-199 supplemented with 10 % fetal calf serum and 50 µg/ml gentamycin at 39 °C in 5 % CO_2 incubator for two periods 21-24 hrs and 25-28 hrs. The nuclear maturation rates were 80.51 % , 88.11 % for cattle oocytes and 72.82 %, 86.93 % for buffalo oocytes at 21-24 hrs and 25-28 hrs respectively. The maturation rate in cattle was significantly increased ($p<0.05$) than buffalo at 21-24 hrs but there were non significant difference between cattle and buffalo at 25-28 hrs. The cattle oocytes matured earlier than buffalo oocytes,as no metaphase I and anaphase I stages (immature oocytes) was recorded at period 25-28 hrs, but in buffalo, 5.02 % metaphase I and 3.01 % anaphase I satges were recorded at the same period. The cattle diploid metaphase II was significantly increased ($p<0.05$) from 2.79 % in the first period to 13.95 % in the second period, while in buffalo the percentage was nearly the same (6.50 % and 6.49 %) and there were non significant difference between the two periods.

Key words: *Meiosis, oocyte, cattle, buffalo.*

INTRODUCTION

Chromosomal abnormalities and abnormal embryonic development have previously been observed with human (Spielmann *et al.*, 1985; Parikh *et al.*, 2001 and Neal *et al.*, 2002) and animals (Hyttel *et al.*, 2000; Villamediana *et al.*, 2001 and Viuff *et al.*, 2001) in vitro fertilization. Chromosomal abnormalities may arise .not only after fertilization but even earlier during meiotic maturation of oocyte in culture. They arise as a result of nondisjunction during the first or second meiotic division in the ovum (Hansmann and El- Nahass, 1979) or spermatozoon (Brahmkshtri *et al.*, 1998).

The chromosome complement of oocytes after in vitro maturation was studied in buffalo (Datta and Goswami, 1999 and Mahmoud, Karima and Nawito, 2003), bovine (Jagiello *et al.*, 1974; Ectors *et al.*, 1995 and Sosnowski *et al.*, 1996) and camel (Mahmoud, Karima *et al.*, 2003). The most common chromosomal abnormality in oocytes was diploid metaphase II. Diploid ova arising from nondisjunction at meiosis II of oogenesis were believed to be responsible for most of the triploid embryos (Mong *et al.*, 1974 and Thorne and Sheldon, 1991). The finding of McFadden *et al.*, 1993) demonstrated

that 75% of the examined human triploid fetuses were of digynic origin while only 25% were diandric.

Oocytes with the diploid number of chromosomes have been observed with various frequencies according to the species, in human: 8.3% (Djalali *et al.*, 1988), and 9.9% (Kunathikom *et al.*, 2001), in pig: 14.4% (Bonneau *et al.*, 1992), in horse: 2.7% (King, 1990), in buffalo: 5.33% (Mahmoud,Karima 2001), in bovine: 10.7% (Yadav *et al.*, 1991), 3.1% (Ectors *et al.*, 1995) and 11.5% (Lechniak *et al.*, 1996). The diploid oocyte has been found to be a more frequent abnormality than the diploid spermatocyte which does not exceed 1%. It was recorded that diploid sperm cells occurred with a frequency of 0.05% in cattle (Hassanane *et al.*, 1999), 0.09% in human (Martin *et al.*, 1995) and 1% in rabbits (Carothers and Beatty, 1975).

The aim of the present investigation is to analyze the meiotic stages of buffalo and cattle oocytes and to determine the incidence of unreduced (diploid) oocytes to evaluate the in vitro maturation process of both species.

MATERIALS and METHODS

Oocytes recovery and selection

Ovaries of cattle (Bos taurus) and buffalo (Bubalus bubalis) were collected allover the year, transported from slaughterhouse to the laboratory in a modified phosphate buffer saline (PH,7.2) containing 100 I.U/ml pencillin and 100 µg/ml streptomycin. Oocytes were aspirated from 2-5 mm follicles using 18 gauge needle attached to a 10 ml syringe. Oocytes were washed 2-3 times in enriched Earle's salt media; TCM 199 with 10% fetal calf serum and 50 µg/ml gentamycin. Only oocytes with at least two layers of compact cumulus cells and homogenous cytoplasm were selected.

In vitro oocytes maturation

The recovered oocytes of cattle and buffaloes with completed or partially invested cumulus cell layers were matured in vitro using the enriched Earl's salt media for 21-24 hrs and 25-28 hrs at 39 C° in atmosphere containing 5% CO_2 and 95% relative humidity.

Chromosome preparation

At the end of the culture period, the cattle oocytes were incubated for short time (30-60 sec) in a solution of 0.25% trypsin and 0.02 M EDTA to help in removing the cumulus cells by pipetting. In buffaloes, cumulus cells were removed mechanically by pipetting.

Chromosome slides were prepared according to the procedure described by Tarkowski (1966). The meiotic stages and ploidy were recorded. The oocytes that reach telophase I and haploid metaphase II stage were considered as matured oocytes. The percentage of diploid oocytes were calculated by dividing the total number of diploid metaphases over total number of metaphase II.

Statistical analysis

All data were statistically analyzed according to Snedecor and Cochran (1982) using Costat computer program version 3.03 : copyright 1986 Cohort softwar,USA.

RESULTS

A total number of 721 together with 989 oocytes from cattle and buffalo were selected for in vitro maturation. Chromosome slides were prepared for 71.84% and 70.47% of cattle and buffalo oocytes. Only 69.31% in cattle and 68.15% in buffalo were available for observation.

The cytogenetic analysis of in vitro matured oocytes revealed that 80.51 %, 88.11 % of cattle oocytes and 72.82 %, 86.93 % of buffalo oocytes were matured (T I or haploid M II stages) at periods 21-24 hrs and 25-28 hrs respectively. The maturation rate in cattle was significantly increased ($p<0.05$) than buffalo at 21-24 hrs but there were non significant difference between cattle and buffalo at 25-28 hrs.

With the progress of in vitro maturation of cattle and buffalo oocytes, as shown in table (1) and fig. (1-2), cattle oocytes were matured earlier than of buffalo.There was no any immature oocytes (metaphase I and anaphase I) at period 25-28 hrs in cattle but in buffalo at this period, there was 5.02 % metaphase 1 and 3.01 % anaphase 1 stages were recorded.

The cattle diploid metaphase II was significantly increased ($p<0.05$) from 2.79 % in the first period to 13.95 % in the second period, while in buffalo the percentage was nearly the same (6.50 % and 6.49 %) and there were non significant difference between the two periods.

DISCUSSION

From the present data, the maturation rate was significantly increased ($p<0.05$) in cattle than buffalo at 21-24 hrs.But the maturation rate was nearly the same at 25-28 hrs for both cattle and buffalo. This coincide with Palta and Chauhan (1998) who found the maturation rates were the same in cattle and buffalo but the fertilization rates and the

yield of blastocysts were much lower in buffalo than in cattle (Boni *et al.*, 1999).

The maturation rate in cattle at 21-24 hrs was within the range of 79 % reported by Ocana Quero *et al.*, (1994), 77.3 % by Sosnowski *et al.*, (1996) and 80.4 % by Hamam *et al.*, (1997). In buffalo at 24 hrs, similar maturation rate (73.3 %) was obtained by Singh and Dhanda (1999), but was higher than 55.9 % reported by Hamam *et al.*, (1997) and lower than 92.10 % obtained by Datta and Goswami (1999).

The cattle oocytes matured earlier than of buffalo as there was no any immature oocytes (MI or A1) were detected at 25-28 hrs in cattle. While in buffalo about 5.02 % MI and 3.01 % A1 stages were recorded. This immature percentage of oocytes in buffalo at 25-28 hrs agrees with the result of (Mahmoud, Karima, 2001).

The incidence of diploid cattle and buffalo oocytes in the present investigation are within the range reported by the previous studies of Ectors *et al.*, (1995) and Lechniak *et al.*, (1996) in cattle and Mahmoud, Karima (2001) in buffalo. In both cattle and buffalo, a higher proportion of oocytes at metaphase II stage was found in oocytes matured for 25-28 hrs when compared with oocytes matured for 21-24 hrs. Unfortunately, in cattle, prolonged duration of in vitro maturation cause significant increased ($p<0.05$) incidence of oocytes with the unreduced (diploid) chromosome number.It may indicate that oocytes that require a longer time to reach metaphase II also more frequently demonstrate polar body extrusion failure. The study of Sosnowski *et al.*, (2003) showed that in vitro maturation lasting 40 or more hours resulted in an increased frequency of pig diploid oocytes (15.0 %), when compared with a shorter (30-36 h) duration of IVM (9.0 %). The effect of the duration of oocytes in vitro maturation was analysed in cattle by Ocana-Quero *et al.*, (1999) and they showed that the prolongation of IVM from 24 to 48 hrs caused a nearly four times increase in the frequency of the diploidy (2.6 % after 24 hr and 11.4 % after 48 hr).

Diploid oocyte is one documented cause of triploid embryos. The embryos formed as a result of fertilization of such oocytes would fail to develop (Hansen, 2002). The occurrence of unreduced oocytes is due to failure of the first polar body extrusion, or disturbance of mitotic cleavage in the germ cell line resulting in the production of tetraploid oogonia followed by normal meiotic division (Funaki and Mikamo, 1980).

With in vitro maturation procedure several factors can be distingushed that influence the process of maturation which could lead to

the development of diploid oocytes. Reports in human by Tarin and Pellicer (1990) suggested that the number of diploid secondary oocytes without extrusion of the first polar body was significantly higher as the increased number of oocytes were obtained with patients following superovulation. They concluded that disruption of endocrine control of meiosis could cause the appearance of diploid oocytes. On the other hand, Ocana -Quero *et al.*, (1999) noticed that high concentration of serum (50%), follicles with a diameter between 11 and 15 mm, lower culture temperature (37 C°) and prolonged incubation time (48 h) were significantly ($P<0.01$) raised the percentage of bovine diploid oocytes. Also a relationship between bovine oocytes diameter and the ploidy after in vitro maturation was recorded. The size of secondary oocytes with unreduced chromosome numbers was significantly smaller ($P< 0.01$) than the haploid ones (Lechniak *et al.*, 2002). Another factor was noticed by Luna *et al.*, (2001) who reported that, the nuclear stage at which bovine oocytes are vitrified may affect the incidence of diploid significantly ($P<0.05$) as more diploid oocytes were detected after vitrification at 0 h (28.5%) or 8 h (35.4%) of maturation. No differences in the incidence of diploid metaphase II oocytes were observed between the control (non - vitrified) group (2.4%) and oocytes vitrified at 12 h (6.9%) or 22 h (2%) of maturation.

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Table 1: Progress of *in vitro* maturation and incidence of diploid oocytes in cattle and buffalo.

Culture period (hrs)	species	No. of cultured oocytes	No. of fixed oocytes	No. of metaphase suitable for observation	Metaphase I (M I)		Anaphase I (A I)		Telophase I (T I)		Metaphase II				Maturation % (Haploid MII)	
					No	%	No	%	No	%	Haploid (normal) No	%	Diploid (abnormal) No	%	No	%
21-24	cattle	521	386	272	39	14.33[a]	9	3.30	40	14.70	179	65.80	5	2.79[a]	219	80.51[a]
	Buffalo	599	399	276	52	18.84[a]	12	4.34	32	11.59	169	61.23	11	6.50[b]	201	72.82[b]
25-28	cattle	200	132	101	0	0.0[a]	0	0.0[a]	3	2.97[a]	86	85.14[a]	12	13.95[a]	89	88.11
	Buffalo	390	298	199	10	5.02[b]	6	3.01[b]	19	9.54[b]	154	77.38[b]	10	6.49[b]	173	86.93
Total	cattle	721	518	373	39	10.45	9	2.41	43	11.52	265	71.04[a]	17	6.41	308	85.79[a]
	Buffalo	989	697	475	62	13.05	18	3.78	51	10.73	323	68.0[b]	21	6.50	374	78.73[b]

Means with different superscript within the same column differ significantly ($p<0.05$).

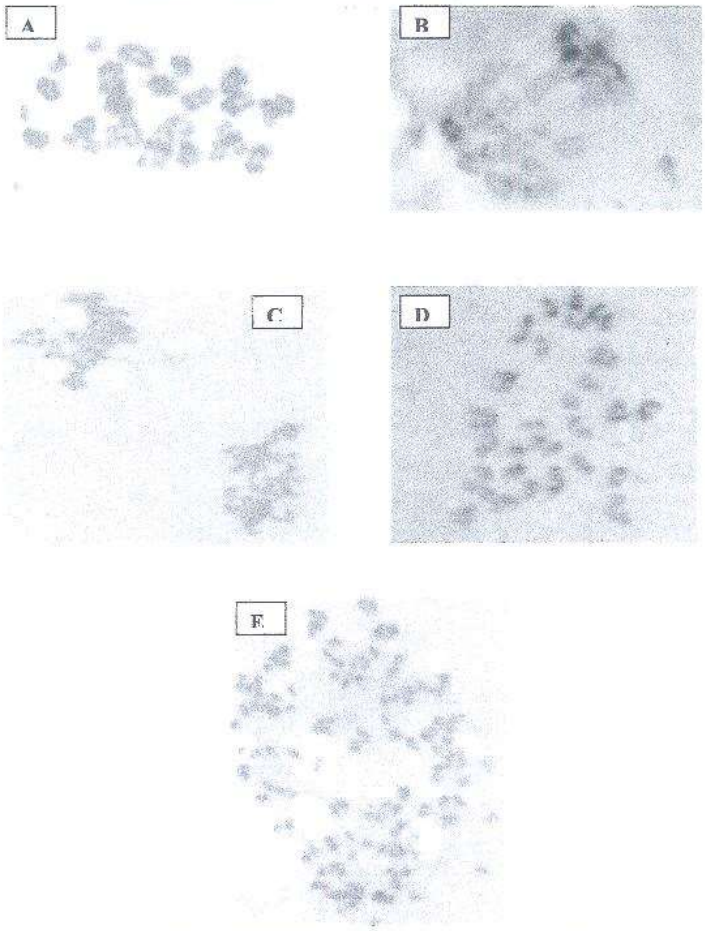

Fig. 1. Cattle oocytes at different stages of meiosis. A) Metaphase I stage. Note the bivalent chromosomes. B) Complete homologous segregation of chromosomes at anaphase I. C) Telophase I stage showing two groups of equally spread homologous chromosomes. D) Metaphase II. Note the normal haploid number. E) Diploid metaphase without extrusion of the first polar body.

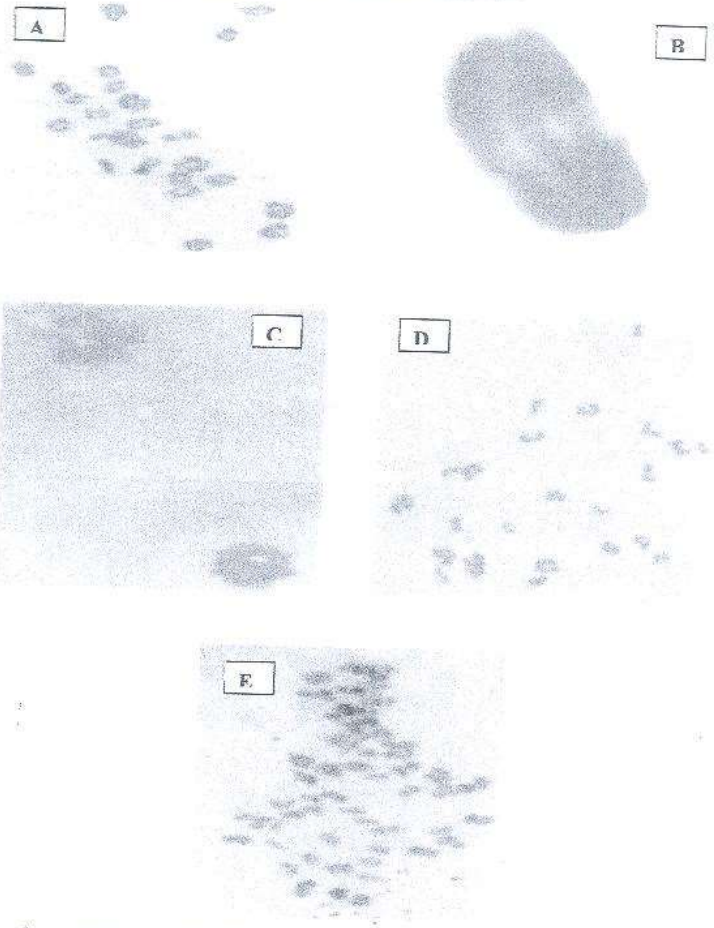

Fig. 2. Buffalo oocytes at different stages of meiosis: A) Metaphase I stage. Note the bivalent chromosomes. B) Complete homologous segregation of chromosomes at anaphase I. C) Telophase I stage showing two groups of equally spread homologous chromosomes. D) Metaphase II. Note the normal haploid number. E) Diploid metaphase without extrusion of the first polar body.