CHAPTER 5

CHROMOSOMAL STUDIES OF DEVELOPING GOAT OOCYTES IN VITRO

5.1. SUMMARY

The aim of this study was to investigate the nuclear changes in goat oocyte cultured in vitro. A total of 836 oocytes, comprising 675 cumulus-oocyte complexes (COCs) and 161 cumulus-free oocytes (CFOs) were incubated in the microdroplet of TCM 199 medium at 39.0°C in 5% CO_2 in air for 0, 20 to 30 and 40 to 48 hr. The stages during nuclear maturation process were determined based on chromosomal configuration changes as germinal vesicle breakdown (GVBD), leptotene, zygotene, pachytene, diplotene, diakinesis, metaphase I (MI) and metaphase II (MII). At 0 hr, most of the oocytes remained at prophase, with the majority of them at pachytene stage (35.1%, 27/77 for COCs and 45.5%, 10/22 for CFOs). At 20 to 30 hr, 55.1% (43/78) COCs attained MII, whereas for CFOs the majority (40%, 8/20) remained in the GVBD stage and only 20% (4/20) attained MII. At 40 to 48 hr, 58.5% (66/113) COCs and 57.6% (19/33) CFOs developed into MII. It is concluded that goat COCs, and not CFOs, incubated between 20 to 30 hr were superior to CFOs and presumably produced higher fertilization rate in the subsequent in vitro fertilization procedure.

5.2. INTRODUCTION

It has been shown that goat oocytes cultured in vitro for 20 hr in TCM 199 medium underwent cytoplasmic ultrastructural changes, indicating such oocytes may have attained fertilizable stage (Chapter 4). In the same study, it has been shown that there was no difference in the ultrastructure of organelles between 20 and 40 hr of incubation in vitro. However, until now there was no information with regards to nuclear changes subjected to the same procedure in oocytes of this species. Both nuclear and cytoplasmic changes are two important criteria to ascertain that the cultured oocytes are fertilizable.

Meiosis proceeds through prophase, metaphase, anaphase and telophase. The prophase of first meiotic division is prolonged and can be divided into leptotene, zygotene, pachytene, diplotene and diakinesis (Baker, 1982). Meiotic prophase is completed to the diplotene stage shortly after birth (Brambell, 1956; Zukermann, 1960). Oocytes then enter prolonged arrested stage of MII which is terminated shortly before ovulation. The subsequent meiotic maturation of oocyte depends on the penetration by spermatozoon during fertilization.

The mammalian primary oocytes are capable to resume spontaneously the process of meiosis when removed from the ovarian follicles and cultured in vitro (Chang, 1955). This knowledge could be beneficial for the understanding of the mechanism of subsequent IVF using oocytes obtained through in vitro maturation (IVM) procedures. However, there is not much information regarding the potentiality of goat oocytes to resume meiosis in vitro. Kim (1981) reported that the goat oocytes collected from ovarian follicles were matured to MII in modified Kreb's (m-Kreb) solution. Song et al. (1985) and Song and Iritani (1987) studied maturation of follicular oocytes of goat treated with gonadotrophic hormones to examine the effect of cumulus cells on maturation process. However, in these studies, cytogenetic analyses of their matured oocytes were not reported. Recently Deb and Goswami (1993) studied the chromosomal configuration of goat oocytes cultured in vitro from ovaries obtained from slaughter house. However, in their studies, only one type of oocyte, COC, was used, and no information regarding the meiotic status of CFO undergoing maturation in vitro was reported.

The purpose of the present study was to investigate the nuclear changes in goat COCs and CFOs cultured at different time intervals in vitro. The results obtained will be as a complement to the data on cytoplasmic changes that occur as

a result of IVM of oocytes previously reported (Chapter 4). The data obtained from both studies (Chapters 4 and 5) will be important for the understanding of mechanism of subsequent IVF procedures reported in Chapter 6.

5.3. MATERIALS AND METHODS

5.3.1. Oocyte Collection

Ovaries from slaughtered goat were washed and kept in phosphate-buffered saline (PBS) at 39.0°C and transported to the Animal Biotechnology-Embryo Laboratory (ABEL) within 45 min of slaughter. The ovaries were sliced to release the oocytes. A total of 836 oocytes from 13 replicates, comprising 675 COCs and 161 CFOs, were obtained. They were washed with hepes-buffered TCM 199 medium (Sigma Chemical Co., Cat. No. M 2520 St. Louis, MO) with Earle's salt and L-glutamine, which was modified by addition of sodium pyruvate, 112 μg/ml and streptomycine sulphate, 50 μg/ml at pH 7.4.

5.3.2. Incubation Medium and Oocyte Treatment

The medium used to incubate the oocytes was modified TCM 199 described by Younis *et al.* (1991). The washed oocytes were transferred to 500-μL droplets of incubation medium. This medium consisted of buffered TCM 199 with

Earle's salt and L-glutamine (b-TCM 199, Sigma Chemical Co., Cat. No. M 4530, St. Louis, MO), which was modified by addition of sodium pyruvate (50 μg/ml); glucose (5.5 mg/ml); streptomycine sulphate (50 μg/ml); bovine serum albumin, BSA (4 mg/ml); and heat-inactivated oestrus goat serum (20% v/v). The oocytes were then placed in a humidified 5% CO_2 in air at 39.0°C for 0, 20 to 30 and 40 to 48 hr after which they were subjected to chromosome analysis.

5.3.3. Analysis of Chromosome

The oocytes were treated with hypotonic tri-sodium citrate (0.88%) solution for 3 to 5 min, then transferred into a microscope slide and fixed in methanol:acetic acid (1:1) followed by 3:1 (King *et al.*, 1979). The slides were subsequently air-dried and stained with Giemsa. The stage of nuclear maturation was determined as germinal vesicle break-down (GVBD), leptotene, zygotene, pachytene, diplotene, diakinesis, metaphase I and metaphase II.

5.4. RESULTS

The progression for meiosis from leptotene to MII are shown in Fig. 5.1-5.7 and Table 1. For both groups of oocytes at all incubation times, the oocytes showed development from GVBD to MII. At 0 hr, most of the oocytes re-

mained at meiotic prophase with the majority of them (35.1%, 27/77 for COCs and 45.5%, 10/22 CFOs) were at pachytene stage. The remaining COCs continued to develop to MI (3/77; 3.9%) and MII (1/77; 1.3%), whereas the percentage of CFOs reached these stages was 9.1% (2/77) for both stages.

At 20 to 30 hr of incubation, further development of oocytes was noted with the majority of COCs (55.1%, 43/78) attained MII, whereas for CFOs the majority of oocytes (40%, 8/20) remained at GVBD stage and only 20% (4/20) of them reached MII . With further incubation (40 to 48 hr), more than half (58.5%, 66/113) of the COCs developed into MII, whilst for CFOs 57.6% (19/33) oocytes reached MII.

5.5.DISCUSSION

The present study showed that the probable incubation time required for breakdown of the germinal vesicle (GVBD) and resumption to MII was from 20 to 48 hr. Although at 0 hr there was maturation of oocytes to MII, the percentage was very low (1.3% in COCs and 9.1% in CFOs). At 20 to 30 hr, 55.1% of COCs were developed to MII, whereas for CFOs it was 20%. Therefore, this finding showed that the incubation time was shorter compared with that reported by Deb and Goswami (1990) who found that the percentage of mature oocytes in vitro reached maximum after 32 hr of culture. In the present

finding there was no information regarding the meiotic status of the oocytes incubated earlier than 20 hr of incubation. Deb and Goswami (1990) showed that no oocytes developed into MII stage earlier than 26 hr of incubation. However, in bovine, King *et al.* (1986) found that MII was most frequent at 18 hr. Thus, the present finding showed some similarity with that of King *et al.* (1986).

The maturation rate of 55.1% obtained in the present study was also far higher than that found by Deb and Goswami (1990) who reported the value was somewhere between 10 to 18.2%. The higher maturation rate found in the present study is interesting despite the fact that the ovaries used were from uncontrolled animals of varying age and probably of low reproductive status, the same reason that Deb and Goswami (1990) put forward. Thus, it is possible to suggest that the difference in the maturation rate between the two studies could be due to the difference in the media and/or the breed of goat used in the respective studies.

The present study has shown that CFOs were able to develop into MII. This was in disagreement with the finding of King *et al.* (1986) who noted that bovine oocytes devoid of cumulus cells had virtually no capacity for nuclear maturation. The present finding was in apparent contradic-

tion with the data reported in Chapter 4. It was known also that cumulus cells have been shown to play an important role in the aquisition of full developmental competence of oocytes (Leibfried and First 1976; Sato *et al.*, 1977; Xu *et al.*, 1986). Dalhausen *et al.* (1981) showed that there was no maturation, or a low rate of maturation of bovine oocytes when the cumulus cells were removed before the oocytes were matured in vitro. Higher rate of embryonic development following in vitro fertilization (IVF) and culture of bovine COC matured in vitro compared with that of nude or corona-enclosed oocytes has also been reported (Critser *et al.*, 1986). Thus, the possible explanation for the maturation of CFOs was that some of these oocytes were those of Graffian follicles after the LH surge still remaining just before ovulation. These oocytes were mixed with those from atretic follicles but no distinction was made regarding the condition of the follicles at the time of slicing of ovaries. Based on the finding on ultrastructure of cytoplasmic changes during in vitro development (Chapter 4) and also in view of the importance of cumulus cells (Leibfried and First, 1976; Sato *et al.*, 1977; Xu *et al.*, 1986; Dalhausen *et al.*, 1981), it is therefore doubtful as to whether such matured CFOs were fertilizable in the subsequent IVF procedures. Even if fertilization does take place in such oocytes, the subsequent embryo produced may not be that viable (Critser *et al.*, 1986).

In conclusion, it is suggested that COCs, incubated between 20 to 30 hr were superior than CFOs, more fertilizable and therefore will presumably produce higher fertilization rate in the subsequent IVF procedures.

Table 5.1: Meiotic progression of goat oocytes at 0, 20 to 30 and 40 to 48 hr of incubation

Time intervals (hr)	0		20 to 30		40 to 48	
Class of oocytes	COC	CFO	COC	CFO	COC	CFO
Total oocytes obtained	235	52	193	48	247	61
Total oocytes with chromosomes	94	28	96	26	131	39
Total oocytes with identified chromosomes	77	22	78	20	113	33
Germinal vesicle breakdown (GVBD)	0	0	10	8	5	5
(%)	(0.0)	(0.0)	(12.8)	(40)	(4.4)	(15.2)
Leptotene	15	2	7	3	2	1
(%)	(19.4)	(9.1)	(9.0)	(15.0)	(1.8)	(3.0)
Zygotene	25	5	2	0	3	2
(%)	(32.5)	(22.7)	(2.6)	(0.0)	(2.6)	(6.0)
Pachytene	27	10	2	2	2	1
(%)	(35.1)	(45.5)	(2.6)	(10.0)	(1.8)	(3.0)
Diplotene	6	1	2	1	5	0
(%)	(7.8)	(4.5)	(2.6)	(5.0)	(4.4)	(0.0)
Diakinesis	0	0	2	0	3	0
(%)	(0.0)	(0.0)	(2.6)	(0.0)	(2.6)	(0.0)
Metaphase I	3	2	10	2	27	5
(%)	(3.9)	(9.1)	(12.8)	(10)	(23.9)	(15.2)
Metaphase II	1	2	43	4	66	19
(%)	(1.3)	(9.1)	(55.1)	(20)	(58.5)	(57.6)

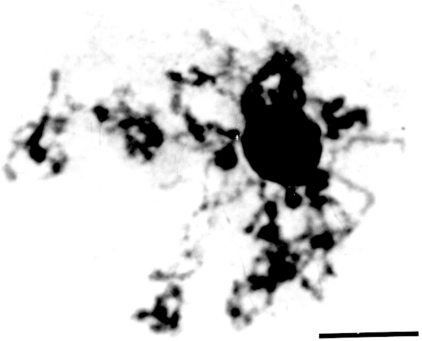

Figure 5.1. Prophase 1, leptotene stage of meiosis in goat oocyte cultured in TCM 199 medium. The chromosomes are long, fine and apparently single thread. Scale bar: 10 μm

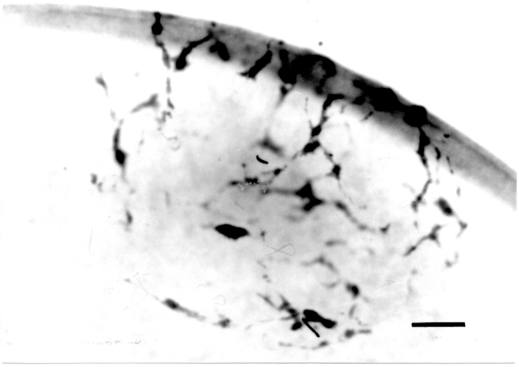

Figure 5.2. Prophase 1, zygotene stage of meiosis in goat oocyte cultured in TCM 199 medium. The chromosomes are visible along the paired homologous autosomes. Scale bar: 10 μm

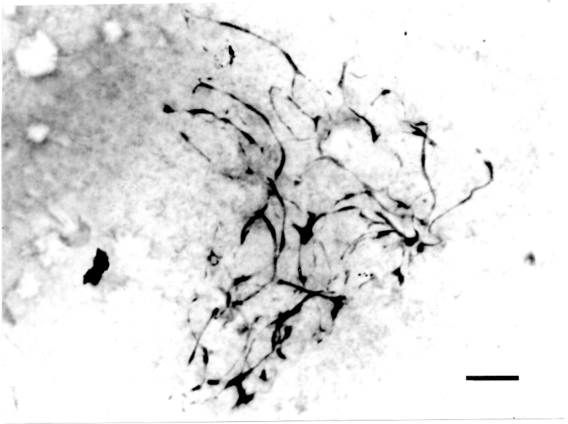

Figure 5.3. Prophase 1, pachytene stage of meiosis in goat oocyte cultured in TCM 199 medium. Scale bar: 10 μm

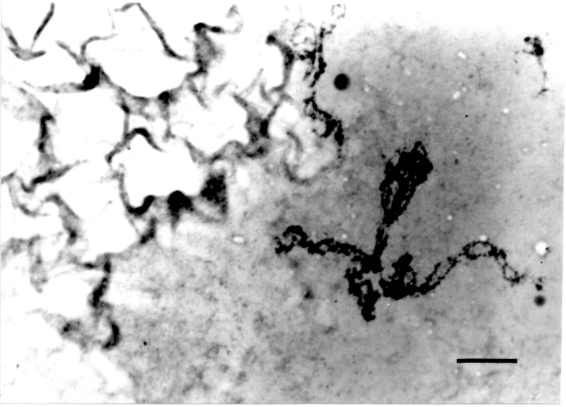

Figure 5.4. Prophase 1, diplotene stage of meiosis of goat oocyte cultured in TCM 199 medium. Within each bivalent, four chromatids are visible. Scale bar: 10 μm

Figure 5.5. Diakinesis, characterized by a more contracted state of chromosomes, by the disappearance of the nucleolus from its associated chromosomes, and by the even distribution of the bivalents throughout the nucleus. Scale bar: 10 μm

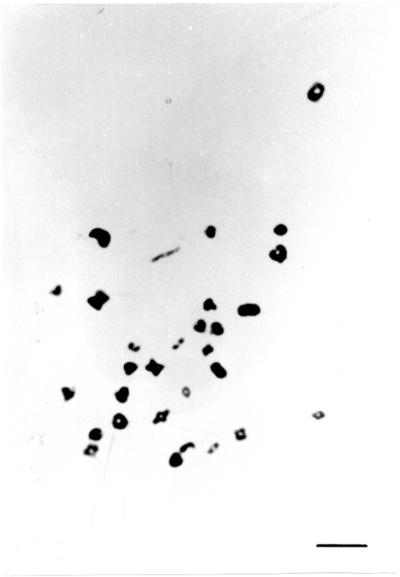

Figure 5.6. Metaphase I. The bivalents have compressed and co-oriented with centromeres at opposite sides of the equator of the spindle. Scale bar: 10 μm

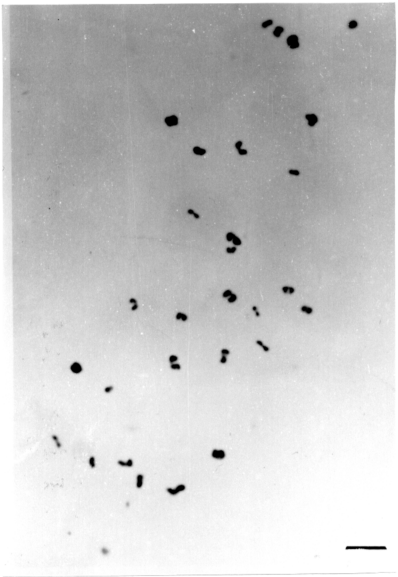

Figure 5.7. Metaphase II. Homologous chromatids are widely separated but centromeres are still undivided and the chromosomes are auto-oriented on the spindle equator. Scale bar: 10 μm