

## Chromosome analysis of bovine oocytes cultured *in vitro*

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**Summary** – Oocytes ( $n = 836$ ) collected from slaughtered cows of unknown reproductive background were matured *in vitro* and subjected to chromosome analysis. In all, 744 (89%) resumed meiosis and 603 (72%) reached metaphase II stage. The non-disjunction rate and dispoloidy rate was 5.8% and 10.7%, respectively. The incidence of non-disjunction in *in vitro* matured bovine oocytes is similar to that reported for other domestic species.

**oocytes / meiosis / non-disjunction / chromosome / bovine**

**Résumé** – Analyse chromosomique d'ovocytes bovins cultivés *in vitro*. Les ovocytes utilisés lors de cette étude furent prélevés sur des ovaires de vaches de statut reproductif inconnu. La maturation de ces ovocytes ( $n = 836$ ) fut effectuée *in vitro* et suivie de leur fixation en vue d'une analyse chromosomique. Au total, 744 (89%) présentèrent une reprise de méiose et 603 (72%) atteignirent le stade de métaphase II. Les taux de non-disjonction et de diploïdie étaient de 5,8% et 10,7% respectivement. L'incidence de la non-disjonction chez les ovocytes bovins maturés *in vitro* est similaire à celles déjà signalées chez d'autres espèces domestiques.

**ovocytes / méiose / non-disjonction / chromosome / bovin**

### INTRODUCTION

Since observations of the chromosomes of domestic cattle were first reported by Krallinger in 1927, extensive studies have yielded a wealth of information pertaining to the normal karyotype. In addition, a large number of individuals with abnormal karyotypes involving anomalies of chromosome structure and number have been reported (for review see Popescu, 1989).

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The study of the post-synaptic stages of meiosis provides information on the chromosome composition of gametes and gives a preview of the embryos that might arise from them. In bulls, the incidence of abnormal second meiotic metaphase has been studied (Gustavsson, 1969; Logue and Harvey, 1978; Popescu, 1978). In cows, the chronological and the morphological aspects of oocyte maturation have been established (Jagiello *et al*, 1974, King *et al*, 1986; Suss *et al*, 1988), however, there is less information on the chromosomal status of the maturing oocyte. With the recent development of reliable techniques for the *in vitro* maturation and fertilization of bovine oocytes (Leibfreid-Rutledge *et al*, 1989) large numbers of oocyte which lead to embryos of known karyotype and developmental potential can be produced and analyzed.

In this paper we report our observations on the chromosomal composition of bovine oocytes matured *in vitro*. The oocytes used for this study were obtained in conjunction with *in vitro* fertilization and culture of bovine oocytes and served as control observations for the oocyte maturation procedure in those studies.

## MATERIALS AND METHODS

The oocytes used for this study were prepared by the method described by Xu *et al* (1987) as follows : ovaries from cows of unknown reproductive histories were collected from a local abattoir. The surface of the ovaries was slashed with a razor blade and rinsed in Hams F-10 supplemented with 2% estrus cow serum, heparin (2 iu/ml) Hepes buffer (10 mM) and antibiotics (100 iu penicillin, 100 µg streptomycin/ml). The cumulus-oocytes complexes (COC) were recovered from this collecting medium and transferred in groups of 30-50 to 1.0 ml of Hams F-10 supplemented with estrus cow serum (20%) and antibiotics. COC cultures were incubated at 39°C in a humid 5% CO<sub>2</sub> atmosphere for 26 to 28 h. COC were then removed from the cultures, the cumulus cells dissociated by mechanical forces and the oocytes were fixed on slides as previously described (King *et al*, 1979). The slides were examined after staining with 4% Giemsa for 4 min and the stage of nuclear maturation assigned as previously described (King *et al*, 1986). The chromosomes at meiotic metaphase I (MI) and meiotic metaphase II (MII) were counted at 1 000× magnification. The rate of non-disjunction (ND) was calculated according to the formula used for bulls (Logue and Harvey, 1978) :

$$ND = \frac{2 \text{ (number of hyperhaploid metaphase)}}{\text{total number of metaphase}}$$

## RESULTS

A total of 836 oocytes were fixed for chromosome analysis. The stage of nuclear maturation could be determined in 744 (89%) oocytes of which 23 (2.6%) were diplotene/diakinesis stage, 118 (14.1%) were MI and 603 (72.1%) were MII. The failure to determine the stage of nuclear maturation in 92 oocytes was due to lack of identifiable chromosomes, clumping and condensation of chromatin or failure

to resume meiosis. The distribution of the number of chromosomes, MI bivalents and MII univalents, of the 389 preparations suitable for counting is summarized in table I. Of the 308 MII spreads suitable for counting, 13 (4.2%) were aneuploid (4 hypohaploid and 9 hyperhaploid) and 33 (10.7%) were diploid. The non-disjunction rate, calculated according to the ND formula above, was 0.058 or 5.8%.

**Table I.** Summary of the stage of meiosis and chromosomal composition of bovine oocytes cultured *in vitro*.

Stage of meiosis	Total observed	Total counted	No of bivalents/univalents					Total diploid
			28	29	30	31	32	
Undetermined	92	—	—	—	—	—	—	—
Pre-MI	23	—	—	—	—	—	—	—
MI	118	81	—	1	78	—	2	—
MII	603	308	1	3	262	3	6	33
Total	836	389	1	4	340	3	8	33

Among the 295 oocytes at the MII stage for which exact chromosome counts could not be made, a further 33 (11.2%) were thought to be diploid. Chromosomes of the polar body were observed in 67 oocytes although they were not possible to analyze due to contraction and clumping.

## DISCUSSION

The high rate of nuclear maturation observed in this study (81%, 603/744) is consistent with other studies on the *in vitro* resumption of meiosis (eg King *et al*, 1986; Suss *et al*, 1988). Since the first observation that oocytes spontaneously resume meiosis once removed from the follicle and cultured *in vitro* (Pincus and Enzmann, 1935), it has been recognized that nuclear maturation is not synonymous with maturation and acquisition of fertilization potential (First *et al*, 1988). IVF data show the rates of fertilization and development are lower than those of nuclear maturation (Leibfried-Rutledge *et al*, 1989). For example, in studies parallel to this, for which some of the present oocytes served as controls, the rates of fertilization (penetration), cleavage and development to the morula/blastocyst stage were 71%, 57% and 28%, respectively (Xu, unpublished observations). This clearly reinforces the observation that not all oocytes which reach MII stage *in vitro* are capable of becoming fertilized and developing into viable embryos. It has yet to be determined if chromosomally abnormal bovine oocytes are less likely to be fertilized than normal ones. In humans, up to two-thirds of oocytes which fail to cleave after IVF attempts have been reported to be chromosomally abnormal (eg Wramsby and Fredga, 1987; Ma *et al*, 1989; Papadopoulos *et al*, 1989). However, it must be remembered that morphological as well as chromosomal abnormalities have been associated with aging of oocytes (Szollosi, 1975). This may account for the high incidence of abnormalities observed in human oocytes.

Chromosomal analysis of oocytes clearly has an advantage over that of spermatocytes in that oocytes can be isolated and prepared individually each containing at most 2 metaphases. However, one technical difficulty common to both oocytes and spermatocytes is chromosomal loss during fixation and slide preparation. Non-disjunction during the first meiotic division is expected to lead to equal portions of hyperhaploid and hypohaploid MII spreads. Expression of the rate of non-disjunction as twice the incidence of hyperhaploid MII spreads compensates for the potential false bias towards hyperhaploidy. However, in the present study technical loss of chromosomes appears to not be a significant factor as hyperhaploids actually out-numbered hypohaploids. The incidence of non-disjunction in the present study (5.8%) is similar to that of horse oocytes (5.5%; King *et al*, 1990) and pig oocytes (3.4%; McGaughey and Polge, 1971). The 10.7% incidence of diploid MII is higher than previously reported in cattle oocytes (2.9%; Jageillo *et al*, 1974) or horse oocyte (2.7%; King *et al*, 1990) although lower than in pig oocytes (12.2%; McGaughey and Polge, 1971). In bulls, the incidence of non-disjunction has been reported as 2.8% (Logue and Harvey, 1978). In a study involving a Robertsonian translocation a 7.7% incidence of diploid MII was observed (Gustavsson, 1969). The similar incidence of aneuploidy and polyploidy in other species suggests a base-line incidence of non-disjunction common to these domestic species.

Fertilization of the oocytes with hypohaploid, hyperhaploid or diploid MII configurations by spermatozoon with a normal chromosome complement would lead to the formation of an aneuploid or polyploid embryo. Chromosome analysis of bovine embryos produced by fertilization *in vivo* and ranging in age from 1 to 18 d has shown an overall incidence of recognizable abnormalities of  $\approx 10\%$ . Included in this group are abnormalities such as mixoploidy which are thought to occur as post-meiotic events during fertilization and cleavage (King, 1990). The most frequently observed abnormality in embryos produced by IVF has been triploidy (10%; Iwasaki *et al*, 1989; 7%; King *et al*, 1988). The incidence of triploidy in bovine embryos due to fertilization of a diploid oocyte (digyni) is not known although the observations of this study suggest that it could be as high as 10.7%. In parallel studies the incidence of dispermic or polyspermic fertilization, as evidenced by the presence of 2 or more male pronuclei, was 5% (Xu, unpublished observations). Studies on triploid human spontaneous abortuses suggest that a greater proportion (85%) arise due to diandry (fertilization by 2 spermatozoa or by a single diploid spermatozoon) than from digyni (15%; Jacob *et al*, 1982).

Only a few examples of pure aneuploidy have been observed in bovine embryos produced by fertilization *in vivo* (King, 1990). Less than 1% of embryos produced by IVF were observed to be aneuploid (Iwasaki *et al*, 1989; King *et al*, unpublished data collected in parallel to the present study). This incidence is substantially lower than what might be extrapolated from the non-disjunction rates observed in oocytes (5.8%; present study) and spermatocytes (2.5%; Logue *et al*, 1978). However, it has been noted that there is a progressive elimination of embryos with abnormal karyotype throughout early stages of development (King, 1990). Furthermore, not all fertilized oocytes are capable of completing even the first cleavage division (Xu *et al*, 1987). Hence, a portion of aneuploid embryos may go undetected in the zygote or early embryo. The possibility that hypo- or hyperhaploid oocytes are less likely to participate in fertilization must also be considered.

In cattle  $\approx 90\%$  of ovulated ova are fertilized, however, calving rates are closer to 50% with embryo loss making up the difference between fertilization and calving rates (Diskin and Sreenan, 1980). While the incidence of chromosome abnormalities is not yet well established in bovine embryos, it is clear that they are a factor contributing to embryonic loss. A small portion of embryos with abnormal karyotype, aneuploidy of the sex chromosomes (*eg* Pinheiro *al*, 1987) or on rare occasions autosomal trisomy (*eg* Mayr *et al*, 1985), do survive to term. No examples of live-born triploid cattle have been reported. Interestingly, gametes that could produce aneuploid individuals are the least frequently observed product of meiosis.

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