Comparative study of bovine oocytes in vitro matured by human medium (SAGE) and TCM-199

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ABSTRACT

Background: In vitro maturation (IVM) of oocytes is a promising technique that has the potential advantages of reducing the costs and averting the side effects of gonadotropin stimulation for IVF. Bovine ovaries contain thousands of oocytes, but fewer than 20 are ovulated per year. Objectives: Till now there are a lot of efforts leading to improve culture mediums. To improve the efficiency, now the research is focused on all factors influencing the parental gametes prior to their collection, and how gametes and embryos react to different culture systems. For example researchers have studied oocyte maturation in different species and the effect of addition of some material such as growth factors, hormones and follicular fluid to the maturation medium. Methods: In order to find the effect of the maturation medium’s kind, the oocytes were divided in to the two trial group. Results: The A group was treated with human mediums and the B group was treated with TCM-199 from 720 bovine oocytes (aspirated from 300 ovaries in 15 repeat, 599(83.19%) oocytes were matured with kit maturation medium of SAGE Company (Group A) And from 620 bovine oocytes (aspirated from 250 ovaries in 15 repeat), 529 (85.32%) oocytes were matured with TCM-199 medium (Group B). Finally the higher percentage of maturation in TCM-199 in comparison to SAGE medium was observed

Key words: In vitro maturation (IVM), bovine oocyte, culture medium
INTRODUCTION

In cattle, the first potential primordial germ cells (PGCs) can be identified in 18-days-old embryos. They migrate to the forming gonad (ovary) and form clusters of dividing cells termed oogonia. After a period of mitotic proliferation, oogonia enter meiotic prophase and differentiate into primary oocytes, which begin their first meiotic division. Bovine ovaries contain thousands of oocytes, but fewer than 20 are ovulated per year. In giving rise to oocytes, oogonia undergo the two cell divisions of meiosis to reduce their diploid chromosome complement (2n) to the haploid state (n), and this is the main difference between meiosis and mitosis. After the initiation of meiosis, the germ cells (primary oocytes) progress through the leptotene, zygotene and pachytene of the first meiotic prophase before arresting at the diplotene (dictyate) stage. As development proceeds towards the diplotene stage, the primary oocytes acquire an outer coat (zona pellucida) and cortical granules, accumulate ribosomes, mRNA, proteins and various nutrients required for further development (Tatjana Smiljaković, W. Tomek, 2006).

In the follicle, the oocyte is surrounded by closely associated granulosa cells (cumulus cells), forming a compact cumulus cell-oocyte complex (COC). Gap junctions formed by different connexins allow a low molecular weight (up to 5000 Da) signal transfer between granulosa cells

And the oocyte which probably is involved in meiotic arrest (Trounson, 2003, DelÀquila et al, 2004).

COCs are embedded in follicular fluid which probably also provides substances which impair meiotic maturation. The preovulatoty LH peak runs a cascade of events in ovarian follicles which include resumption of meiosis by oocyte, cumulus expansion, causing crack in follicular wall and exiting of coc (Richards et al., 2002).

After the LH surge, the oocyte within the dominant follicle is committed to resume meiosis. However, the mechanisms responsible for this event have not yet been determined. Currently two hypotheses have been proposed:

1) Resumption of meiosis is due to decreased effects of meiotic inhibitor(s), mediated by a reduction in the number of gap junctions between granulose cells (Larsen WJ, Wert SE, Brunner GD, 1986).

2) a meiotic stimulator generated within the follicular milieu overcomes meiotic arrest (Eppig JJ, Downs SM, 1987; Downs SM, Daniels SAJ, Epping JJ, 1988).

During resumption of meiosis, oocytes undergo a series of nuclear and cytosolic changes that prepare them for fertilization and are referred to as oocyte maturation. These events include germinal vesicle breakdown (GVB), chromatin condensation and spindle formation and, among cytosolic changes, organelle redistribution and maturation of Ca2 release mechanisms) Jin-Tae Chung, 1999).

Follicles that do not reach ovulation, become atresic and are eliminated.

Generally, the main problem of the procedure of in vitro embryo cultivation is the reduced viability of in vitro embryos compared with in vivo counterparts. To improve the efficiency, now the research is focused on all factors influencing the parental gametes prior to their collection, and how gametes and embryos react to different culture systems.

Oocytes released from their follicle persist in the germinal vesicle stage (GV stage, diplotene of first meiotic division 0 hours of maturation), and bovine oocytes in culture pass through germinal vesicle break down (GVBD) after 10 hours of maturation followed by the first meiotic division and finally reach the metaphase II at 20 to 24 hours of maturation (Tatjana Smiljaković, W. Tomek, 2006).

By developing culture condition, as a result imitation of follicular environment before ovulation and cytoplasmic and nuclear maturation will improve.
History

In vitro maturation (IVM) of oocytes is a promising technique that has the potential advantages of reducing the costs and averting the side effects of gonadotropin stimulation for IVF. However, the efficiency of the current IVM techniques in terms of pregnancy and live birth rates do not match those reported for IVF cycles using full hormonal protocols with triggered maturation in vivo (Banwell and Thompson, 2008). Many reports demonstrated differences in morphology (Van Soom et al., 2003), metabolism (Khurana and Niemann, 2000), the incidence of chromosomal abnormalities (Viuff et al., 1999), blastocyst yield and quality (Rizos et al., 2002b; Knijn et al., 2003), apoptotic index (Gjorret et al., 2003) and so on. It is known that oocytes matured in vivo are more competent to develop to the blastocyst stage than those matured in vitro (Piquette, 2006).

In terms of efficiency, 30–40% of bovine oocytes matured and fertilized in vitro reach the blastocyst stage. In horse by use of IVM maturation more than 60% of oocytes reach metaphase II and then they release the first polar body. Although post-fertilization embryo culture is the most critical period influencing the blastocyst quality (Lonergan et al., 2003), the conditions of oocyte maturation (IVM) and fertilization should not be disregarded (Piquette, 2006).

Till now there are a lot of efforts leading to improve culture mediums. For example researchers have studied oocyte maturation in different species and the effect of addition of some material such as growth factors, hormones and follicular fluid to the maturation medium. Studies have shown that the addition of follicular fluid of big follicles to IVM medium will support cytoplasmic and nuclear maturation of oocytes in cows and pigs (E Sato, M Matsuo and H Miyamoto, 1990). Investigators have observed maturation of mammalian oocytes in media containing either serum or a more defined protein source such as BSA (A. Romero-Arredondo and G.E. Seidel, 1996).also it has been clearly demonstrated that nuclear events of maturation (i.e. resumption of meiosis) will occur in the absence of serum fertilizability of oocytes was superior after in vitro maturation in serum-containing media (Kurt A. Zuelke & Benjamin G. Brackett, 1990).

It is known that the oocyte diameter is one of the factors influencing its ability to resume and complete meiosis in vitro. Fair et al. (1995) showed that oocytes smaller than 110 mm have a reduced ability to resume meiosis. Recently a correlation between oocyte size and disturbances in meiotic division in vitro, especially for the failure of the first polar body extrusion.pigs is reported (Lechniak et al., 2002).

The physiological factors responsible for acquisition of oocyte fertilizability remain ill-defined.reports of increased fertilizability of bovine oocytes following IVM in media supplemented with proestrous or estrous cow sera compared to diestrous or postovulatory sera underscore the importance of hormonal influences on acquisition of oocyte fertilizability during IVM. Hormonal conditioning of oocyte is of great importance in achieving the cytoplasmic maturation required for initiating normal development (Younis AL, Brackett BG, Fayrer-Hosken RA, 1989 ; Moor RM, Polge C, Willadsen SM, 1980 ; Stubbings RB,Battridge KJ, Brsrur PK,1988).

So for increasing the efficiency of IVM, at first we should focus on creating an optimal maturation medium.

Material and method

A) In This study the oocytes were divided in to the two trial grupe for finding the effect of Maturation Medium on their maturation. Maturation Medium A: maturation medium of human oocyte was used in this part and we bought it from sage company. This medium composed of: Nacl, kcl, Nahco3, Glucose, sodium pyruvate, Gentamycin, phenol red, essential and nonessential amino acids and vitamins and we add HCG (5 IU/ML) and FCS (20%) to it.

B) Maturation Medium B: medium TCM-199 was used (sigma, St Louis ,MO, USA ),with addition of
FBS(20%), NaHCO3(2.1 mg/ml), sodium pyruvate(25 Mm), gentamycin (2.5mg), cysteamin (0.007 mg), PMSG (0.25 mg), HCG (1 ul).

**Washing medium**

For A maturation medium, washing medium of human oocytes was used (SAGE), by addition of FCS (10%). but in medium maturation B, Hepes TCM199 (sigma) was used as washing medium with addition of FCS (10%), sodium pyruvate(0.25 Mm) and kanamycin (75 ug).

**Collection and maturation oocytes**

Bovine ovaries were collected at a local abattoir and transported in 30-35 c within 1 h to the laboratory in sterile 0.9% NaCl containing 100IU Gentamycin. Cumulus-oocyte complexes were aspirated from antral follicles (2-8 mm in diameter) with a washed and sterilized plastic syringe (5 or 10 ml) and an 17-gauge needle containing 0.5-1.0 ml of sterile Ham’s F10. Aspirated follicular fluid was expelled into Petri dishes (Falcon Plastics, Los Angeles, CA) on a thermo plate at 38°C. A stereomicroscope was used to select oocytes with 3-4 or more cumulus cell layers. They were washed three times in washing medium. then co’s were transmitted 100 μl of IVM droplets below oil mineral for 24-30 h, at 38.5°C in 5% CO2. IVM droplets were equilibrated for at least 4 h prior of oocyte culture.

**Survey of maturation oocytes**

After passing 24-30 h of maturation, oocytes were assessed for cumulus expansion. Then co’s were transmitted to droplet of hepes TCM199 by addition of hyaloronydase 80 IU for vortex of co’s. finally by use of a stereomicroscope polar body and pre vitelline space were surveyed as symptoms of oocyte maturation.

**Results and Discussion**

Oocyte maturation is often divided into two Cytoplasmic and nuclear processes. Nuclear maturation is a term that refers to the resumption of meiosis and progression to metaphase II. Cytoplasmic maturation, refers to other puberty events that indirectly related to meiosis progression which makes the oocyte ready for fertilization and preimplantation development (De La Fuente R, J.O.Brien M, Eppig JJ, 1999). The ability of oocytes during fertilization, cleavage and also successful development of embryo is determined by meiotic maturation (Trounson A, Anderiesz C, Jones G, 2001).

Meiotic maturation is a complex process that consist of the formation GVBD, Chromosome condensation, formation of metaphase plate, completion of meiosis I, release of the first polar body and stopping in Metaphase II (Scasi L, Lauria A, Gandolfi F, 2001).

from 720 bovine oocytes (aspirated from 300 ovaries in 15 repeat, 599(83.19%) oocytes were matured with kit maturation medium of SAGE Company (Groupe A) And from 620 bovine oocytes (aspirated from 250 ovaries in 15 repeat), 529 (85.32%) oocytes were matured with TCM-199 medium (Group B).

Statistical analysis of the obtained results by using SPSS software and paired t test was performed (Table 1).

**Table 1: Comparing of TCM-199 and SAGE output**
Figure 1: matured and non matured oocytes with SAGE and TCM-199 mediums

Khatir et al. showed that bovine oocytes in response to supplementation with serum or follicular fluid for subsequent growth and development during in vitro maturation were disable. They guessed that lack of receptors for gonadotropins or growth factors is the reason of this disability. The results of this study indicate that although the used mediums for maturing did not cause any significant difference in the number of matured oocytes, more mature oocytes, however, was observed by use of TCM-199 medium.

References

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