Genetic and Biochemical Effects of Natural Extracts on *in Vitro* Maturation of Egyptian Buffalo Oocytes

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Received February 17, 2010; accepted July 3, 2010

Summary Preparation of oocytes is one of the critical factors that determine the developmental competence of embryos produced by *in vitro* fertilization (IVF). The present study was conducted to evaluate the effect of different maturation media on the nuclear and cytoplasmic maturation of buffalo oocytes. Also, to investigate if the addition of ginseng and fenugreek to the maturation media during *in vitro* maturation (IVM) would influence on IVM, and developmental competence of buffalo oocytes as well as intracellular GSH level. Three experiments were conducted to achieve the goals of present study. In the first experiment, the buffalo oocytes were cultured in different media without any supplementations to detect the most suitable effective medium for IVM. The second experiment compared between different culture media for IVM of oocytes through measurement of intracellular glutathione level. The third experiment examined the effect of ginseng and fenugreek additives during the IVM of Egyptian buffalo oocytes. The results of this study proved that the TCM-199 medium significantly increased the percentage of cumulus expansion, nuclear maturation and intracellular GSH concentration after IVM of buffalo oocytes, while CR1aa was the least effective medium. Treatment with ginseng and fenugreek extracts during IVM culture efficiently improves the development competence of buffalo oocytes, and this improvement is correlated with increase of intracellular GSH concentration. In conclusion, TCM-199 was the most effective medium and is desirable for *in vitro* maturation of Egyptian buffalo oocytes. Ginseng and fenugreek additives enhanced the quality of less effective medium (CR1aa), as well as optimized the effect of TCM-199 medium.

Key words Buffalo, IVM, Cytogenetic, Glutathione, Fenugreek, Ginseng.

Water buffalo (*Bubalus Bubalis*) is a distinct species within the family *Bovidae* and it has a distinguished position in the Egyptian agriculture system. Decreases in the buffalo population, as well as increases in the demand for buffalo meat, have led to urgent requirements for the improvement of breeding strategies and the genetics of this domestic livestock. Modern reproductive biotechnologies, such as embryo transfer and *in vitro* embryo production (IVEP), have been used as adjuncts to conventional breeding programs, with limited success (Palta and Chauhan 1998). Despite this intrinsic limitation, improvements are possible through the optimization of the different steps of the procedures that can be achieved by taking into account species-specific differences, as indicated by the higher blastocyst yields reported over recent years (Gasparrini *et al.* 2006). Major factors that might affect the IVEP efficiency are inadequate oocyte maturation, an inappropriate timing of insemination and a non-optimal time of gamete co-incubation.

Oocyte developmental competence, an important factor that affected the IVEP, which involves the ability of a germ cell to produce a normal and viable embryo after fertilization, is a condition that results from both nuclear and cytoplasmic maturation (Niemann and Rath 2001). Many factors influence the karyoplasmic and cytoplasmic maturation of oocytes *in vitro*, including co-culturing

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with follicular cells such as cumulus cells or granulosa cells, duration of maturation, and type of media (de Matos and Furnus 2007, Dilip *et al.* 2006). The process of oocyte cytoplasmic maturation involves numerous molecular events, including synthesis of biochemical compounds, protein phosphorylation and activation of particular metabolically pathways (Eppig 1996, Krisher and Bavister 1999). The synthesis of intracellular glutathione (GSH) is a critical part of oocyte cytoplasmic maturation (Eppig 1996). In bovine oocytes, synthesis of GSH during IVM has been reported (Miyamura *et al.* 1995, de Matos *et al.* 2002), and GSH participates in the male pronucleus formation after fertilization (Miyamura *et al.* 1995, de Matos *et al.* 1996). Therefore, GSH is a potentially highly relevant biochemical marker of the viability and developmental capacity of mammalian oocytes (Zuelke *et al.* 2003). However, the developmental ability of oocytes matured in defined media still tends to be lower than that of oocytes matured in media supplemented with chemicals such as amino acids or antioxidants (Herrick *et al.* 2004, Hong *et al.* 2004).

The maturation medium, selection of protein supplements, and hormones for IVM play an important role in the subsequent fertilization and development of mammalian oocytes during *in vitro* culture (Bavister and Rose-Hellekant 1992). However, the addition of steroids (estrogen and progesterone) improves the completion of maturation (Moor *et al.* 1980) and plays an important role not only for nuclear maturation but also for cytoplasmic maturation of sheep (Guler *et al.* 2000), cattle (Ali and Sirard 2002) and human oocytes (Tesarik and Mendoza 1997).

Phytoestrogens are natural plant substances similar to endogenous estrogens by their chemical structure. Due to this similarity they can bind to estrogen receptors (Cassidy 2003). Fenugreek (*Trigonella foenum graecum*) is one such plant whose termed phytoestrogens, its seeds and leaves are used not only as food but also as an ingredient in traditional medicine. Its seeds contain diosgenin which is used in synthetic estrogen (Billaud 2001). Diosgenin, a steroid sapogenin, is the starting compound for over 60% of the total steroid production by the pharmaceutical industry (Jayaweera 1981, Petit *et al.* 1995). Administration with fenugreek extract significantly increased the total number of the oocytes as well as improved the quality of oocytes in mice (Hassan *et al.* 2006). A steroid sapogenin constituent of fenugreek seeds is a precursor of steroid hormones, such as progesterone and anti-inflammatory steroids, such as cortisone (Norton 1998).

Ginseng is a popular herb with a long history of medicinal use; ginseng roots contain various pharmaceutical components, ginsenosides are the most pharmaceutically active (Kim *et al.* 2005). Traditionally, ginseng is considered a safe herb with few adverse effects. Estrogen-like effects after the use of ginseng products have been reported (Palop *et al.* 1999). Patients treated with ginseng extract had increased the total and free plasma follicle-stimulating hormone (FSH), and luteinizing hormone (LH) level (Tsai *et al.* 2003) but decreased prolactin levels (Salvati *et al.* 1996). Similarity of the ginseng molecule to the structure of some steroid hormones may contribute to the plant's pharmacological actions (Gray *et al.* 2004). According to the available literature, there are no reports where the fenugreek or ginseng has been used as phyto-estrogenic agents on *in vitro* maturation of buffalo oocytes. The aims of the present study were to evaluate the effect of different maturation media on the nuclear and cytoplasmic maturation of buffalo oocytes. Also, to investigate the effect of ginseng and fenugreek supplementation to the maturation media during IVM on the *in vitro* maturation, developmental competence of buffalo oocytes as well as intracellular GSH level. To our knowledge, the present study is the first one to show the stimulatory effect of ginseng and fenugreek addition to maturation medium on the improvement of buffalo oocytes developmental competence.

Materials and methods

Reagents and media

All the chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless indicated otherwise. Folligon was purchased from Intervet International BV. (Boxmeer-Holland). Fetal calf serum (FCS) (GIBCO, Grand Island, NY). The IVM media were; 1) TCM199 buffered with 25 mM sodium bicarbonate; 2) Ham's F10 and 3) CR1aa (prepared as described by Rosenkrans and First 1994). All maturation media were supplemented with 40 IU/ml Folligon, 1 μ g/ml estradiol-17 β , 50 μ g/ml gentamicin sulfates, and 10% (v/v) fetal calf serum. Ginseng extract used in this study was standardized Panax ginseng extract EFLA400 (Phoenix ginseng) (Batch No. 303298) supplied from Lotte Group R & D Centre (Seoul, Korea). Water extracts of fenugreek were presented as a yellow freeze dried crystalline form.

Collection of oocytes and in vitro maturation

Immediately after the slaughter of adult female buffalo, ovaries were collected and placed into Dulbecco's phosphate buffered saline (DPBS) at 37°C and transported to the laboratory, within 2–3 h from slaughter. Cumulus-oocyte complexes (COCs) were recovered by aspiration of 2–8 mm follicles using a 20-gauge. The COCs were evaluated on the basis of their morphology. Only oocytes with compact layers of cumulus cells and intact cytoplasm were used for the experiments. The selected COCs were washed thoroughly in phosphate buffer saline (PBS) supplemented with 0.3% bovine serum albumin and once in the final maturation medium and cultured in the same medium. IVM was carried out at 38.5°C for 24 h in a controlled gas atmosphere of 5% CO₂ in humidified air. After maturation period, half of matured oocytes were frozen at –80°C in microfuge tubes after removal of cumulus cells until the day of GSH assay. Each tube containing 20–30 oocytes in 5 μ l of PBS. The other half of matured oocytes was subjected to nuclear staining to evaluate the nuclear maturation rate.

Cytogenetic studies

At the end of the *in vitro* culture period, oocytes were firstly evaluated depending on expansion of cumulus cells, then transferred into 400 μ l of PBS solution and stripped from their cumulus cells by mechanical displacement by gentle mouth pipetting using a small-bore glass pipette. Matured oocytes were fixed with acetic alcohol (methanol and acetic acid, 3 : 1, v/v) for 24–48 h and stained with 1% aceto-orcein in 45% (v/v) acetic acid. The stages of oocytes were classified according to Santos *et al.* (2006) as follows, intact germinal vesicle (GV; unidentifiable nucleolus and very fine filaments of chromatin); germinal vesicle breakdown (GVBD; presence of different patterns of chromatin condensation, chromosomes coiled up and no visible individual chromosomes); metaphase I (MI), anaphase I (AI), metaphase II (MII), once the formation of bivalents was completed and appeared on 1 or 2 sets of chromosomes.

Glutathione assay

Intracellular content of GSH was measured as described by Funahashi *et al.* (1994). The frozen samples were thawed at room temperature; this procedure was repeated three times. Five microliters of 0.2 M sodium phosphate containing 10 mM EDTA (Sigma, assay buffer) and 5 μ l of 1.25 M phosphoric acid (Fluka, Buchs, Switzerland) were added to a 1.5 ml microfuge tube containing 30 oocytes. Samples were stored at –80°C until use. Concentrations of GSH in the oocytes were determined by the dithionitrobenzoic acid-glutathione disulphide (DTNB-GSSG) reductase-recycling assay (Anderson 1985). Briefly, 700 μ l of 0.33 mg NADPH/ml in stock buffer, 100 μ l of 6 mM DTNB in the stock buffer and 190 μ l water were added with mixing into the microfuge tube. To initiate the reaction, 10 μ l glutathione reductase was added with mixing. The

formation of 5-thio-2-nitrobenzoic acid was followed continuously and the absorbance was monitored at 412 nm with a spectrophotometer (Ultraspec-2000, Pharmacia Biotech Ltd., Cambridge, UK) and recorded at 30 s and 5 min. Both the reagent blank and GSH standards (0.1–1.0 nmol) were assayed under the same conditions. The amount of GSH in each sample was determined by comparing it with a standard curve prepared at the same time.

Experimental design

Egyptian buffalo oocytes were *in vitro* matured, as described before, for 22–24 h. After this period, samples of oocytes were examined for nuclear maturation stage and the rest of oocytes were processed for GSH assay. Replicates of experiments were performed on different days with different batches of oocytes.

Experiment 1 The first experiment was carried out to determine the effects of maturation media (TCM-199, Ham's F10 and CR1aa) on maturation rate depending on nuclear progression.

Experiment 2 The second experiment was carried out to measure the concentration of intracellular glutathione of buffalo oocytes matured in different culture media (TCM-199, Ham's F10 and CR1aa).

Experiment 3 This experiment was conducted based on the results obtained from experiments 1 and 2. In this experiment we studied the effect of the addition of 0.1 µg/ml (fenugreek or ginseng extracts) and 0.6 mM cysteine, as a positive control, on the *in vitro* maturation media (TCM-199 and CR1aa) on maturation rate of buffalo oocytes with respect to cumulus expansion and intracellular glutathione. The nuclear progression of oocytes matured in less effective medium supplemented with the extracts was evaluated to confirm the improving effect of extracts.

Statistical analysis

Statistical analyses for all data were carried out using analysis of variance (ANOVA) and Duncan's test using the SPSS program. Data in the text were expressed as mean ± SE. Analysis was used to compare the rate of maturation and GSH concentration in matured oocytes. A probability of $p < 0.05$ was considered statistically significant.

Results

Experiment 1: Effect of different maturation media on in vitro maturation of buffalo oocytes

To determine the effect of different maturation media on the developmental competence of buffalo oocytes, we compared the proportions of matured oocytes and the cumulus expansion percentage after 24 h of IVM. The data concerning the influence of different maturation media on the percentage of cumulus expansion is shown in Figs. 1 and 2. The percentage of oocytes with expanded cumulus was highly significant ($p < 0.5$) in TCM-199 (90%) medium when compared with that in CR1aa (82.5%).

Regarding to the nuclear maturation, the results obtained in Table 1 showed that there were no significant differences in oocytes stages; GV, GVBD, AI, MI and MII; in TCM-199 and Ham's F10 media (Fig. 3). CR1aa medium showed significantly difference when compared with TCM-199 and Ham's F10 media in all oocytes stages, with the exception in GV stage which did not show any significant difference between all media. The number of oocytes developed to MI and MII in TCM-199 medium (30.6 ± 2.8 and 26.0 ± 2.9 , respectively) was significantly higher than those in CR1aa medium (16.6 ± 1.2 and 18.2 ± 1.2 , respectively). The percentage of nuclear maturation in TCM-199 and Ham's media (59% and 46%, respectively) was significantly higher compared to CR1aa medium (37%).

Experiment 2: Effect of different maturation media on intracellular GSH concentration in buffalo oocytes during IVM

Results of GSH concentrations are shown in Fig. 4 and revealed a significant increase in GSH levels in oocytes matured in TCM-199 medium (6.2 ± 0.4 pmol/oocytes) compared to the other media; Ham's F10 and CR1aa (5.3 ± 0.4 and 3.9 ± 0.3 pmol/oocytes, respectively).

Fig. 1. Effect of different maturation media on the percentage of cumulus expansion of buffalo oocytes matured *in vitro*.

Experiment 3: Effect of fenugreek and ginseng extracts during IVM on oocytes maturation

The results obtained from the first and second experiments revealed that CR1aa medium was the least effective for maturation of buffalo oocytes while TCM-199 was most effective. So, the third experiment was conducted to determine the effects of treatment with fenugreek, ginseng extracts, and cysteine, as a positive control, in CR1aa and TCM-199 media on *in vitro* maturation of buffalo oocytes. As shown in Fig. 5 the concentration of intracellular GSH significantly increased in TCM-199 medium when supplemented with ginseng and fenugreek (8.8 ± 0.3 and 7.8 ± 0.3 pmol/oocyte, respectively) compared

Fig. 2. Compact cumulus (A): tightly attached cells surrounding the oocyte with a smooth surface over the cumulus hillock. Expanded cumulus (B): cells detached from the oocyte with matrix visible between cumulus cells.

Table 1. Effects of different maturation media on nuclear maturation and meiotic progression of buffalo oocytes during IVM

Groups	No. of oocytes	Nuclear maturation %	Meiotic progression				
			GV	GVBD	MI	AI	MII
TCM-199	519	305/519 59%	6.2 ± 1.7^a	19.6 ± 1.1^a	30.6 ± 2.8^a	6.6 ± 0.5^a	26.0 ± 2.9^a
Ham's F10	651	302/651 46%	7.2 ± 0.5^a	18.2 ± 1.1^a	28.0 ± 3.0^a	5.4 ± 0.5^a	25.4 ± 1.2^a
CR1aa	560	200/560 37%	6.2 ± 0.5^a	13.2 ± 0.6^b	16.6 ± 1.2^b	2.2 ± 0.7^b	18.2 ± 1.2^b

Data are presented as the mean $\pm$ S.E.M. Values with different superscripts are significantly different ($p < 0.05$).

Table 2. Effects of ginseng and fenugreek during IVM on nuclear maturation and meiotic progression in buffalo oocytes matured in CR1aa medium

Groups	No. of oocytes	Nuclear maturation %	Meiotic progression				
			GV	GVBD	MI	AI	MII
CR1aa	560	200/560 37%	6.2±0.5 ^{ab}	13.2±0.58 ^a	16.6±1.2 ^b	2.2±0.7 ^b	18.2±1.2 ^c
CR1aa+Ginseng	320	167/320 52.5%	5.6±0.75 ^b	8.6±1.5 ^b	20.0±3.5 ^a	3.9±0.51 ^a	28.6±0.68 ^a
CR1aa+Fenugreek	370	156/370 41.8%	7.2±0.58 ^a	9.8±0.37 ^b	19.0±1.2 ^a	3.0±0.89 ^a	26.2±0.58 ^b
CR1aa+Cysteine	300	129/300 43%	6.6±0.81 ^{ab}	10.8±1.66 ^{ab}	15.6±1.47 ^b	1.4±0.51 ^b	26.0±0.71 ^b

Data are presented as the mean±S.E.M. Values with different superscripts are significantly different ($p<0.05$).

Fig. 3. Photomicrographs of stained buffalo oocytes represent various nuclear stages during meiotic progression. **Fig. 4.** Effect of different maturation media on the concentration of the intracellular GSH in buffalo oocytes during IVM.

to those matured in TCM-199 medium without supplementation (6.0 ± 0.4 pmol/oocyte). Moreover, GSH concentrations were significantly increased in oocytes matured in CR1aa medium supplemented with ginseng and fenugreek (6.0 ± 0.28 and 5.4 ± 0.22 pmol/oocyte, respectively) compared to those matured in CR1aa medium alone (3.9 ± 0.31 pmol/oocyte). Supplementation with ginseng and fenugreek during IVM significantly increased the concentration of GSH in oocytes matured in TCM-199 compared to CR1aa media.

The proportion of oocytes with expanded cumulus cells was significantly higher in all supplemented media compared to TCM-199 and CR1aa without supplementation ($p<0.5$) as shown in Fig. 6. Ginseng and fenugreek supplementation to culture media during IVM

increase the proportions of oocytes with expanded cumulus cells in TCM-199 than CR1aa medium. However, no significant difference was observed between TCM-199 and CR1aa supplemented with cysteine.

The progression through the meiotic stages of buffalo oocytes matured in CR1aa medium

Fig. 5. Effect of ginseng and fenugreek addition to TCM-199 and CR1aa media on the concentration of the intracellular GSH in buffalo oocytes.

Fig. 6. Effect of ginseng and fenugreek addition to TCM-199 and CR1aa media on the percentage of cumulus expansion.

supplemented with ginseng, fenugreek and cysteine is presented in Table 2. The highest ($p < 0.5$) proportion of oocytes completed nuclear maturation (*i.e.* reached the MII stage) were observed in CR1aa plus ginseng compared to all other groups. Furthermore, all the supplemented media showed an increase in the rate of oocytes that reached the MII stage compared to the non-supplemented CR1aa medium.

Discussion

Development of a suitable culture system for IVM of oocytes is a major component of *in vitro*

embryo production procedures, as it is not only affects the proportion of oocytes that reach the metaphase II, but also influences subsequent embryonic development (Rizos *et al.* 2002).

In this study, TCM-199, Ham's F-10 and CR1aa media were used for *in vitro* maturation of buffalo oocytes and were compared for their efficiency in enhancing both nuclear and cytoplasmic maturation. The results revealed that using the TCM-199 medium during IVM of buffalo oocytes significantly increased the nuclear maturation and induced greater cumulus expansion in comparison with Ham's F-10 and CR1aa media. Our results are in agreement with other studies, which also showed higher maturation rates in TCM-199 than in Ham's F-10 (Pawshe *et al.* 1996, Smetanina *et al.* 2000). Similar findings have been reported by Jamil *et al.* (2007), who found a significant difference for maturation rate of buffalo oocytes matured in both media. A variety of media have been used for *in vitro* maturation of follicular oocytes, however TCM-199 medium was the basic maturation medium in many recent studies (Li *et al.* 2004, Jamil *et al.* 2007, Amer *et al.* 2008). Roushandeh *et al.* (2007) found that TCM-199 medium improved GVBD and MII development better than MEME medium. TCM-199 has a lower glucose concentration, both essential and non-essential amino-acids, a large range of vitamins and several other components such as cholesterol and ribose. Amino acids are known to be beneficial for embryo development, and oocyte maturation (Ruth *et al.* 2002).

Glutathione plays many important biological roles and it has been suggested that GSH synthesis is critical for the acquisition of developmental competence of oocytes at the cytoplasmic level (Eppig 1996). This is reinforced by the observation that the intracellular GSH concentration of porcine oocytes following IVM reflects the degree of cytoplasmic maturation (Funahashi *et al.* 1994). Furthermore, de Matos and Furnus (2000) suggested that the measurement of GSH levels at the end of IVM may represent a reliable indicator of the cytoplasmic maturation and its concentration could be affected by maturation media (Yoshida *et al.* 1993). In the present study, the highest intracytoplasmic GSH concentration was found in oocytes matured in TCM-199 medium followed by Ham's F-10 then CR1aa. These findings are consistent with the report of Yoshida *et al.* (1992) who reported that glutathione level in pig oocytes matured in TCM-199 was significantly higher than those matured in TLP-PVA and the finding of Hibasami *et al.* (2003) who concluded that TCM-199 is the basic maturation medium used in the *in vitro* oocytes maturation.

Among its other multiple roles, previously described, glutathione is also critically involved in the cellular defense against oxidative stress, which is one of the most important factors interfering with a proper cell function (Gasparrini *et al.* 2003). The addition of antioxidants to IVM media significantly increases the glutathione content and improved the developmental competence of oocytes (Wang *et al.* 2007). The present study demonstrated that the addition of ginseng and fenugreek to TCM-199 and CR1aa maturation media during IVM significantly increased the level of GSH compared with control media (non-supplemented groups) and cysteine supplemented groups, which indicated that the improvement of developmental competence of buffalo oocytes may be partly due to increase of intracellular GSH concentration after IVM of oocytes. Several authors have postulated that the measurements of GSH concentration in oocytes after IVM may be a valuable indicator of the oocyte cytoplasmic maturation in bovine (de Matos and Furnus 2000), ovine (de Matos *et al.* 2002) and caprine (Cognié *et al.* 2003). In addition, fenugreek leaves are a rich source of calcium, iron, B-carotene and other vitamins (Sharma *et al.* 1996) which have been found to have antioxidant and anticarcinogenic potency in different settings (Sur *et al.* 2001, Hibasami *et al.* 2003). Moreover, ginseng roots contain various pharmaceutical components like ginsenosides (saponins), polyacetylenes and polyphenolic compounds which have strong antioxidant activity (Higdon and Frei 2003, Kim *et al.* 2005). Hassan and Abdel-Wahhab (2006) reported that ginseng has an antioxidant effect and improved sperm quality in mice. Both fenugreek and ginseng have antioxidant activity, and it may be attributed to stimulate synthesis of glutathione in oocytes during IVM.

In this study, the addition of ginseng or fenugreek extracts to CR1aa medium significantly enhanced the maturation rate. This finding suggested that the enhancement in the maturation rate may be attributed to the steroid compound in both extracts. In IVM, the addition of steroids improved the completion of maturation (Moor *et al.* 1980). However, *in vivo* the balance between the gonadotropins (FSH and LH) and steroids (estrogen and progesterone) might be the important criteria in the initiation of follicle growth and good-quality oocytes (Amer *et al.* 2008). Administration with fenugreek extract significantly increased the total number of the oocytes as well as improved the quality of oocytes in mice (Hassan *et al.* 2006). Fenugreek seeds contain diosgenin which is used to make synthetic estrogen (Billaud 2001). Diosgenin, a steroid sapogenin, is the initial compound for over 60% of the total steroid production by the pharmaceutical industry (Jayaweera 1981, Petit *et al.* 1995). While the similarity of the ginsenoside molecule, in ginseng, to the structure of some steroid hormones may contribute to the plant's pharmacological actions (Gray *et al.* 2004). In another study the interaction of this ginsenoside with the estrogen receptor was reported (Lee *et al.* 2003). Also estrogen-like effects after use of ginseng products have been reported by Palop *et al.* (1999). Our results demonstrated that the addition of ginseng extract during IVM had a significantly higher ($p < 0.05$) effect on maturation rate than fenugreek extract. Oocytes matured *in vitro* in the presence of gonadotrophins and steroid had higher maturation rates compared with maturation protocols, in which no hormones were added (Choi *et al.* 2001). Gonadotrophins and steroid cause synergistic enhancement of nuclear maturation in mammalian oocytes *in vitro* (Pawshe *et al.* 1996). Also, oocytes matured *in vitro* in the presence of gonadotrophins and steroid especially estradiol showed higher maturation and fertilization rates compared with those matured in the absence of hormones (Abdoon *et al.* 2001). Hence, supplementation of the media with ginseng extract had a biphasic favorable effect on maturation.

In conclusion, the results of the current study showed that TCM-199 medium was most effective during IVM of buffalo oocytes while CR1aa was less effective one. Addition of ginseng or fenugreek extracts during IVM efficiently improves the development competence of buffalo oocytes, and this improvement is correlated with increase of intracellular GSH concentration after IVM of oocytes. Ginseng and fenugreek enhanced the quality of CR1aa medium as well as optimized the effect of TCM-199 medium. Use of alternative concentrations and culture conditions to manipulate supplementation of culture media with natural extracts will be useful in further elucidating the mechanisms of these actions. This initial hypothesis needs further proof, but it may explain why different phyto-estrogens exert a more or less inhibitory influence on the maturation processes of oocytes.

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