

Effects of Fenugreek Seed Extract on *in vitro* Maturation and Subsequent Development of Sheep Oocytes

Ibrahim A.H. Barakat^{1,2} and Ahmed R. Al-Himaidi¹

¹ Zoology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Kingdom of Saudi Arabia.

² Cell Biology Department, National Research Center, Dokki, Giza, Egypt.

Abstract.- The present study was conducted to determine the role and optimum concentration of fenugreek seed extract during *in-vitro* maturation on maturation rate and developmental competence of Neaimi sheep oocytes following *in-vitro* fertilization. The Cumulus oocyte complexes (COCs) collected from sheep slaughterhouse ovaries were randomly divided into three groups, and they were matured for 24 hrs in maturation medium containing fenugreek seed extract (0, 1 and 10 $\mu\text{g ml}^{-1}$). Oocytes of a control group were matured in a medium containing 1 $\mu\text{g ml}^{-1}$ estradiol 17 β . After maturation, half of oocytes were fixed and stained for evaluation of nuclear maturation. The rest of oocytes were fertilized *in vitro* with fresh semen, then cultured for 9 days for the assessment of the developmental capacity of the oocytes. The results showed that the mean values of oocytes with expanded cumulus cells percentage were not significantly different among all groups ($P < 0.05$), but nuclear maturation rate of oocytes matured with 10 $\mu\text{g ml}^{-1}$ fenugreek seed extract was significantly higher than that of the control group. The maturation rate and development to morula and blastocyst stage for oocytes matured at 10 $\mu\text{g ml}^{-1}$ fenugreek seed extract was significantly higher than those matured at 1 $\mu\text{g ml}^{-1}$ of fenugreek seed extract and the control group. In conclusion, better maturation and developmental capacity rate to morula and blastocyst stage were obtained by the addition of 10 $\mu\text{g ml}^{-1}$ fenugreek seed extract to maturation medium than addition of 1 $\mu\text{g ml}^{-1}$ estradiol-17 β ($P < 0.05$).

Keywords: Fenugreek seed extract, *in vitro* maturation, sheep oocytes, *in vitro* fertilization, embryo development.

INTRODUCTION

Sheep are an important livestock that acts as a source of wool, meat and milk to millions around the globe. Sheep are usually seasonal breeders and they do not yield sufficient lambs to meet demand. The *in vitro* embryo production system includes three major steps, namely *in vitro* maturation of the primary oocytes, *in vitro* fertilization of the matured oocytes and *in vitro* culture of presumptive embryos, until transferred or cryopreserved for future use (Cognie *et al.*, 2003; Gandolfi *et al.*, 2005; Zhu *et al.*, 2007). Hence assisted reproduction technologies have been developed over the past few decades to produce high-yielding lambs in large numbers. As with other technologies, *in vitro* embryo production technologies have their share of problems and failures (Camargo *et al.*, 2006) and therefore need to be optimized to produce healthy and viable lamb.

Medicinal plants have been useful in the development of new drugs and continue to play an invaluable role in the drug discovery processes (Amer *et al.*, 2013). Fenugreek (*Trigonella foenum graecum* L.) is an annual plant from the family Papilionaceae–Leguminosae and is extensively cultivated in India, Mediterranean region, North Africa and Yemen (Kassem *et al.*, 2006). Phytoestrogens are natural plant substances which act as endogenous estrogens because of the similarity of their chemical structure. Therefore, they can bind to estrogen receptors of mammalian cells (Cassidy, 2003). In addition, Fenugreek seed contain diosgenin which is used in synthetic estrogen (Billaud, 2001). A steroid sapogenin constituent of fenugreek seed is a precursor of steroid hormones, such as progesterone and anti-inflammatory steroids, such as cortisone (Norton, 1998). Studies show that fenugreek seed have antioxidant properties (Hibasami *et al.*, 2003). Further fenugreek seed polyphenols prevented oxidative hemolysis and lipid peroxidation induced by H_2O_2 *in vitro* in human erythrocytes (Kaviarasan *et al.*, 2004). In our previous study (Barakat *et al.*, 2010) addition of fenugreek extract to maturation

* Corresponding author: ibrahimahb@yahoo.com, ibarakat@ksu.edu.sa

0030-9923/2013/0006-1679 \$ 8.00/0

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medium enhanced the quality of less effective medium (CR1aa), as well as optimized the effect of TCM-199 medium for *in vitro* maturation of Egyptian buffalo oocytes.

Moreover, oral administration of fenugreek extract significantly increased the total number and quality of collected mice oocytes as mentioned by Hassan *et al.* (2006).

In vitro maturation is one of the essential steps in the *in vitro* fertilization process. Addition of hormones into maturation medium for *in vitro* maturation of oocytes plays an important role in subsequent *in vitro* fertilization and *in vitro* development in goats (Pawshé *et al.*, 1996) and cattle (Saeki *et al.*, 1991). The addition of steroids (estrogen and progesterone) improves the completion of maturation (Moor *et al.*, 1980) and plays an important role not only in nuclear maturation, but also in cytoplasmic maturation of sheep (Guler *et al.*, 2000), cattle (Ali and Sirard, 2002) and human oocytes (Tesarik and Mendoza, 1997). The presence of steroids in the follicular fluid before and during maturation may play a role in oocyte maturation (Ainsworth *et al.*, 1980). In fact, it has been shown that estradiols as well as other steroids are involved in keeping the oocytes in meiotic arrest (Mingoti *et al.*, 1995). Furthermore, it has been suggested that estradiol is important in the oocyte acquisition of the fertilization competence (Pellicer, 1997). However, the specific role of estrogens in follicular and oocyte maturation as well as ovulation and embryo development seems to be species dependent (Moudgal *et al.*, 1996) and is currently unknown in various species. Pig cumulus oocyte complexes secrete estradiol during cultivation in a steroid free medium, probably as a consequence of the action of gonadotropins (Dode and Graves, 2002). Secretion of estradiol by human and bovine cumulus cells has also been reported (Mingoti *et al.*, 2002).

In vitro matured oocytes which were collected from slaughterhouse ovaries may be easily and cheaply obtained and their use may become a useful tool for basic research of the reproductive biology and for application of advanced biotechnologies such as somatic cell cloning and transgenic livestock (Nadi *et al.*, 2002). However, there are some problems encountered with *in vitro*

production of sheep embryos such as, still 60% failure of IVM/IVF oocytes reaching the blastocyst stage (Gilchrist and Thompson 2007; Katska-Ksiazkiewicz *et al.*, 2007); the progress in the *in vitro* fertilization techniques, embryo culture procedures and oocytes quality was inadequate over the past years (Cognie *et al.*, 2004; Russo *et al.*, 2007; Morton *et al.*, 2008).

To our knowledge, there are no reports where the fenugreek seed extract has been used as phytoestrogenic agent in the *in vitro* maturation of sheep oocytes and the subsequent development of the matured oocytes to the blastocyst stage following the *in vitro* fertilization. Hence, the aim of the present study was to examine the role and the optimum concentration of fenugreek seed extract during the *in vitro* maturation of Neaimi sheep oocytes and the subsequent IVP.

MATERIAL AND METHODS

Chemicals

Unless otherwise mentioned, all chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of fenugreek seed extract

Fenugreek seed extract was extracted with hot water for once time in the beginning of the experiments. Briefly, fenugreek seed were heated in water (10 g/100 ml) until boiling and evaporate the 75% of the water, then spreading the suspension and leave to dry completely (Maghsoud *et al.*, 2013).

Oocytes maturation

Neaimi sheep ovaries were collected from a local slaughterhouse and transported to the laboratory in saline (30 to 35°C) within 1 to 3 hrs. from collection. Ovaries were washed three times with pre-warmed fresh saline (37°C), and all visible follicles with a diameter from 2 to 8 mm were aspirated using a 20 gauge needle. After aspiration, only cumulus oocytes complex (COCs) surrounded by more than 3 layers of cumulus cells were selected for *in vitro* maturation (IVM) (Fig.1). Selected oocytes were washed three times with maturation medium. The basic maturation medium consists of TCM-199 with Earle's (Sigma, m4530)

supplemented with 10 % Fetal bovine serum (FBS, GIBCO, Grand Island, NY), 40 IU/ml equine chronic gonadotropin (eCG, Folligon, Intervet International BV, Boxmeer, Holland), 10 μ l penicillin sodium salt and 50 μ g ml⁻¹ gentamycin sulphate. Ten to fifteen selected COCs were transferred into 50 μ l droplet of maturation medium supplemented with estradiol 17 β or fenugreek seed extract for 24 h. and were cultivated at 38.5°C at 5% CO₂ and high humidity.

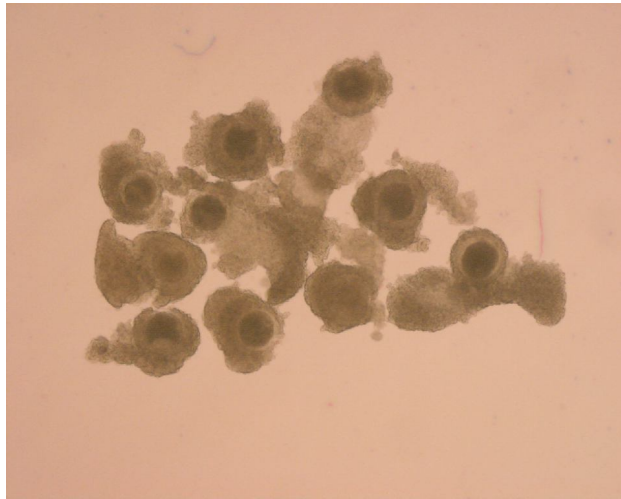


Fig. 1. Sheep oocytes used in *in vitro* production of sheep embryos (200X).

Experimental design

To evaluate the role and optimum concentration of fenugreek seed extract on *in vitro* maturation and developmental competence of Neaimi sheep oocytes, *in vitro* fertilization has been followed. Three thousands and two hundred sixty two oocytes were used, 1391 oocytes were used for nuclear maturation assay and 1871 oocytes were used for IVF. Each treatment was repeated five times. According to the culture medium used oocytes were divided into 3 groups: group I (n= 943) – basic maturation medium + 1 μ g estradiol 17 β /ml media, group II (n= 1143) – basic maturation medium + 1 μ g fenugreek seed extract/ml media and group III (n= 1180) – basic maturation medium + 10 μ g fenugreek seed extract/ml. Fenugreek seed extract doses were selected depending on the recommended estradiol 17 β (1 μ g estradiol 17 β /ml medium) dose which has

been reported used in IVM cultivation medium for most animals (Lv *et al.*, 2010). Group I served as the control group and groups II and III were considered as treatment groups.

Nuclear maturation assay

After maturation, cumulus cells were removed from matured oocytes using 0.1% hyaluronidase and mechanical displacement by gentle mouth pipetting using a small-bore glass pipette. The cumulus free oocytes were then fixed in acetic:ethanol (1:3 v/v) for 24–48 h and were stained with 1% aceto-orcein in 45% (v/v) acetic acid (Rao *et al.*, 2002). The nuclear status of the matured oocytes was classified according to Santos *et al.* (2006).

Preparation of sperm and in vitro fertilization

For IVF, 35 μ L Bracket and Oliphant (B.O.) IVF medium (Bracket and Oliphant, 1975) drops were made in petri dishes. The drops were covered with paraffin oil and equilibrated at 39°C under 5% CO₂ in humidified air in the incubator for at least 2 h prior to use. Fresh semen was collected from sheep ram of proven fertility. For swim up, 80 to 100 μ l of semen was kept under 1 ml of B.O. medium supplemented with 5 mg BSA/ml and 0.2 mg Heparin/ml in a 15 ml conical Falcon tube at 38.5°C for up to 45 min. After swim up, the 700 to 800 μ l of the supernatant were added to 3 ml of BSA-B.O. medium, centrifuged twice at 1800 rpm for 5 min and the final pellet was re-suspended with BSA-B.O. medium. Insemination was carried out by adding 15 μ l of sperm suspension, containing 1.0×10^6 sperm/ml, into the fertilization drop (10 to 15 oocytes per 45 μ l fertilization drop). The gametes were co-incubated with sperm for 18–20 h at 38.5°C, 5% CO₂ and high humidified.

In vitro culture of embryos

After fertilization, oocytes were denuded by successive pipetting, then 15 to 20 oocytes were transferred into 50 μ l droplets of SOF medium (Maraa *et al.*, 2013) supplemented with 10% FBS, 0.0027g sodium pyruvate, 100 IU/ml penicillin sodium salt and 50 μ g ml⁻¹ gentamycin sulphate and were then covered with mineral oil. The fertilized oocytes were cultured for 9 days under 5% CO₂, 5%

O₂, 90 % N₂, 38.5°C with high humidity. The day of IVF was considered as day 0. The final numbers of embryos at various stages (2-, 4-, 8-, 16-, morula, and blastocyst) were recorded at day 9.

Statistical analysis

Replicates of experiments were performed on different days with different batches of oocytes and semen. Statistical analyses for all data were carried out using analysis of variance (ANOVA). Statistical differences were considered significantly at $P \leq 0.05$ levels by using Duncan's Multiple Range Test procedure (Duncan, 1955). Results were expressed as mean \pm SEM (standard error of mean). Analysis of data was used to compare the maturation and different stages of embryo development *in vitro* in different treatments. All the calculations were performed using the SPSS statistical program.

RESULTS

In vitro maturation

Effects of fenugreek seed extract on cumulus expansion and nuclear maturation of sheep oocytes are presented in Table I. The mean value of oocytes with expanded cumulus cells percentage was not significantly different ($P \leq 0.05$) among all groups (Fig. 2). Regarding nuclear maturation, there were no significant differences in oocytes stages; GV, GVBD, MI, AI, and MII; in group II and group III, group I and group II for GV and M II stages only, and group I and group III for GV only (Fig. 3). The number of oocytes developed to M I and Anaph. I in group II and group III was significantly higher than those in group I, while there was no significant difference between group II and group III. Concerning oocytes with M II stage, it was significant higher in group III compared to group I, but there is no significant difference ($P \leq 0.05$) between group II and group III (Table I).

In vitro embryo development

To determine the effect of fenugreek seed extract on the developmental competence of sheep oocytes, we compared between oocytes matured *in vitro* in medium supplemented with or without the extract. The analysis of embryo development stages (Fig. 4) data in Table II showed significant

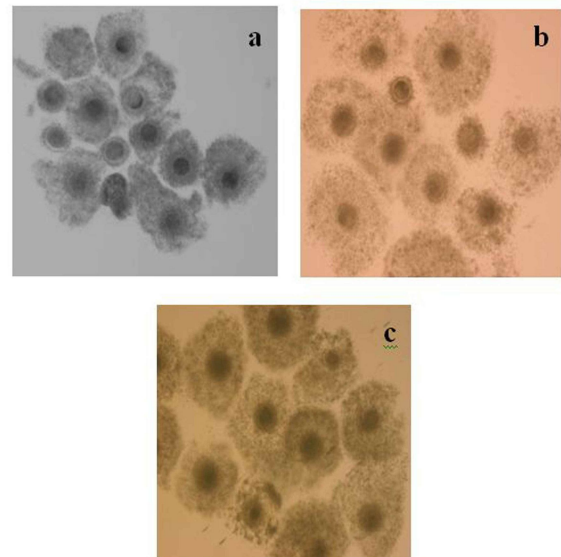


Fig. 2. Expansion of cumulus cells as a result of exposing the sheep oocytes to different concentrations of fenugreek extract. a) Control, b) 10 µg/ml, c) 1 µg/ml (200X).

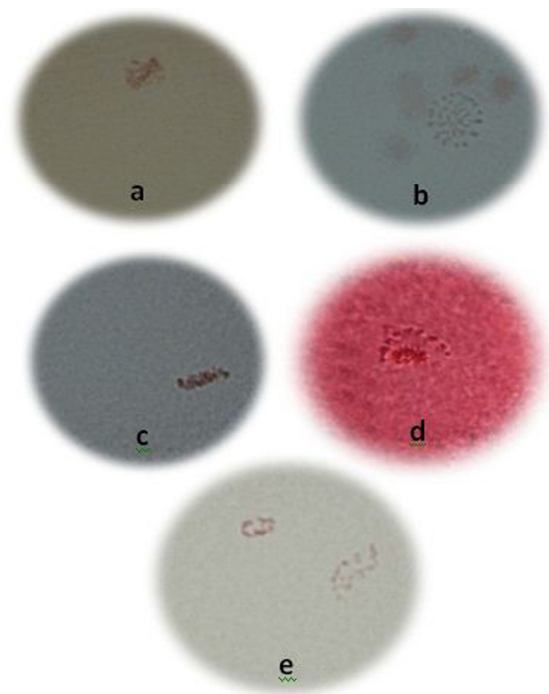


Fig. 3. Stained of Neami sheep oocytes with aceto-orcein stain represented nuclear stages during meiosis progression. a) Germinal Vesicle (GV). b) Germinal vesicle break down (GVBD). c) Metaphase I (M I). d) Anaphase. e) Metaphase II (M II) (400X).

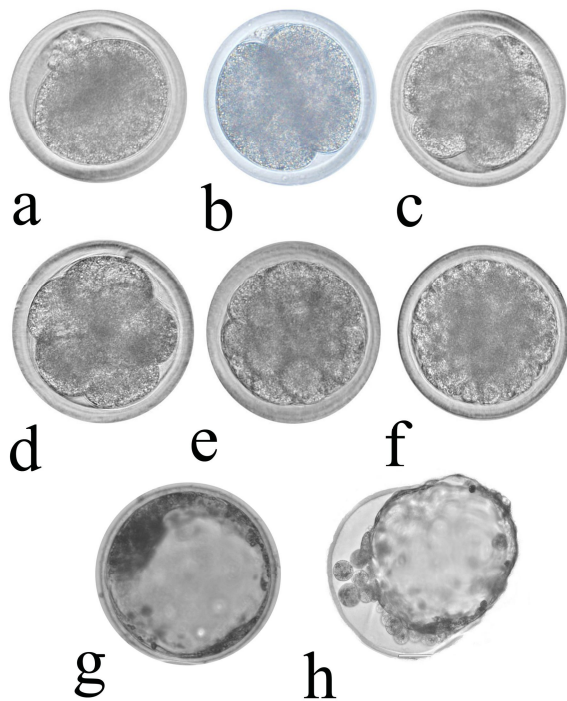


Fig. 4. Neaimi sheep embryos developmental stages; a) Fertilized oocyte, b) 2-cell stage, c) 4 cell stage, d) 8 cell stage, e) 16 cell stage, f) Morula stage, g) Expanded blastocyst, and h) Hatching blastocyst (200X).

differences ($P \leq 0.05$) between group III and group I and group II for degenerated and fragmented embryos, 4-, 16-, and blastocyst stages, while there was no significant differences for 2- and 8- stages. Concerning the morula stage, data analysis showed that the mean value significantly differ between group III and group I, while there is no significant difference between group I and group II. The mean value of oocytes matured *in vitro* in medium supplemented with fenugreek seed extract at $10 \mu\text{g ml}^{-1}$ and developed to blastocyst stage (28.0 ± 0.7) was significantly higher ($P \leq 0.05$) than those oocytes matured *in vitro* in medium supplemented with $1 \mu\text{g ml}^{-1}$ estradiol 17β (23.8 ± 0.7) and $1 \mu\text{g ml}^{-1}$ fenugreek seed extract (25.4 ± 0.5) as shown in Table II. Data analysis of embryo development showed that $10 \mu\text{g ml}^{-1}$ fenugreek seed extract (group III) is better than other treatments (group I and group II).

DISCUSSION

As far as we know, there are no reports on the effects of addition of fenugreek seed extract into maturation medium used in IVP of sheep oocytes. In this study, addition of fenugreek seed extract to maturation medium of sheep oocytes enhanced the rates of maturation and subsequent development to morula and blastocyst stage after *in vitro* fertilization. So, the discussion will be written from the perspective of the impact of estrogen or estradiol- 17β on the maturation of oocytes and embryo growth, where the fenugreek extract has an estrogenic effect (Billaud, 2001; Norton, 1998). Improvement of an IVM system is a major step of *in vitro* embryo production procedures, because it does not only affects the maturation rate of oocytes, but also has a role in subsequent embryonic development (Rizos *et al.*, 2002). Several researchers have studied different aspects of IVM in mammalian oocytes (Rao *et al.*, 2002; Kharche *et al.*, 2005). The maturation media with the selection of protein supplements and hormones play an important role for IVM, subsequent IVF and *in vitro* development (Motlagh *et al.*, 2008). In previous studies reported that the addition of fenugreek extract to maturation medium significantly enhanced the maturation rate and embryo development (Barakat *et al.*, 2010; Choi *et al.*, 2001; Roushandeh *et al.*, 2007) as found in our results. This study showed that the developmental rate of oocytes to morula and blastocyst stage significantly increased when the fenugreek seed extract was added into maturation medium compared to estradiol- 17β .

These results may be attributed to the steroid compound in the seed extract, since the addition of steroids to a maturation medium improved the completion of maturation (Moor *et al.*, 1980). Also, *in vivo* oocyte growth and maturation are directly regulated by intra-ovarian factors such as steroids, cytokines and other growth factors acting at key points during the process of follicle development (Campbell and McNeilly, 1996). Among these factors, estradiol (E2) may be of great importance (Zheng *et al.*, 2003).

Our results demonstrated that the addition of fenugreek seed extract to maturation medium

Table I. Effect of fenugreek seed extract on cumulus expansion and nuclear maturation (mean \pm SEM) of sheep oocytes.

Groups	Traits	Nuclear maturation					
		Oocytes with expanded cumulus cells (%) [§]	GV	GVBD	M I	Anaph. I	M II
Estradiol 17 β (Control)	Group I	97.8 \pm 0.4 a	12.6 \pm 0.8a	8.4 \pm 1.0a	8.4 \pm 1.1a	5.2 \pm 0.7a	44.8 \pm 6.3a
Fenugreek seed extract	1 μ g/ml (Group II)	98.0 \pm 0.3a	12.2 \pm 0.9a	12.2 \pm 0.7b	11.8 \pm 1.1b	7.8 \pm 0.9b	48.4 \pm 2.7 ab
	10 μ g/ml (Group III)	98.0 \pm 0.3a	10.2 \pm 0.7a	13.2 \pm 1.3b	12.4 \pm 1.0b	8.4 \pm 0.9b	62.2 \pm 3.8 b

* Mean values in the same columns with different superscripts (a, b) differ significantly ($p \leq 0.05$).

GV, germinal vesicle; GVBD, germinal vesicle breakdown M-I, metaphase-I; Anaph. I, anaphase I; M-II, metaphase-II.

[§] Mean maturation rate % depending on expansion of cumulus cells

Table II. Embryo developments (mean \pm SEM) after *in vitro* maturation of sheep oocytes in medium supplemented with fenugreek seed extract.

Groups	Traits	Embryo developed to							
		Degenerated embryos	Fragmented embryos	2-cell stage	4-cell stage	8-cell stage	16-cell stage	Morula	Blastocysts
Estradiol 17 β (Control)	Group I	12.6 \pm 0.5a	5.6 \pm 0.4a	2.2 \pm 0.4a	1.2 \pm 0.4a	1.8 \pm 0.4a	1.4 \pm 0.2a	60.6 \pm 1.8a	23.8 \pm 0.7a
Fenugreek seed extract	1 μ g/ml (Group II)	11.4 \pm 0.5b	19.4 \pm 0.8c	4.2 \pm 0.6b	4.0 \pm 0.5c	3.2 \pm 0.4b	1.6 \pm 0.2a	66.2 \pm 1.9ab	25.4 \pm 0.5a
	10 μ g/ml (Group III)	9.0 \pm 0.7a	10.2 \pm 0.9b	3.4 \pm 0.5ab	2.4 \pm 0.2b	2.2 \pm 0.4ab	2.6 \pm 0.2b	71.4 \pm 2.0b	28.0 \pm 0.7b

* Mean values in the same columns with different superscripts (a, b, c) differ significantly ($p \leq 0.05$).

enhance the maturation rate and embryonic development of Neaimi sheep oocytes. This finding suggested that the enhancement may be attributed to the steroid compound in the extract (Billaud, 2001), as well as, the fact that antioxidants present in the extract may enhance the maturation rate and embryonic development (Sur *et al.*, 2001; Hibasami *et al.*, 2003).

CONCLUSION

According to our results, an alternative natural source of estradiol-17 β with fenugreek seed extract at 10 μ g/ml concentration seems to improve the *in vitro* maturation rate of Neaimi sheep oocytes and the morula and blastocyst rate. However,

additional research is needed to prove the positive effects of using natural sources of extracts in IVP media.

ACKNOWLEDGMENTS

We wish to thank the National Plan for Sciences and Technology (NPST) at King Saud University for support. This research is granted by NPST Project No: BIO-1019-2-10.

REFERENCES

- AINSWORTH, L., TSANG, B.K., DOWNEY, B.R., MARCUS, G.J. AND ARMSTRONG, D.T., 1980. Interrelationship between follicular fluid steroid levels, gonadotrophic stimuli and oocyte maturation during

- preovulatory development of porcine follicles. *Biol. Reprod.*, **23**: 621 – 627.
- AMER O.S., DKHIL M.A. AND AL-QURAIHY S 2013. Antischistosomal and hepatoprotective activity of *Morus alba* leaves extract. *Pakistan J. Zool.*, **45**: 387-393.
- ALI, A. AND SIRARD, M.A., 2002. The effects of 17 β -estradiol and protein supplement on the response to purified and recombinant follicle stimulating hormone in bovine oocytes. *Zygote*, **10**: 65–71.
- BARAKAT, I.A.H., HASSAN, A.M., ALAM, S.S. AND KHALIL, W.K.B., 2010. Genetic and biochemical effects of natural extracts on *in vitro* maturation of Egyptian buffalo oocytes. *Cytologia*, **75**: 243–253.
- BILLAUD, C., 2001. Composition, nutritional value and physiological properties. *Adrian J Fenugreek Sci.-ail.*, **21**: 3–26.
- BRACKET, B.G. AND OLIPHANT, G., 1975. Capacitation of rabbit spermatozoa *in vitro*. *Biol. Reprod.*, **12**: 260 – 274
- CAMARGO, L.S.A., VIANA, J.H.M., SA, W.F., FERREIRA, A.M., RAMOS, A.A. AND FILHO, V.R.V., 2006. Factors influencing *in vitro* embryo production. *Anim. Reprod.*, **3**: 19–28.
- CAMPBELL, B.K. AND MCNEILLY, A.S., 1996. Follicular dominance and oocyte maturation. *Zygote*, **4**: 327 – 334.
- CASSIDY, A., 2003. Potential risks and benefits of phytoestrogen-rich diets. *Int. J. Vitam. Nutr. Res.*, **73**: 120–126.
- CHOI, Y.H., CARNEVALE, E.M., SIEDEL, G.E. AND SQUIRES, E.L., 2001. Effect of gonadotropins on bovine oocyte matured in TCM-199. *Theriogenology*, **56**: 661–670.
- COGNIE, Y., BARIL, G., POULIN, N. AND MERMILLOD, P., 2003. Current status of embryo technologies in sheep and goat. *Theriogenology*, **59**: 171-188.
- COGNIE, Y., POULIN, N., LOCATELLI, Y. AND MERMILLOD, P., 2004. State-of-the-art production, conservation and transfer of *in vitro* produced embryos in small ruminants. *Reprod. Fert. Develop.*, **16**: 437-445.
- DODE, M.A.N. AND GRAVES, C.N., 2002. Involvement of steroid hormones on *in vitro* maturation of pig oocytes. *Theriogenology*, **57**: 811–821.
- DUNCAN, D.B., 1955. Multiple ranges and multiple F test. *Biometrics*, **11**: 1-42.
- GANDOLFI, F., BREVINI, T.A.L., CILLO, F. AND ANTONINI, S., 2005. Cellular and molecular mechanisms regulating oocyte quality and the relevance for farm animal reproductive efficiency. *Rev. Sci. Tech. Oie.*, **24**: 413-423.
- GILCHRIST, R.B. AND THOMPSON, J.G., 2007. Oocyte maturation: Emerging concepts and technologies to improve developmental potential *in vitro*. *Theriogenology*, **67**: 6-15.
- GULER, A., POULIN, N., MERMILLOD, P., TERQUI, M. AND COGNIE, Y., 2000. Effect of growth factors, EGF and IGF-1 and estradiol on *in vitro* maturation of sheep oocytes. *Theriogenology*, **54**: 209–218.
- HASSAN, A.M. AND ABDEL-WAHAB, M.A., 2006. Antioxidant effect of parsley and panax ginseng extract against alteration induced in reproductive functions in male mice. *Egypt. J. Hosp. Med.*, **22**: 60–72.
- HIBASAMI, H., MOTEKI, H. AND ISHIKAWA, K., 2003. Protodioscin isolated from fenugreek (*Trigonella foenum graecum* L.) induces cell death and morphological change indicative of apoptosis in leukemic cell line H-60, but not in gastric cancer cell line KATO III. *Int. J. mol. Med.*, **11**: 23–26.
- KASSEM, A., ABDULWALI, A., MOLHAM, A. AND MOHAMMED, A., 2006. Evaluation of the potential antifertility effect of fenugreek seed in male and female rabbits. *Contraception*, **73**: 301–306.
- KATSKA-KSIAZKIEWICZ, L., OPIELA, J. AND RYNSKA, B., 2007. Effects of oocytes quality, semen donor and embryo co-culture system on the efficiency of blastosyst production in goats. *Theriogenology*, **68**: 736-744.
- KAVIARASAN, S., VIJAYALAKSHMI, K. AND ANURADHA, C.V., 2004. A polyphenol-rich extract of fenugreek seed protect erythrocytes from oxidative damage. *Pl. Foods Hum. Nutr.*, **59**: 143–147.
- KHARCHE, S.D., SHARMA, G.T. AND MAJUMDAR, A.C., 2005. *In vitro* maturation and fertilization of goat oocytes vitrified at germinal vesicle stage. *Small Rumin. Res.*, **57**: 81-84.
- LV, L., YUE, W., LIU, W., REN, Y., LI, F., LEE, K. AND SMITH, G.W. 2010. Effect of oocyte selection, estradiol and antioxidant treatment on *in vitro* maturation of oocytes collected from prepubertal Boer goats. *Ital. J. Anim. Sci.*, **9**: 50 – 54.
- MAGHSOUD, P., SAEEDAH, Y.S., ALI, A.M. AND SAJAD, A., 2013. The effect of endurance training and extract of fenugreek seed on serum visfatin and vaspin levels in diabetic rats. *Annls. Biol. Res.*, **4**: 301-306.
- MARAA, L., SANNAA, D., CASUA, S., DATTENAA, M. AND MAYORGA MUÑOZ, I.M., 2013. Blastocyst rate of *in vitro* embryo production in sheep is affected by season. *Zygote*, Mar **5**: 1-6.
- MINGOTI, G.Z., GARCIA, J.M. AND ROSA-E-SILVA, A.A., 1995. The effect of serum on *in vitro* maturation, *in vitro* fertilization and steroidogenesis of bovine oocytes co-cultured with granulosa cells. *Braz. J med. biol. Res.*, **28**: 213–217.
- MINGOTI, G.Z., GARCIA, J.M. AND ROSA-E-SILVA, A.A., 2002. Steroidogenesis in cumulus cells of bovine cumulus oocyte complexes matured *in vitro* with BSA and different concentrations of steroids. *Anim. Reprod. Sci.*, **69**: 175–186.

- MOOR, R., POLGE, C. AND WILADSEN, S., 1980. Effect of follicular steroids on the maturation and fertilization of mammalian oocytes. *J. Embryol. exp. Morph.*, **56**: 319–335.
- MORTON, K.M., MAXWELL, W.M.C. AND EVANS, G., 2008. Effect of aspiration pressure during oocyte harvesting on oocyte recovery and *in vitro* development of ovine oocytes. *Reprod. Domest. Anim.*, **43**: 106–110.
- MOTLAGH, M.K., SHAHNEH, A.Z., DALIRI, M., KOHRAM, H. AND GHRAGOZLOU, F., 2008. *in vitro* maturation of sheep oocytes in different concentrations of mare serum. *Afr. J. Biotechnol.*, **7**: 3380–3382.
- MOUDGAL, N.R., SHETTY, G., SELVARAJ, N. AND BHATNAGAR, A.S., 1996. Use of a specific aromatase inhibitor for determining whether there is a role for oestrogen in follicle/oocyte maturation, ovulation and preimplantation embryo development. *J. Reprod. Fertil.*, **50**: 69–81.
- NADI, S., RAVINDRANATHA, B.M., GUPTA, P.S.P. AND SARMA, P.V., 2002. Timing of sequential changes in cumulus cells and first polar body extrusion during *in vitro* maturation of buffalo oocytes. *Theriogenology*, **57**: 1151–1159.
- NORTON, S.A., 1998. Useful plants of dermatology. III. Corticosteroids, strophanthus, and dioscorea. *J. Am. Acad. Dermatol.*, **38**: 256–259.
- PAWSHE, C.H., PALANISAMY, A., TANEJA, M., JAIN, S.K. AND TOTEY, S.M., 1996. Comparison of various maturation treatments on *in vitro* maturation of goat oocytes and their early embryonic development and cell numbers. *Theriogenology*, **46**: 971–982.
- PELLICER, A., 1997. Oestrogens and follicular and oocyte development. *Hum. Reprod.*, **3**: 93–94.
- RAO, B.S., NAIDU, K.S., AMARNATH, D., VAGDEVI, R., RAO, A.S., BRAHMAIAH, K.V. AND RAO, V.H., 2002. *in vitro* maturation of sheep oocytes in different media during breeding and non-breeding seasons. *Small Ruminant. Res.*, **43**: 31–36.
- RIZOS, D., LONERGAN, P., BOLAND, M.P., ARROYO-GARCIA, R., PINTADO, B., DELA-FUENTE, J. AND GUTIERREZ-ADAN, A., 2002. Analysis of differential messenger RNA expression between bovine blastocyst produce in different culture system: implication for blastocyst quality. *Biol. Reprod.*, **66**: 589–595.
- ROUSHANDEH, A.M., PASBAKHSH, P., ALIZADEH, Z. AND ROUDKENAR, M.H., 2007. *In vitro* maturation media, cysteamine concentration and glutathione level affect blastocysts development in mouse. *Iran. J. Reprod. Med.*, **5**: 159–163.
- RUSSO, V., MARTELLI, M., MAURO, A., DI GIACINTO, O., NARDINOCCHI, D. AND BERARDINELLI, P., 2007. Nuclear remodelling in growing oocytes of sheep. *Vet. Res. Commun.*, **31**: 201–204.
- SAEKI, K., KATO, H., HOSOI, Y., MIYAKE, M., UTSUMI, K. AND IRITANI, A., 1991. Early morphological events of *in vitro* fertilized bovine oocytes with frozen-thawed spermatozoa. *Theriogenology*, **35**: 1051–1058.
- SANTOS, L.C., RODRIGUES, B.A. AND RODRIGUES, J.L., 2006. *In vitro* nuclear maturation of bitch oocytes in the presence of polyvinyl-pyrrolidone. *Anim. Reprod.*, **3**: 70–75.
- SUR, P., DAS, M. AND GOMES, A., 2001. *Trigonella foenum graecum* (fenugreek) seed extract as an antineoplastic agent. *Phytother. Res.*, **15**: 257–259.
- TESARIK, J. AND MENDOZA, C., 1997. Direct non-genomic effects of follicular steroids on maturing human oocytes. *Hum. Reprod.*, **3**: 95–100.
- ZHENG, P., WEI, S., BARRY, D.B., JIFENG, Y., CHENGHUI, D. AND WEIZHI, J., 2003. Estradiol 17 β and progesterone improve *in vitro* cytoplasmic maturation of oocytes from unstimulated prepubertal and adult rhesus monkeys. *Hum. Reprod.*, **18**: 2137 – 2144.
- ZHU, S.X., SUN, Z. AND ZHANG, J.P., 2007. Ovine (*Ovis aries*) blastula from an *in vitro* production system and isolation of primary embryonic stem cells. *Zygote*, **15**, 35–41.

(Received 30 August 2013, revised 24 September 2013)