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Effects of Growth Hormone on Nuclear Maturation of Ovine Oocytes and Subsequent Embryo Development

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Contents

The objective of this study was to determine the effect of the presence of recombinant ovine growth hormone either alone or together with follicle stimulating hormone (FSH) during ovine oocyte *in vitro* maturation (IVM) on nuclear maturation and subsequent embryo development. Moreover, the effect of growth hormine (GH) on embryo development whether influenced by the presence of foetal bovine serum (FBS) was assessed. The abattoir-derived oocytes were randomly divided into four treatment groups and cultured in maturation medium supplemented with: (i) 0.05 IU/ml FSH; (ii) 300 ng/ml roGH; (iii) FSH + roGH; and (iv) no FSH and GH (control). The percentages of germinal vesicle-stage oocytes in GH-treated group after 8 h of culture was significantly higher than the FSH and FSH + GH groups and lower than control (22.4%, 8.7%, 9.1%, and 32% respectively). The percentage of MII-stage oocytes was significantly increased in the presence of GH after 16 and 24 h of culture compared to the control (44.7% and 83.1% vs 32.6% and 73.6% respectively). There was no significant synergism between GH and FSH in terms of nuclear maturation. The blastocyst rates in serum-supplemented groups were enhanced by the presence of FSH and GH compared to the control (35.4% and 31.3 vs 11.4% respectively). Compared with either GH or FSH alone, the subsequent embryo development (blastocyst rate), however, was negatively influenced by co-presence of both hormones (22.8%). In contrast, the corresponding values were not affected in the absence of serum. In conclusion, GH had positive effect on nuclear maturation of sheep oocytes. Moreover, the pattern of the effect of GH on embryo development was influenced by the presence of FBS during IVM.

Introduction

Over recent years, there have been many studies done in an attempt to determine what conditions are needed during the *in vitro* maturation, fertilization and culture processes to maximize embryo production. The maturational (cytoplasmic and nuclear) and developmental (fertilization, pronuclei formation and cleavage) competencies of oocytes are influenced by various factors. Regarding to that, follicular and oviductal fluids, many types of serum, steroids, gonadotropins and the size of the follicle from which the cumulus-oocyte complexes (COCs) is harvested (Vatzias and Hagen 1999) have been of great concern. Besides these factors, the role of growth hormone (GH) supplementation in the maturation media has been studied in several species including the bovine, porcine, equine and canine in *in vitro* embryo production systems (Izadyar et al. 1996, 1997, 1998a; Songsasen and Leibo 2002; Marchal et al. 2003). It has been indicated that the stimulatory effects of GH on oocyte maturation are correlated with changes in the synthesis of gap junction proteins (Kolle et al. 2003) and that its stimulatory effects are exerted through cumulus cells (Yoshimura et al. 1993, 1994). Based on *in vitro* studies, GH may enhance oocyte quality by accelerating and coordinating cytoplasmic (e.g. the ability to decondense sperm chromatin, sub-oolemal migration of cortical granules, sperm aster formation and blastocyst formation) and nuclear maturation (complete meiosis I and undergo zygote cleavage and blastocyst formation more frequently) in GH-treated cell populations (Hyttel et al. 1989; Van der Westerlaken et al. 1994; Izadyar et al. 1996, 1997, 1998a,b). Accordingly, the fertilization rate in bovine oocytes *in vitro* is increased by incubation with GH (Izadyar et al. 1998a). Growth hormone also stimulates nuclear maturation of fox, rabbit, equine, and pig oocytes (Hagen and Graboski 1990; Apa et al. 1994b; Kalous et al. 1998) but had no effect on porcine fertilization and embryo development (Marchal et al. 2003).

In human, oocytes harvested from follicles with high antral fluid GH concentrations are more fertile than those from follicles with low GH concentrations and amongst fertilized oocytes, intrafollicular GH concentration is positively related to normal subsequent cleavage and morphological functions of the cleaved embryos by enhancing or acting in synergy with gonadotrophin-controlled developmental processes (Mendoza et al. 1999).

In ewe, there is evidence indicating that GH may play an important role in early stages of folliculogenesis and that it is involved in the maintenance of sensitivity to gonadotropins. In this species, the mRNA for GH receptor has been detected in the membrana granulosa and oocytes of small antral and preantral follicles (Eckery et al. 1997).

The stimulatory and acceleratory effects of FSH and LH on the meiotic maturation of the oocyte, and selective improvement of cytoplasmic maturation by LH have been well documented (Moor and Trounson 1997; Galli and Moor 1991; Mattioli et al. 1991).

To date, no study has been done regarding to the effect of GH on maturational and developmental competence of the ovine oocytes cultured *in vitro*. The aim of the current study was to determine the effect of GH supplementation during *in vitro* maturation of ovine oocytes on nuclear maturation and subsequent embryo development. Since gonadotrophins are routinely added to maturation media, and because of evidence available on the possible interaction between gonadotrophins and GH, the possible synergism between FSH and GH on ovine oocytes nuclear maturation and subsequent embryo development was also evaluated. Since the

beneficial effect of GH on embryo development has been evaluated in chemically defined medium (Izadyar et al. 2000), the effect of GH on ovine embryo development whether was influenced by the presence of FBS, was also assessed.

Materials and Methods

Except where otherwise indicated, all chemicals were obtained from the Sigma (St Louis, MO, USA).

Oocyte collection

Adult and pre-pubertal sheep ovaries were collected during the non-breeding season (June to August) from a local slaughterhouse (Latitude: 32′17″N; Longitude 50′51″E; Altitude 2049 m) and transported to the laboratory in saline (30–35°C) in a thermos flask, within 1–3 h following collection. Ovaries were washed three times with pre-warmed fresh saline (37°C), and all visible follicles with a diameter of 2–6 mm were aspirated using gentle vacuum (30 mmHg) via a 20 gauge short beveled needle connected to a vacuum pump.

Prior to aspiration, the collecting tube was filled with 2 ml pre-incubated Hepes-modified tissue culture medium (TCM), supplemented with 50 IU/ml heparin.

In vitro maturation

After aspiration, only oocytes with evenly granulated cytoplasm surrounded by more than three layers of unexpanded cumulus cells (COCs) were recovered and selected for *in vitro* maturation (IVM). From totally 3680 ovaries, 5518 COCs were selected for IVM (1.5 COCs/ovary). Before culturing, oocytes were washed in Hepes-buffered TCM199 (H-TCM199) supplemented with 5% FBS (Fetal bovine serum, Gibco 10270; Gibco, Paisley, UK), and 2 mM glutamine. The oocyte culture medium (OCM) consisted of bicarbonate-buffered TCM 199 with 2 mM L-glutamine supplemented with 0.02 mg/ml cysteamine, 1 IU/ml hCG, 1 μg/ml E2, 100 IU/ml penicillin, 100 μg/ml streptomycin, 10% FBS (Fetal bovine serum, Gibco 10270), and 0.2 mM Na-Pyrovate. The medium was adjusted to 275 mOsm. The selected COCs were pooled and randomly distributed in four experimental groups. In each experimental group the selected COCs were placed in maturation droplets (15 oocytes/50 μl) and covered by sterile paraffin oil in a 60-mm Petri dish (Falcon 3004; Becton & Dickinson, Franklin Lakes, NJ, USA) and were then incubated under an atmosphere of 5%CO_2 95% air with 100% humidity at 39°C for 24 h.

Experimental groups

The selected oocytes were randomly allocated to four treatment groups. Each treatment was consisted of at least six replicates.

Group control: Cumulus enclosed oocytes cultured in OCM without FSH and GH Group FSH: Cumulus enclosed oocytes cultured in OCM supplemented with 0.05 IU/ml oFSH (From sheep pituitary, F8174) Group GH: Cumulus enclosed oocytes cultured in OCM supplemented with 300 ng/ml recombinant ovine growth hormone (roGH) (ProSpec-Tany Techno Gene Ltd, Rehovot, Israel) Group FSH + GH: Cumulus enclosed oocytes cultured in OCM supplemented with oFSH +roGH In the first course of experiment, the assessment of nuclear maturation in each experimental group was carried out in serum-supplemented OCM and in the second round the assessments of subsequent *in vitro* embryo development was done in both serum-supplemented and serum-free OCM.

Preparation of sperm and *in vitro* fertilization

After IVM, the oocytes were washed four times in HSOF [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-synthetic oviductal fluid] and once in fertilization medium and were then transferred into the fertilization droplets. Fresh semen was collected from a Lori-Bakhtiari ram of proven fertility. For swim up, 80–100 μl of semen was kept under 1 ml of BSA-HSOF in 15 ml conical tube at 39°C for up to 45 min. After swim up, the 700–800 μl of the supernatant were then added to 3 ml of BSA-HSOF, centrifuged twice at $200 \times g$ for 3 min and the final pellet was re-suspended with BSA-HSOF. Oocytes were inseminated with 1.0×10^6 normal, motile spermatozoa/ml. The fertilization medium was synthetic oviductal fluid (SOF), as originally described by Tervit et al. (1972), enriched with 20% heat inactivated estrous sheep serum. A 5 μl aliquot of sperm suspension, 1.0×10^6 sperm/ml, was added into the fertilization droplets (45 μl) containing 10 oocytes. Fertilization was carried out by co-incubation of sperm and oocytes in an atmosphere of 5% CO_2 in humidified air at 39°C for 22 h.

In vitro culture

After *in vitro* fertilization, presumptive zygotes were vortexed for 2–3 min to remove the cumulus cells and then washed in H-SOF to remove spermatozoa and cellular debris. They were then allocated to the 20 μl culture drops (five to six embryos/drop) containing SOF supplemented with 2% (v/v) basal medium eagle-essential amino acids, 1% (v/v) minimum essential medium-non-essential amino acids, 1mM glutamine and 8 mg/ml fatty acid free BSA and subsequently were cultured at 39°C under conditions of 7% O_2, 5% CO_2 and 88% N_2 in humidified air. On the third and fifth day of culture (Day 0 defined as the day of fertilization) 10% charcoal stripped FBS was added to the medium. The osmolarity was maintained at 270–285 mOsmol. The percentage of cleaved embryos at day 3 and the percentage of blastocysts at day 7 were expressed on the basis of the number of oocytes at the onset of culture, and the percentage of hatched blastocysts at day 8 expressed on the basis of the total number of blastocysts present at day 7.

Assessment of nuclear maturation

After culture, the nuclear status of the oocytes was determined by 4,6-diamino-2-phenylindole (DAPI) staining as described by Mori et al. (1988). Briefly, COCs were denuded by vortexing for 3 min and then fixed for

15 min in 2.5% (w/v) glutaraldehyde, washed with phosphate buffered saline, stained with 1 μg/ml DAPI, and mounted on slides. The nuclear state of the stained oocytes was assessed under a fluorescence microscope (Axiolab, Carl Zeiss, Germany). Oocytes in which diffuse chromatin could be identified were classified as being in the germinal vesicle (GV) stage. Oocytes possessing slightly condensed or clumped chromatin were classified as being in the germinal vesicle breakdown (GVBD) stage. Oocytes with strongly condensed chromatin that formed an irregular network of individual bivalents (prometaphase), or a metaphase plate but no polar body, were classified as being in metaphase-I (MI) stage, and oocytes with either a polar body or two shiny chromatin spots were classified as being in metaphase-II (MII) stage of the maturation process. The damages oocytes were excluded from analysis.

Statistical analysis

Data were collected over at least six replicates. Differences among groups were analysed using one-way ANOVA by SPSS (version 11.5) after arcsine transformation of the proportional data of nuclear maturation, cleavage, developing to the blastocyst stage and hatched blastocyst. Comparison of means among groups was performed using Fisher least significant difference method. When equal variance test was failed, the treatments were compared by Student–Newman–Keuls Method. When normality test was failed the Kruskal–Wallis on ANOVA ranks was applied. Differences were considered significant when $p < 0.05$. Data were expressed as mean ± SEM.

Results

The effect of the addition of 300 ng/ml roGH to the maturation media alone or in combination with FSH on nuclear maturation compared to the control group is depicted on Tables 1, 2, 3. At 8 h of culture, though the percentage of GV stage oocytes in GH treated group was significantly lower than control, the percentage was significantly higher than the FSH and FSH + GH groups ($p < 0.001$). The high percentage of GV oocytes was reflected the slightly lower percentage of condensed oocytes in GH treated group compared with FSH and FSH + GH groups (Table 1). When COCs were cultured for 16 and 24 h, the percentage of oocytes that

Table 1. Nuclear maturation status of sheep oocytes after 8 h culture in four different groups

Experimental groups	Cultured oocytes no.	Nuclear division stage			
		GV	GVBD	Condensed	MI
		n (mean ± SEM)			
Control	173	55(32.0 ± 3.8)[a]	26(15.0 ± 2.2)[a]	91(52.5 ± 5.1)[a]	1(0.5 ± 0.4)
FSH	170	14(8.7 ± 2.5)[b]	24(14.6 ± 1.2)[a,b]	130(76.2 ± 2.4)[b,c]	2(1.1 ± 0.5)
GH	192	45(22.4 ± 3.8)[c]	17(9.1 ± 2.0)[b,c]	126(66.4 ± 4.8)[b]	4(2.0 ± 0.7)
FSH + GH	142	13(9.1 ± 2.1)[b]	10(6.7 ± 2.5)[c]	117(82.8 ± 4.0)[c]	2(1.3 ± 0.9)

GV, germinal vesicle; GVBD, germinal vesicle breakdown; MI, metaphase I; GH, growth hormone.
Numbers with different superscripts letters in the same column differ significantly ($p < 0.001$).

Table 2. Nuclear maturation status of sheep oocytes after 16 h culture in four different groups

Experimental groups	Cultured oocytes no.	Nuclear division stage			
		GV	GVBD	MI	MII
		n (mean ± SEM)			
Control	143	29(21.2 ± 5.0)	9(5.8 ± 1.3)	57(40.3 ± 1.5)	48(32.6 ± 4.9)[a]
FSH	250	26(10.9 ± 1.6)	27(10.3 ± 2.4)	78(31.2 ± 2.4)	19(47.7 ± 2.8)[b]
GH	281	36(12.6 ± 1.5)	31(10.9 ± 2.6)	88(31.6 ± 2.6)	126(44.7 ± 3.1)[b]
FSH + GH	207	16(7.5 ± 1.3)	15(8.0 ± 2.7)	66(32.1 ± 1.7)	110(52.4 ± 3.3)[b]

GV, germinal vesicle; GVBD, germinal vesicle breakdown; MI, metaphase I; MII, metaphase II; GH, growth hormone.
Numbers with different superscripts letters in the same column differ significantly ($p < 0.01$).

Table 3. Nuclear maturation status of sheep oocytes after 24 h culture in four different groups

Experimental groups	Cultured oocytes no.	Nuclear division stage			
		GV	GVBD	MI	MII
		n (mean ± SEM)			
Control	188	22(11.9 ± 2.7)	8(4.4 ± 1.3)	9(10.1 ± 6.1)	139(73.6 ± 3.8)[a]
FSH	195	8(3.50 ± 1.2)	7(3.8 ± 0.9)	12(5.5 ± 2.0)	168(87.5 ± 2.9)[b]
GH	206	13(6.20 ± 2.6)	8(4.1 ± 1.5)	14(6.6 ± 3.2)	171(83.1 ± 2.7)[b]
FSH + GH	177	7(3.80 ± 1.2)	4(2.3 ± 0.8)	10(5.6 ± 5.5)	156(88.9 ± 2.7)[b]

GV, germinal vesicle; GVBD, germinal vesicle breakdown; MI, metaphase I; MII, metaphase II; GH, growth hormone.
Numbers with different superscripts letters in the same column differ significantly ($p < 0.01$).

Table 4. Effect of growth hormone in serum-containing oocyte maturation medium on subsequent *in vitro* ovine embryo development.

Experimental groups	Presumptive zygotes no.	Embryos in developmental stage		
		Cleaved	Blastocyst	Hatched blastocyst*
		n (mean ± SEM)		
Control	447	222(50.3 ± 2.7)[a]	50(11.4 ± 1.8)[a]	24(42.3 ± 6.8)[a]
FSH	628	508(80.0 ± 1.5)[b]	227(35.4 ± 1.4)[b]	173(75.7 ± 1.4)[b]
GH	618	415(67.2 ± 2.9)[c]	192(31.3 ± 1.1)[b]	144(73.4 ± 3.2)[b]
FSH + GH	446	272(60.5 ± 1.7)[c]	103(22.8 ± 1.1)[c]	76(73.2 ± 3.2)[b]

Numbers with different superscripts letters in the same column differ significantly ($p < 0.001$).
*The percentage of hatched blastocysts on day 8 expressed on the basis of the total number of blastocysts present at day 7.

Table 5. Effect of growth hormone in serum-free oocyte maturation medium on subsequent *in vitro* ovine embryo development

Experimental groups	Presumptive zygotes no.	Embryos in developmental stage		
		Cleaved	Blastocyst	Hatched blastocyst*
		n (mean ± SEM)		
Control	318	177(56.1 ± 1.3)[a]	65(20.4 ± 1.5)[a]	29(48.3 ± 5.5)
FSH	314	198(62.7 ± 3.5)[a,b]	81(26.0 ± 1.7)[b]	45(53.5 ± 8.7)
GH	314	181(57.7 ± 1.2)[a,b]	72(23.1 ± 1.7)[a,b]	35(52.2 ± 6.2)
FSH + GH	334	213(63.8 ± 3.6)[b]	90(27.0 ± 1.5)[b]	55(60.5 ± 6.9)

Numbers with different superscripts letters in the same column differ significantly ($p < 0.05$).
*The percentage of hatched blastocysts on day 8 expressed on the basis of the total number of blastocysts present at day 7.

reached MII stage was significantly increased in the all hormonally treated groups compared with control oocytes ($p < 0.01$, Tables 2, 3).

Addition of 300 ng/ml GH in serum-containing maturation medium during IVM was significantly increased embryo development in terms of cleavage, blastocyst, and hatching rates compared to the control (Table 4). In contrast, GH supplementation, 300 ng/ml, in serum-free oocyte maturation medium had no effect on subsequent cleavage, blastocyst, and hatching rate compared to the control (Table 5).

Discussion

As demonstrated, in the present study the presence of GH in the culture media, either alone or in combination with FSH, had a positive effect on the *in vitro* nuclear maturation of ovine oocytes. The pattern of this positive effect became more evident as the time exposure of the oocytes to GH was increased. Exposure of ovine oocytes to GH during the first 8 h of IVM could significantly enhance the nuclear maturation in GH-treated oocytes compared with control. The percentage of oocytes progressing beyond the GV, however, was greater in FSH treated oocyte after 8 h compared with GH and control groups ($p < 0.001$). This indicates that in ovine oocytes, FSH is more effective in acceleration of nuclear maturation than GH, in terms of passage beyond GV and progression to GVBD and condensed chromatin. Although, the difference in positive effect of GH and FSH on sheep oocyte nuclear maturation (MII stage oocytes) was insignificant after 16 and 24 h of culture. The nuclear maturation of oocytes, however, was significantly increased in both GH and FSH groups compared with control. While this finding confirms the positive effect of GH on nuclear maturation of bovine oocytes (Izadyar et al. 1997, 1998a; Iga et al. 1998), it is in contrast to the transient inhibitory role of FSH on bovine nuclear maturation (Izadyar et al. 1998b). The type of FSH used in the current study (pituitary oFSH) which could be contaminated with other adenohypophyseal hormones and what had been used by Izadyar (rhFSH) could be the reason for the observed discrepancy between the current result and what has been reported in bovine oocytes. Indeed, contamination with the other adenohypophyseal hormones, probably, could neutralize the transient inhibitory effect of FSH on nuclear maturation. Whether there is species specific difference between ovine and bovine oocytes in response to the FSH, in term of nuclear maturation, could be another possibility. Meanwhile, the source and the amount of GH used in the current study (roGH, 300 ng/ml) was different from that of used in cattle (pituitary bovine GH, 100 ng/ml; Izadyar et al. 1996). Based on our preliminary experiment, compared with 100, 200, 300 and 400 ng/ml of roGH (A. Shirazi, N. Shams-Esfandabadi, E. Ahmadi and B. Heidari, unpublished data); 300 ng/ml was the optimal dose. Furthermore, despite of the presence of the mRNA for GH receptor in the membrana granulosa and oocytes of small antral and preantral follicles (Eckery et al. 1997), the pattern of positive effect of GH on oocyte maturation whether exerted directly on oocyte or indirectly through cumulus cells has yet to be determined in ovine species.

In the current study the positive effect of GH on nuclear maturation of sheep oocytes was also in agreement with the results in rat (Apa et al. 1994b), Blue fox (Srsen et al. 1998), and equine oocytes (Marchal et al. 2003). The synergistic action between GH and gonadotrophs, as reported in nuclear maturation rate of porcine oocytes *in vitro* was not evident in nuclear maturation of sheep oocytes which was in agreement with bovine oocytes (Izadyar et al. 1998a).

In serum supplemented groups, the cleavage rate was significantly higher in all those hormonally treated groups, including GH-treated group, compared to the control. This was in agreement with the results in cattle and human (Izadyar et al. 1996; Hassan et al. 2001) and was in contrast to the results in domestic cat (Schramm and Bavister 1995). When this comparison was carried out in the absence of serum, the cleavage rate was not influenced by the presence of GH, while GH together with FSH was significantly increased the cleavage rate compared to the control. Since the percentage of cleavage in FSH-treated group was similar to the corresponding percentage in FSH + GH group, to draw a conclusion that whether there is a synergistic effects between two hormones should cautiously be noticed. In contrast, in the presence of serum not only there was no evidence of synergistic effect between two hormones but also the percentage was decreased to less than the corresponding value in either FSH-treated ($p < 0.001$) or GH-treated groups. Indeed, in serum containing medium, either FSH or GH alone was more effective on cleavage rate than in combination.

In serum supplemented groups while the difference in blastocyst percentages between FSH and GH-treated groups was insignificant, the corresponding value in GH-treated group was higher than FSH + GH group ($p < 0.001$). In serum free groups the blastocyst rates were almost followed the same pattern as cleavage rate, and there was no significant difference among hormonally treated groups. While the difference in hatchability rates of blastocysts in serum free groups was insignificant, which was in accordance with bovine oocytes (Izadyar et al. 1996), the hatchability rates were positively ($p < 0.001$) influenced by the presence of serum in hormonally treated groups compared to the control.

In serum supplemented groups, the lower rates of cleavage and blastocyst in FSH + GH-treated oocytes compared with FSH and GH groups could be explained by the hypothesis that GH may induce more steroidogenesis by potentiating gonadotrophin action on cumulus cells which in turn negatively influence the embryo development. There is evidence indicating the presence of synergistic action between GH and gonadotrophs in human (Carlsson et al. 1992; Lanzone et al. 1992) and in pig (Hsu and Hammond 1987) granulosa cell steroidogenesis. Gonadotrophins and GH act synergistically to increase oestradiol and progesterone synthesis in human (Carlsson et al. 1992; Lanzone et al. 1992) and rat granulosa cells, respectively (Hsu and Hammond 1987). This synergy may reflect upregulation of gonadotrophin receptors by GH or the upregulation of growth hormone releasing hormones by gonadotrophin induced cAMP (Adashi et al. 1994; Hull and Harvey 2001). Progesterone production from human (Lanzone et al. 1992; Doldi et al. 1996), rat (Jia et al. 1986; Hong and Herington 1991; Apa et al. 1994a), bovine (Langhout et al. 1991; Spicer et al. 1993; Liebermann and Schams 1994) and sheep (Wathes et al. 1995) luteal cells are similarly enhanced by GH. Although it is recognized that oocytes require a specific steroid environment to achieve full maturation and developmental competence *in vivo* (Silva and Knight 2000), it seems in *in vitro* condition the importance of presence, absence and/or precise balance of steroids during maturation of oocyte, is more critical for its normal maturation and subsequent development. For instance, in bovine, the presence of E2 during IVM had a detrimental effect (both in denuded and cumulus enclosed oocytes) on the nuclear maturation (abnormal dispersion of chromosomes), and on subsequent embryo development (Beker et al. 2002). In contrast, in rhesus monkey, formation of morula and blastocysts is greatest in oocytes matured in medium containing estradiol and/or progesterone (Zheng et al. 2003).

In canine E_2 or P_4 alone significantly increased maturation of oocyte to MII and that P_4 supplementation with E_2 further promote or decrease oocyte maturation compared to E_2 alone depending on P_4 concentration (Kim et al. 2005). There is also evidence to indicate that in bovine, testosterone increases oocyte cleavage while progesterone reduces by approximately 40% ($p < 0.05$) the proportions of both total oocytes and cleaved oocytes that form blastocysts (Silva and Knight 2000).

In the current study, this question arise that why the negative synergism between GH and FSH on embryo development is exerted in serum supplemented groups. Whether in serum there is factor(s) which could potentiate the effect of both hormones in term of steroidogenesis or production of other factors that could finally derange the precise balance of components in culture medium and led to the improper embryo development, need to be further investigated.

Concerning to the molecular aspects of the effect of GH on oocyte maturation, it has been reported that insulin-like growth factor-I (IGF-I), produced by the cumulus cells, mediate several GH functions (Iga et al. 1998). There is, however, evidence indicating that in cattle the positive effect of GH is mediated via cAMP but not IGF-I (Izadyar et al. 1997). Considering the mediatory role of cAMP and the fact that FSH and GH use cAMP as a second messenger (Izadyar et al. 1998b), two questions could be propounded. Whether the production capacity of cellular cAMP is limited and in co-presence of FSH and GH, how extent the effects of those hormones on subsequent embryo development could be influenced. Meanwhile, whether in co-presence of both hormones, there is a synergistic effect as an extra production of cellular cAMP, which in turn could negatively influence the oocyte maturation processes.

It could be inferred that in the absence of serum the proposed synergistic effects between two hormones is less pronounced and just lead to the moderate increase in production of cellular cAMP which is beneficial rather than detrimental on oocyte maturation and subsequent embryo development. In contrast, in the presence of serum the proposed synergistic effects on production of cAMP is quite pronounced which in turn would be detrimental rather than beneficial on oocyte maturation and subsequent embryo development.

Concerning to the probable interaction between hCG and GH, there is evidence indicating the synergistic interaction between two hormones in production of progesterone by cultured human luteal cells (Lanzone et al. 1992) and stimulation of androgen synthesis by

GH in luteinizing hormone (LH) responsive, but not LH-resistant, bovine thecal cells (Spicer and Stewart 1996). In the current study, however, because of the presence of the hCG in all treatment groups and the absence of luteal cells, the difference in cleavage and blastocyst formation rates in serum-containing groups could not be due to the interaction between roGH and hCG. Taken together, it seems the improper microenvironment of COCs in serum-containing maturation medium induced by synergistic action between GH and FSH, has a negative influence on embryo development in terms of cleavage and blastocyst rate in FSH + GH-treated oocytes compared with either FSH or GH groups.

In conclusion, we infer that IMV of ovine oocytes in the presence of GH enhances nuclear maturation and subsequent embryo development. In addition, supplementation of GH and FSH during IMV not only does not result in an extra increase in the number of matured oocytes and blastocysts but also decreases the number of blastocysts in serum supplemented medium.

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Author contribution

A. Shirazi responsible for the design of study and analysing the data. E. Ahmadi and B. Heidari conducted the study. A. Shirazi and N. Shams-Esfandabadi helped in paper drafting.

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