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Effects of Sperm Pretreatments and *In vitro* Culture Systems on Development of *In vitro* Fertilized Embryos Derived from Prepubertal Boer Goat Oocytes in China*

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ABSTRACT : Use of oocytes from prepubertal animals for *in vitro* embryo production holds potential application for reducing generation intervals and increasing genetic progress through embryo transfer. The objective of these studies was to compare the effect of three sperm pretreatments (prior to *in vitro* fertilization) and seven embryo culture protocols on fertilization rate and (or) subsequent development of *in vitro* fertilized embryos derived from oocytes harvested from ovaries of 1-6 month old prepubertal Boer goats in China. Cleavage rates were highest for embryos fertilized with heparin-treated versus calcium ionophore- or caffeine-treated sperm. Similar rates of blastocyst development were observed using heparin- and ionophore-treated sperm, which were higher than obtained with caffeine-treated sperm. No differences in cleavage or blastocyst rates were observed following embryo culture in basal medias (synthetic oviductal fluid (SOF), Charles Rosenkrans 1 (CR1) or tissue culture medium-199 (TCM-199)) containing 10% fetal bovine serum (FBS). Cumulus or oviductal cell co-culture did not enhance cleavage or blastocyst rates relative to culture in SOF+10% FBS. Replacement of FBS in SOF medium with 0.3% BSA increased cleavage rates, but did not increase rates of blastocyst development. Sequential culture in SOF+0.3% BSA followed by SOF+10% FBS increased blastocyst yield versus continuous culture in SOF+10% FBS and tended to increase blastocyst yield versus continuous culture in SOF+0.3% BSA. These results demonstrate a pronounced effects of sperm pretreatments and *in vitro* embryo culture systems on rates of blastocyst development and provide a potential protocol (sperm pretreatment with heparin and sequential embryo culture in SOF+0.3% BSA followed by SOF+10% FBS) for generation of the significant numbers of *in vitro* produced blastocysts from oocytes of prepubertal Boer goats necessary for application of embryo transfer in rural regions of China for distribution of Boer goat genetics. (**Key Words** : Embryo Culture, *In vitro* Fertilization, Oocyte, Prepubertal Goat, Sperm Pretreatment)

INTRODUCTION

Goats are a prominent food source in many developing countries, including China. Goat inventories in China are estimated at over 1×10^9 animals (Yonghong, 2002). The vast majority of such animals represent indigenous breeds. Boer goats were introduced into China in 1995 and offer advantages in terms of size, meat quality, disease resistance and other production traits relative to native indigenous breeds (Yonghong, 2002). Indigenous breeds are predominant in the rural, less developed agricultural areas in China where introduction of Boer goat genetics would have significant impact. However, practical and effective strategies for distribution of Boer goat genetics in China have not been widely implemented.

Previous studies have focused on the use of MOET (multiple ovulation embryo transfer) technology (Mishra et al., 2003; Mishra et al., 2004; Chang et al., 2006) as a means of propagation of Boer goat genetics. Cloning of high genetic merit goats by somatic cell nuclear transfer represents another potential approach (Park et al., 2007). However, our long-term goal is to develop effective procedures for *in vitro* production and subsequent transfer of embryos derived from oocytes of prepubertal Boer goats

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in China. The use of prepubertal animals as embryo donors holds significant potential for reducing generation intervals and increasing the rate of genetic gain through embryo transfer (Duby et al., 1996). Ovaries collected from such prepubertal animals at slaughter represent a readily available source of oocytes for *in vitro* embryo production that would otherwise be discarded. Production of Boer goat embryos *in vitro* and transfer into recipient females of indigenous breeds in rural areas represents one potential strategy for genetic improvement.

Despite the fact that initial reports of live births following *in vitro* oocyte maturation, *in vitro* fertilization and *in vitro* culture of resulting goat embryos occurred over 14 years ago (Keskintepe et al., 1994a), and common use of goat embryos for biotechnological applications (Baldassarre et al., 2004; Vajta and Gjerris, 2006), studies of *in vitro* embryo production in the goat are less prevalent than other food animal species (Cognie et al., 2003) and the efficiency of *in vitro* embryo production in general is far from ideal (Lonergan and Fair, 2008). Evidence indicates that *in vitro* embryo developmental potential in the goat and other species can be significantly impacted by sperm pretreatments prior to *in vitro* fertilization and *in vitro* culture systems (Cognie et al., 2003; Marquez and Suarez, 2004), but efficient systems for *in vitro* embryo production from prepubertal goat oocytes are not well established. For example, previous studies demonstrated that pretreatment of caprine spermatozoa with calcium ionophore treatment was more effective than heparin or caffeine pretreatment in promoting ability of spermatozoa to undergo the acrosome reaction (Pereira et al., 2000; Ho et al., 2001). Furthermore, differences in composition of the basal embryo culture medium (Congie et al., 2003; Gardner, 2008), coculture with somatic cells (Kane et al., 1992), and addition of serum (Gutierrez-Adan et al., 2001) have been shown to positively influence rates of embryonic development *in vitro* in various species. However, to our knowledge, direct side by side comparisons of the impact of above treatments on fertilization rates and (or) cleavage rates and subsequent blastocyst rates following *in vitro* fertilization of oocytes from prepubertal goats have not been reported. We hypothesize that different sperm pretreatments prior to *in vitro* fertilization, composition of basal embryo culture medium and embryo culture in the presence of somatic cells or serum supplementation will influence fertilization rates and (or) *in vitro* development of embryos derived from prepubertal Boer goat oocytes. The objectives of the present studies were to directly compare the impact of three sperm pretreatments (heparin, versus caffeine versus calcium ionophore A23187) prior to *in vitro* fertilization, composition of basal culture medium (synthetic oviductal fluid (SOF) versus, Charles Rosenkrans 1 (CR1) versus tissue culture medium-199 (TCM-199) and somatic cell (cumulus versus oviductal) coculture versus serum supplementation on fertilization and (or) cleavage and blastocyst rates following *in vitro* fertilization of oocytes from prepubertal Chinese Boer goats that were matured *in vitro*.

MATERIALS AND METHODS

Collection of cumulus-oocyte complexes (COCs)

Ovaries of 1-6 month old Boer goats with no visible corpora lutea were harvested at an abattoir and transported to the laboratory at 20-30°C in sterile 0.9% NaCl containing 0.3 mg/ml gentamicin (Hua Bei Medicine Factory, Shijiazhuang City, China). Upon return to the laboratory (within 3 h), ovaries were washed three times in sterile 0.9% NaCl and two times in phosphate buffered saline (DPBS, Ca^{2+} and Mg^{2+} free) containing 1 mg/ml L-glucose, 0.1 mg/ml penicillin (Hua Bei Medicine Factory), 0.1 mg/ml streptomycin (Hua Bei Medicine Factory) and 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) at 38.5°C. Oocytes were liberated from all visible 1-6 mm follicles. Grade 1 cumulus oocyte complexes (COCs) with at least three complete and compact layers of cumulus cells and an evenly granulated cytoplasm were selected for *in vitro* maturation under a stereomicroscope. The COCs were then washed three times in DPBS and two times in maturation medium (TCM 199/Hepes; Invitrogen) containing 2.2 mg/ml sodium bicarbonate, 0.05 mg/ml gentamicin, 10 μg/ml FSH (Ning Bo Hormone Factory, China), 10 μg/ml LH (Ning Bo Hormone Factory), 1 μg/ml estradiol (Sigma; St. Louis, MO USA) and 10% FBS and incubated under a humidified atmosphere of 5% CO_2 at 38.5°C for 27 h. Only COCs with an expanded cumulus layer were selected for subsequent *in vitro* fertilization (IVF) and used in described experiments.

Sperm preparation

All experiments were conducted using frozen/thawed semen from mature male Boer goats. Straws of semen were thawed in a water bath at 37°C for 20 s and evaluated for motility, viability and acrosome integrity under an inverted microscope. For each IVF run, approximately 50 μl of frozen/thawed semen were layered onto a 45-90% discontinuous Percoll gradient in Brackett and Oliphant (BO) medium (Brackett and Oliphant, 1975) supplemented with 50 μg/ml gentamicin and 6 mg/ml bovine serum albumin (BSA; Sigma) and centrifuged (200 g) for 15 min at 25°C. The sperm pellet was then washed three times in BO medium prior to initiation of sperm pretreatments.

Pretreatment of spermatozoa prior to *in vitro* fertilization

Upon completion of Percoll gradient purification, the

resulting sperm pellets were re-suspended in BO medium and diluted 1:1 in BO medium containing 50 μg/ml heparin (Sigma; Experiments 1-3), 10 mM caffeine (Sigma; Exp. 1) or 0.1 μM of the calcium ionophore A23187 (Sigma; Exp. 1), respectively. Sperm were incubated for 1-2 h at 38.5°C under a humidified atmosphere of 5% CO_2 until used in IVF.

***In vitro* fertilization**

After sperm pretreatment, sperm concentration was assessed using a hemocytometer and groups of 14-16 COCs transferred to 100 μl drops of BO medium (under paraffin oil). Aliquots of sperm suspension were added to drops to achieve a final sperm concentration of 5×10^6 live cells/ml. *In vitro* fertilization was allowed to occur under a humidified atmosphere of 5% CO_2 at 38.5°C for 24 h.

Preparation of goat cumulus and oviduct cell monolayers for embryo co-culture

For preparation of cumulus cell monolayers for embryo co-culture, COCs were aspirated from 1-6 mm follicles of ovaries of prepubertal animals and placed in droplets of 0.1% hyaluronidase (Sigma) in DPBS for three minutes. Repeated pipetting through a fine glass pipette was then used to release and monodisperse cumulus cells. After removal of oocytes from droplets, cell suspensions were collected and cells washed three times in synthetic oviductal fluid (SOF) medium containing 10% FBS. After washing, cell numbers were determined, and cells plated at a concentration of 3×10^6 cells/ml in 50 μl drops under paraffin oil in a humidified atmosphere of 5% CO_2 at 38.5°C. Cumulus cells were cultured for two days before addition of embryos for co-culture experiments.

For oviductal cell co-cultures, oviductal tissue that remained attached to ovaries at collection was dissected free of surrounding mesosalpinx, washed four times in DPBS and opened with fine scissors to expose the lumen. Epithelial cells were harvested by scraping the luminal surface of oviductal tissue and cells monodispersed in 0.25% trypsin (Sigma) for 3-5 minutes. Cells lysates were harvested and cell pellets washed three times in SOF medium containing 10% FBS. Cell numbers were then determined and cells plated at a concentration of 3×10^6 cells/ml in 50 μl drops under paraffin oil in a humidified atmosphere of 5% CO_2 at 38.5°C. Oviductal cells were cultured for three days before addition of embryos for co-culture experiments.

Embryo culture

Following 24 h of sperm exposure, oocytes were washed in DPBS three times to remove corona cells and attached sperm cells by repeated pipetting, washed two times in embryo culture medium, then 12 to 14 presumptive zygotes were transferred into 50 μl drops of embryo culture medium or 50 μl drops containing cumulus or oviductal cells for embryo co-culture (treatments described below under individual experiments). All drops were covered by paraffin oil. In experiment 1, presumptive zygotes were examined under a stereomicroscope for evidence of fertilization (presence of two pronuclei, exclusion of second polar body) and fertilization rates documented prior to initiation of embryo culture.

At every 48 h post-insemination, culture medium was changed by extracting 40 μl old medium from the drops and adding 40 μl of fresh medium. Cleavage rates were determined 48 h post insemination and blastocyst rates on day 9 of embryo culture.

Experimental design

In experiment 1, the effects of sperm pretreatment with heparin (50 μg/ml), versus caffeine (10 mM) versus calcium ionophore (0.1 μM) on subsequent early embryonic development following culture in SOF+10% FBS were compared. After sperm pretreatment, a total of 112, 95 and 109 oocytes per treatment group were subjected to IVF, respectively and effects of treatments on fertilization rate, cleavage rate and blastocyst rate determined. In experiment 2, the effects of embryo culture in the following medias: SOF versus Charles Rosenkrans 1 (CR1) versus tissue culture medium-199 (TCM-199) containing 10% FBS were compared (n = 121, 110 and 113 presumptive zygotes per treatment, respectively). Effects of treatments on cleavage rate and blastocyst rate were determined as described above. In experiment 3, the effects of embryo culture in basal medium (SOF+10% FBS) versus SOF medium containing 0.3% BSA versus sequential culture in SOF medium+0.3% BSA for 48 h followed by SOF medium+10% FBS versus culture in medium conditioned by cumulus cells (cumulus cell co-culture) versus culture in medium conditioned by oviductal cells (oviductal cell co-culture) were compared. Effects of treatments on cleavage rate and blastocyst rate were determined (n = 135, 147, 139, 110 and 98 presumptive zygotes per treatment, respectively).

Statistical analysis

Effects of treatments on fertilization rate (Exp. 1), cleavage rate and blastocyst rate (Exp. 1-3) were determined by analysis of variance with percent data arcsine transformed prior to analysis. Blastocyst rates in Exp. 1 and 2 were calculated as blastocyst number/number of cleaved embryos and in Exp. 3 as blastocyst number/number of cleaved embryos and as blastocyst number/number of oocytes subjected to IVF. Differences between treatment means were determined using Fisher's protected least significant differences test.

Table 1. Effect of sperm capacitation treatments on fertilization and *in vitro* development of embryos derived from *in vitro* matured prepubertal Boer goat oocytes

Treatment	Total number of oocytes	% oocytes fertilized	% fertilized embryos that cleaved	% blastocysts from cleaved embryos
Heparin (50 mg/ml)	112	56.3±2.3[b]	51.0±2.1[c]	35.3±4.5[b]
Caffeine (10 mM)	95	20.6±1.0[a]	20.0±0.0[a]	0[a]
Ca^{2+} ionophore (0.1 μM)	109	67.1±0.7[c]	36.2±0.2[b]	31.7±6.0[b]

[a, b, c] Within same column; $p<0.01$.

RESULTS

Effects of heparin, caffeine and calcium ionophore pretreatment of spermatozoa on *in vitro* fertilization and subsequent development of embryos derived from *in vitro* matured prepubertal goat oocytes.

Results in Table 1 illustrate that fertilization, cleavage and blastocyst rates were lower when spermatozoa pretreated with caffeine were utilized for IVF relative to spermatozoa capacitated with heparin and calcium ionophore ($p<0.001$). Cleavage rates were higher following IVF with heparin versus calcium ionophore treated sperm ($p<0.05$), but no difference in blastocyst rates was observed between the two groups. Results support use of heparin for sperm pretreatment prior to IVF resulting in higher cleavage and (or) blastocyst rates for *in vitro* produced embryos derived from prepubertal goat oocytes and indicate that caffeine pretreatment of goat spermatozoa is detrimental to fertilization and subsequent development of IVF embryos.

Effects of *in vitro* embryo culture in SOF, CR1 and TCM-199 media on embryonic development

Results of studies designed to compare indices of embryonic development when *in vitro* fertilized embryos derived from prepubertal goat oocytes were cultured in three different basal media containing 10% FBS (SOF, CR1 and TCM-199) are illustrated in Table 2. No differences in cleavage rates and blastocyst rates were observed, so SOF medium was used in subsequent experiments.

Effects of cumulus and oviductal cell co-culture, and BSA versus FBS supplementation of SOF medium on development of embryos derived from oocytes of prepubertal goats *in vitro*

Results of studies designed to further optimize a system for *in vitro* culture of embryos derived from *in vitro* matured oocytes harvested from prepubertal goats are outlined in Table 3. Relative to embryos cultured in basal medium (SOF+10% FBS), cleavage rates and rates of development to the blastocyst stage (blastocysts/cleaved embryo and blastocysts/oocyte subjected to IVF) were not enhanced by co-culture with cumulus cells or oviductal cells. Furthermore, culture of embryos for the first 48 h in SOF medium containing 0.3% BSA (SOF+0.3% BSA or SOF+0.3% BSA followed by SOF+10% FBS) resulted in higher cleavage rates ($p<0.01$) than embryos cultured continuously in SOF+10% FBS. However, continuous culture in SOF+0.3% BSA did not increase resulting rates

Table 2. Effect of basal culture medium on *in vitro* development of embryos derived from *in vitro* matured prepubertal Boer goat oocytes

Treatment (medium)	Total number of oocytes	% oocytes that cleaved	% blastocysts from cleaved embryos
Synthetic oviductal fluid	121	50.4±4.5	16.6±1.7
Charles Rosenkrans 1	110	50.0±2.2	16.2±1.9
Tissue culture medium-199	113	46.0±4.5	15.1±0.8

Table 3. Effect of cumulus and oviductal cell co-culture and supplementation with BSA versus serum on *in vitro* development of embryos derived from *in vitro* matured prepubertal Boer goat oocytes

Treatment	Total number of oocytes	% oocytes that cleaved	% blastocysts from oocytes	% blastocysts from cleaved embryos
Cumulus cell co-culture	110	46.3±1.8[ab]	8.7±1.3[a]	18.6±2.5[a]
Oviductal cell co-culture	98	40.6±4.0[a]	7.8±2.4[a]	20.4±6.9[ab]
Synthetic oviductal fluid+10% FBS	135	41.2±0.7[a]	8.4±2.2[a]	20.3±5.4[a]
Synthetic oviductal fluid+0.3% BSA	147	53.1±2.0[b]	12.2±1.0[ab]	22.9±1.0[ab]
Sequential culture	139	51.7±1.4[b]	17.9±0.2[b]	34.7±0.9[b]

[a, b] Within same column; $p<0.05$.

of blastocyst development (blastocyst number/number of cleaved embryos or blastocyst number/number of oocytes subjected to IVF) relative to embryos cultured continuously in SOF+10% FBS. Sequential culture in SOF+0.3% BSA followed by SOF+10% FBS resulted in the largest yield of blastocysts of all culture systems evaluated. The percent blastocysts obtained relative to number of cleaved embryos was greater following sequential culture than observed with either co-culture system (cumulus or oviductal cell; $p< 0.05$) or with continuous culture in SOF+10% FBS ($p< 0.06$), but did not differ from that observed following continuous culture in SOF+0.3% BSA ($p<0.12$). When blastocyst development was expressed as a percentage of oocytes subjected to IVF, blastocyst rates tended to be greater for embryos exposed to sequential culture than for embryos exposed to continuous culture in SOF+0.3% BSA ($p<0.1$). Results indicate that use of SOF+0.3% BSA for first 48 h of embryo culture results in increased cleavage rates and increased cleavage rates are manifest with higher yields of blastocysts stage embryos if initial 48 h of culture in SOF+0.3% BSA is followed by culture in SOF containing 10% FBS (sequential culture).

DISCUSSION

Application of *in vitro* embryo production using oocytes harvested from prepubertal animals coupled with embryo transfer holds potential for propagation of Boer goat genetics in rural regions of China to enhance rates of genetic progress. Practical application of this strategy for genetic improvement is dependent upon a multitude of factors, including establishment of efficient strategies for *in vitro* embryo production. The present studies compared the effects of different sperm pretreatments and embryo culture systems, which are key determinants of efficiency of *in vitro* embryo production (Cognie et al., 2004), on multiple indices of development of *in vitro* fertilized embryos derived from prepubertal goat oocytes. Results demonstrate beneficial effects of sperm pretreatment with heparin prior to IVF and sequential embryo culture in SOF medium+ 0.3% BSA followed by SOF medium+10% FBS on fertilization and (or) cleavage rates and blastocyst rates relative to other experimental paradigms tested and enhance progress towards development of a platform suitable for generation of *in vitro* produced embryos for subsequent embryo transfer in China.

Sperm pretreatment is a common component of *in vitro* embryo production systems in the goat (Younis et al., 1991; Keskintepe et al., 1994b; Cognie et al., 2004). Such treatments are generally believed to primarily promote sperm capacitation. Capacitation is a crucial process that mammalian sperm must undergo in order to achieve fertilizing ability (Salicioni et al., 2007). The present studies compared the effects of sperm pretreatment with heparin versus caffeine versus ionophore on fertilization, cleavage and blastocyst rates for embryos derived from prepubertal Boer goat oocytes. Results indicate that heparin treatment of caprine spermatozoa prior to IVF results in higher cleavage rates relative to ionophore or caffeine treatment. Because effects of treatments on capacitation were not directly measured in the current studies, it is unclear whether beneficial effects of heparin on cleavage rates are solely due to increased sperm capacitation, However, it has been shown that sperm capacitation in multiple species including cattle (Parrish et al., 1988), pigs (Dapino et al., 2006) and goats (Cox et al., 1995) can be enhanced by heparin treatment. Beneficial effects of heparin pretreatment of caprine sperm prior to IVF on cleavage of resulting embryos have been reported previously (Cognie et al., 2004; Katska-Ksiazkiewicz et al., 2004).

To our knowledge, effects of calcium ionophore and caffeine pretreatment of caprine spermatozoa on fertilization, cleavage and blastocyst rates for embryos derived from prepubertal goat oocytes have not been reported previously. Treatment with calcium ionophore A23187 induces an increase in intracellular calcium and increased intracellular calcium has been functionally linked to sperm capacitation (Visconti and Kopf, 1998). While calcium ionophore treatment was more effective than heparin or caffeine in inducing the acrosome reaction in goat sperm *in vitro* (Pereira et al., 2000), no differences in blastocyst rates were observed following IVF using sperm preincubated with heparin versus calcium ionophore in the current studies. However, because of the greater cleavage rates observed, heparin pretreatment was used prior to IVF in subsequent experiments.

Caffeine pretreatment of sperm prior to IVF was found to be inhibitory to fertilization and subsequent embryonic development in the current studies. The reasons for observed inhibitory effects of caffeine treatment are not understood. Treatment of goat spermatozoa with similar concentrations of caffeine during IVF did not reduce sperm penetration rates in previous studies, but higher caffeine concentrations were inhibitory to sperm penetration (Cox et al., 1994). Thus, a reduction in sperm penetration linked to caffeine pretreatment (as evidenced by a 2.5 fold reduction in fertilization rates) may explain, at least in part, the inhibitory effects of caffeine treatment on blastocyst rates observed here.

In addition to sperm pretreatments, composition of the semen extender or characteristics of the semen donor can also influence success rates of *in vitro* embryo production in other species. Supplementation of taurine as an antioxidant in boar semen extender can improve the *in vitro*

development of porcine embryos generated by *in vitro* oocyte maturation and IVF (Jang et al., 2008). Serum testosterone concentrations of boars at time of semen collection can also influence *in vitro* embryo production (Yi et al., 2004). However, to our knowledge, the impact of taurine supplementation after collection or variation in serum testosterone concentrations in semen donors on success of *in vitro* embryo production in Boer goats has not been examined.

The composition of the basal embryo culture medium can directly influence embryo developmental capacity (Cognie et al., 2003; Gardner, 2008) and blastocyst gene expression (Rinaudo and Schultz, 2004). However, differences in cleavage rates and rates of blastocyst development for embryos cultured in SOF, CR1 or TCM-199 media containing 10% FBS were not observed in the present studies. Co-culture with various somatic cell types is also sometimes utilized for *in vitro* embryo production systems and believed to provide necessary growth factors and (or) metabolize or sequester embryotoxic factors inhibitory to development (Kane et al., 1992). Beneficial effects of co-culture with oviductal epithelial cells on blastocyst rates have been reported previously (Sakkas et al., 1989; Prichard et al., 1992; Yadav et al., 1998). While total cleavage rates were not different, higher blastocyst rates following co-culture with goat oviductal epithelial cells versus cumulus cells were also reported previously (Izquierdo et al., 1998). However, cleavage rates and blastocyst rates were similar for embryos co-cultured with cumulus cells versus oviductal cells in the current studies. Keskintepe et al. (1994a) obtained a higher number of embryos reaching the morula stage following cumulus versus oviductal cell co-culture, but a different oxygen tension was used during embryo culture. Results of the present studies do not support a beneficial effect of cumulus or oviductal cell co-culture on efficiency of *in vitro* embryo production from prepubertal goat oocytes.

Stimulatory effects of serum supplementation on rates of embryonic development are well described (Gutierrez-Adan et al., 2001), but serum supplementation can dramatically alter phenotype of resulting blastocysts, including tolerance to freezing and blastocyst gene expression (Rizos et al., 2003) and can result in excessive fetal growth rates and birth of large offspring in cattle and sheep (Young et al., 1998). Initial culture of caprine embryos in SOF medium containing 0.3% BSA resulted in higher total cleavage rates at 48 h post insemination than observed for embryos cultured continuously in SOF+10% FBS. The higher cleavage rates were not manifest by a greater percentage of embryos developing to the blastocyst stage, unless the remainder of *in vitro* embryo culture was conducted in SOF medium containing 10% FBS. Effects of serum on bovine embryonic development have previously been shown to be biphasic, with presence of serum inhibiting initial cleavage divisions prior to genome activation, but then accelerating subsequent rates of blastocyst development (Pinyopummintr and Bavister, 1994; Van Langendonckt et al., 1997; Thompson et al., 1998).

Collectively, we conclude that preincubation of caprine sperm in the presence of heparin prior to IVF and culture of resulting embryos in SOF medium containing 0.3% BSA for the first 48 h followed by culture in SOF medium containing 10% FBS results in higher rates of cleavage and (or) blastocyst development versus other treatments tested and represents a potentially viable approach for *in vitro* embryo production using *in vitro* matured oocytes from prepubertal Boer goats. To our knowledge, the large offspring syndrome phenotype has not been reported following transfer of goat embryos cultured in serum. However, given the relative lack of information on *in vitro* embryo production in the goat relative to other farm animal species, further investigation of effects of embryo culture in the presence of serum on indices of caprine blastocyst quality (cryotolerance, gene expression etc.) and pilot embryo transfer studies using above protocol may be of merit prior to widespread application of above strategy for *in vitro* embryo production and transfer of Boer goat embryos in rural regions of China as a means of genetic improvement.

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