The Suppression of Maturational Competence by Streptomycin during *In vitro* Maturation of Goat Follicular Oocytes

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ABSTRACT : Antibiotics are common additives in culture media during *in vitro* embryo development, but their effects on oocyte maturation *in vitro* have not been tested. The effects of penicillin, streptomycin and gentamicin on the maturational competence and subsequent development potential of goat follicular oocytes were examined after parthenogenetic activation *in vitro*. Maturation rates at 24 h after *in vitro* maturation, and parthenogenetic development at 48 h after activation, were evaluated by observing the protruding first polar body and the 4 cell stage cleavage, respectively. When streptomycin was present in the maturation medium, the percentages of matured oocytes 24 h after activation were significantly ($p<0.01$) lower than those from the other groups (42.5-45.7% vs. 69.1-73.8%). Penicillin and gentamicin treatment did not affect the maturation rates or the percentages reaching the 4 cell stage 48 h after activation. There was no significant difference in cleavage rates among the different antibiotic treatments 48 h after activation. Therefore, streptomycin suppresses the *in vitro* maturation of immature goat oocytes, but does not influence their subsequent development. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 8 : 1076-1079)***

Key Words : Antibiotics, Streptomycin, *In vitro* Maturation, Goat Oocytes

INTRODUCTION

Embryos can be produced on a large scale *in vitro* by using ovaries collected from slaughterhouse animals as a source of oocytes (Yang et al., 2003). These embryos are valuable for studying preimplantation development, and for the application of new animal biotechnologies such as embryo cloning and production of transgenic offspring. The domestic goat is an important species for agriculture and biomedical technology but the *in vitro* production and development of its embryos are poorly characterized (Malakar and Majumdar, 2002; Bormann et al., 2003). Recently, however, culture media have been formulated to improve the *in vitro* development of goat embryos (Martino et al., 1995; Yadav et al., 1998; Mayor et al., 2001; Rho et al., 2001; Teotia et al., 2001; Bormann et al., 2003).

Antibiotics are frequently added to embryo culture media to prevent bacterial and fungal contamination. Penicillin and streptomycin are most commonly used, but gentamicin is an alternative (Zhou et al., 2000). Justification for the use of these antibiotics in culture media is mainly based on tests using somatic cell or organ culture systems, not preimplantation stage embryos (Amonn et al., 1978; Moss et al., 1984; Shimizu et al., 1991). Media for embryo development *in vitro* are generally formulated by considering the embryo's nutritional requirements, but the effects of antibiotic supplements have not been evaluated thoroughly. Two previous studies reported an adverse effect of penicillin and streptomycin on the development of human (Magli et al., 1996) and hamster (Zhou et al., 2000) embryos.

Oocyte maturation and activation are key steps in the *in vitro* production of reconstituted embryos, such as cloned or IVF embryos. In this study, we investigated whether penicillin, streptomycin or gentamycin affect the maturational competence of goat follicular oocytes and their subsequent developmental potential *in vitro* following parthenogenetic activation.

MATERIALS AND MATHODS

Oocyte collection

Goat ovaries were obtained from a local abattoir and transported to the laboratory within 3-5 h of collection at 30-35°C in 0.9% saline containing 100 IU/ml penicillin and 50 μg/ml streptomycin. The ovaries were rinsed twice with 0.9% saline. Cumulus oocyte complexes (COCs) were aspirated from 2-8 mm diameter follicles with a 20 gauge needle fixed to a 10 ml disposable syringe. Only COCs having at least one layer of non-expanded cumulus and an even distribution of cytoplasm were selected. COCs were washed three times in tissue culture medium (TCM) 199 (Gibco Grand Island, NY) supplemented with 10% (v:v) fetal bovine serum (FBS; Gibco), and randomly distributed among the treatment groups.

In vitro maturation

COCs were placed in 500 μl of maturation medium

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Table 1. Formulation of goat oocyte maturaton medium

Basic meium	Component	Unit
TCM 199	bLH	0.02 U
	bFSH	0.02 U
	Estradiol-β17	1 μg/mL
	Sodium pyruvate	0.2 mM
	Cysteamine	0.1 mM
	FBS	10%
	Antibiotics[a]	

[a] 100 IU/ml penicillin; 50 μg/ml streptomycin; 50 μg/ml gentamicin; 100 IU/ml. Penicillin and 50 μg/ml streptomycin.

Table 2. Effect of antibiotics on maturational competence of goat follicular oocytes and subsequent developmental potential *in vitro* following parthenogenetic activation

Treatment	N[a]	Matured oocytes		Cleaved	
		n	% ±SEM[b]	n	% ±SEM[c]
Control	84	62	73.8±7.2[d]	50	79.9±8.4
Penicillin	88	62	69.1±11.1[d]	50	83.5±11.8
Streptomycin	84	36	42.5±8.3[e]	30	80.4±14.2
Gentamicin	84	60	71.3±10.1[d]	50	81.9±0.5
Pen+strep	82	38	45.7±8.5[e]	30	80.2±16.9

[a] Number of goat follicular oocytes cultured in 4 replicate experiments.
[b] Percentage of matured oocytes=(no. of matured oocytes/no. of cultured oocytes)×100.
[c] Percentage of cleaved=(no. of 4 cell stage embryos/no. of matured oocytes)×100.
[d, e] Different superscripts within columns indicate significant differences, $p<0.01$.

which had been covered with mineral oil in a 4 well multidish (Nunc, Roskilde, Denmark) and incubated for 24 h at 38.5°C in an atmosphere of 5% CO_2 in air with maximum humidity. The maturation medium was TCM 199 supplemented with 10% FBS, 5 μg/ml FSH (Sigma Chemical Co, St. Louis, MO), 10 μg/ml LH (Sigma) and 1 μg/ml 17 β-estadiol (Sigma) as listed in Table 1.

To examine the effects of antibiotics, the COCs were divided among five treatment groups: 1) Control: maturation medium with no antibiotics; 2) maturation medium with 100 IU/ml penicillin; 3) maturation medium with 50 μg/ml streptomycin; 4) maturation medium with 50 μg/ml gentamicin; 5) maturation medium with both 100 IU/ml penicillin and 50 μg/ml streptomycin. After 24 h culture, oocytes were freed from the cumulus cells by vigorous vortexing for 1-2 min in TCM 199 supplemented with 0.15% hyaluronidase. Matured oocytes were evaluated by the appearance of the first polar body. Metaphase II plate in the cytoplasm was observed following staining with 1 μg/ml Hoechst 33258 (Sigma).

Oocyte activation

Matured oocytes were chemically activated using the ionomycin (Sigma) and 6-diethlaminopurine (6-DMAP; Sigma) methods of Susko-Parrish et al. (1994). Briefly, oocytes were incubated for 5 min in TCM 199 containing 5 μM ionomycin at room temperature and then washed three times in TCM 199 supplemented with 10% FBS. The activated oocytes were cultured for 3.5 h at 38.5°C, 5% CO_2 in 2 mM 6-DMAP, then placed in culture drops (50 μl) consisting of TCM 199 supplemented with 10% FBS under an oil overlay.

(a) (b)

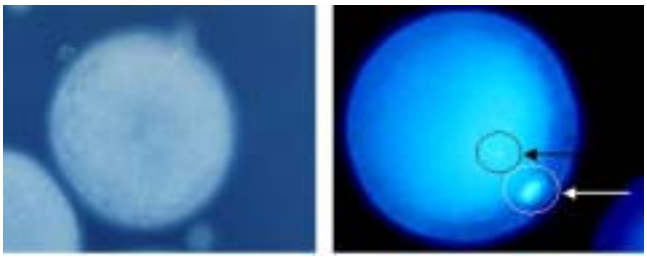

Figure 1. Oocyte maturation *in vitro*. (a) Incompletely matured oocyte. (b) Matured oocyte with first polar body (white circle and arrow) and metaphase II plate (black circle and arrow).

In vitro culture

After activation, oocytes were washed and placed in 500 μl of culture medium that had been covered with mineral oil in a 4 well multidish and were incubated for 48 h at 38.5°C, 100% humidity in a 5% CO_2 atmosphere. The culture medium was TCM 199 supplemented with 10% FBS and antibiotic. The treatment groups were: 1) Control: culture medium with no antibiotics; 2) culture medium with 100 IU/ml penicillin; 3) culture medium with 50 μg/ml streptomycin; 4) culture medium with 50 μg/ml gentamicin; and 5) culture medium with both 100 IU/ml penicillin and 50 μg/ml streptomycin.

Parthenogenetic cleavage development 48 h after activation was evaluated by the appearance of normal morphology at the 4 cell cleavage stage.

Statistical analysis

Maturation and cleavage rates were recorded as percentages of total and matured oocytes. The data were analyzed by ANOVA. Percentage data were subjected to an arcsine transformation before analysis. Differences among treatments were compared using Student's t-test. Differences with a probability value of 0.01 or less were considered significant. Results were reported as % ±SEM.

RESULTS

The effects of the antibiotics on the maturation and development *in vitro* of goat follicular oocytes are shown in Table 2. Maturation of the oocytes was evaluated by the extrusion of first polar body and metaphase II formation (Figure 1). Four replicate experiments were conducted for each treatment. When streptomycin was present in the

maturation medium, the percentages of matured oocytes (42.5 and 45.8%, respectively, in streptomycin only and in penicillin plus streptomycin) 24 h after activation were significantly lower ($p<0.01$) than those from the other groups (69.1-73.8%). However, penicillin and gentamicin treatments did not significantly affect the maturation rates of immature goat oocytes compared to control. When oocytes matured under the five different treatment regimes were cultured *in vitro* for 48 h following chemically induced activation, no significant differences were observed among the subsequent development rates to the 4 cell stage.

DISCUSSION

The present study showed that streptomycin interfered with the maturation of immature goat oocytes, but did not affect the subsequent development of mature goat oocytes. In media for somatic cell culture, streptomycin or streptomycin plus penicillin retard or depress protein and DNA synthesis (Amonn et al., 1978; Moss et al., 1984). Streptomycin may also depress protein and DNA synthesis in cultures of immature goat oocytes. A number of antibiotics have been reported to disturb prokaryotic translation and to inhibit the functions of various ribozymes. Hertweck et al. (2002) reported that erythromycin, Cl-tetracycline and streptomycin inhibited splicing in HeLa cell nuclear extract. We do not know why streptomycin interfered with the maturation of immature goat oocytes, but differences in gene expression between immature and mature oocytes are likely to be involved. This matter requires further investigation.

As shown in Table 1, penicillin and gentamicin did not interfere with the maturation of goat follicular oocytes. Gentamicin is known to be stable over a wider pH range than either penicillin or streptomycin, and maintains its biological activity in the presence of serum (Schafer et al., 1972). The present study suggests that gentamicin is the antibiotic of choice for preventing bacterial contamination of goat oocyte maturation media. However, gentamicin at 40μg/ml can inhibit enzyme activities in primary fetal cells (Shimizu et al., 1991). Zhou et al. (2000) reported that penicillin and streptomycin used in conjunction significantly inhibited the development of hamster embryos to the 8 cell and blastocyst stages. In the human, significantly higher cleavage rates were obtained in antibiotic-free medium, including at the blastocyst stage (Magli et al., 1996). Therefore, there is a need to examine the choice of antibiotics for *in vitro* development of embryos from various species.

In conclusion, this result showed that streptomycin suppressed the maturation of immature goat oocytes but did not affect the subsequent development of matured goat oocytes, suggesting that streptomycin can be detrimental to *in vitro* maturation of goat follicular oocytes.

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