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Development of embryos after in vitro fertilization of bovine oocytes with sperm from either yaks (*Bos grunniens*) or cattle (*Bos taurus*)

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Abstract

The objectives of this study were to investigate differences in fertilization and development of embryos after in vitro fertilization of *Bos taurus* (cow) oocytes with sperm from either yaks (*Bos grunniens*) or Holstein bulls. Frozen–thawed spermatozoa (Holstein $n = 5$ sires; yak $n = 5$ sires) were evaluated for motility (forward progression) and acrosomal status immediately post-thaw and then 1, 2, 3, and 8 h later. In vitro-matured cow oocytes ($n = 1652$) were inseminated with either Holstein bull or yak spermatozoa and after an 18-h co-incubation period, a proportion of the oocytes were fixed and examined for sperm penetration, polyspermy, and male pronuclear formation. The remaining oocytes were cultured in vitro and evaluated for cleavage and blastocyst production rates. Overall, there were species differences ($P < 0.05$) and an effect of time ($P < 0.01$) in sperm motility and acrosome integrity. An effect ($P < 0.01$) of a species-by-time interaction was detected for motility, but not for acrosome integrity. The percentage of oocytes penetrated and the formation of two pronuclei when cow oocytes were inseminated with yak spermatozoa (97.4% and 81.6%, respectively) were greater ($P < 0.01$) than that achieved with Holstein bull spermatozoa (77.8% and 65.9%, respectively), but the incidence of polyspermy (>2 pronuclei) was similar ($P > 0.05$; 10.8% vs. 15.8%). The yak ♂ × cow combination gave a higher cleavage rate than the Holstein ♂ × cow combination ($P < 0.05$; 76.3% vs. 63.3%), but there was no difference in the blastocyst rate (17.9% vs. 14.5%). It is concluded that yak spermatozoa could successfully fertilize cattle oocytes and their hybrid embryos had normal competence to develop to blastocysts.

Keywords: Yak; Bovine; In vitro fertilization; In vitro development; Sperm motility

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1. Introduction

The yak (*Bos grunniens*) is one of the world's most remarkable domestic animals—a herbivore living on the "roof of the world", in and around the Himalayas and north, in areas of altitude ranging from 2500 to 5500 m with no frost-free periods, and mostly above the tree line. Yaks provide their owners with meat and milk, where few other animals will survive, although their production traits are inferior to those of improved cattle breeds (Cai and Wiener, 1995; Zi et al., 2004). Crossbreeding with cattle (*Bos taurus*) is advocated as a means of increasing milk and meat output from the mountainous regions. The first cross progeny from dairy cattle breeds such as Holstein Friesian and Jersey have yielded a much higher daily amount of milk (4–5-fold) than the yak. When slaughter at 17 months old, the first crosses from beef cattle breeds such as Simmental, Charollais and Hereford are 50% heavier than yaks at the same age (Cai, 1984).

However, several factors militate against such crossbreeding programs despite their apparent benefits. The first is that F1 males are sterile because there are no sperms in the seminal fluid. The F1 females are always back-crossed to either the yak or the desired *B. taurus* breed but the productivity of both types of back-crosses is often little better than that of the yak and this constrains the economic potential of the F1 females (Cai and Wiener, 1995). A second important factor is the lower conception rate and higher fetal loss in the crossbred animals compared with either the yak or cow (Cai, 1979; Zi et al., 2006). The underlying physiology of these reductions is not fully understood. In vitro fertilization (IVF) has been widely used in embryo production for domestic species and offers the best method for studying sperm–egg interactions and the development of pre-implantation embryos. The use of a heterologous IVF system between the yak and the cow will provide hybrid F1 embryos not only to produce valuable offsprings but also to provide a means of studying the underlying reproductive physiology associated with reduced conception rates in F1 progeny. There are two heterologous IVF systems to produce hybrid embryos namely *B. taurus* spermatozoa × *B. grunniens* oocytes and *B. grunniens* spermatozoa × *B. taurus* oocytes. The former would produce genetic superior offspring because of the superior production traits of *B. taurus* compared with *B. grunniens* but the use of this system is limited by seasonal breeding (June to November) and the seasonal supply of yak ovaries (September to December) (Zi, 2003; Zi et al., 2004). On the other hand, the alternative system can provide hybrid embryos year-round although genetic merit is limited by oocytes being harvested from genetically inferior indigenous breeds of cattle.

The objectives of the present study were to (1) characterize post-thaw yak sperm motility and acrosome integrity over time, (2) evaluate sperm penetration, and polyspermy in cow oocytes after co-incubation with yak sperm, (3) determine the cleavage rate and the blastocyst rate of yak-cow hybrid embryos, and (4) compare results with those from parallel studies with domestic bull and yak spermatozoa.

2. Materials and methods

2.1. Chemicals and reagents

Dulbecco's phosphate-buffered saline (DPBS) was purchased from Hyclone Laboratories Inc. (Logan, UT), Folltropin (FSH) from Bioniche Inc. (Belleville, Ontario, Canada) and fetal calf serum (FCS) from Gibco (Grand Island, NY). All other chemicals and reagents used for in vitro maturation (IVM), IVF and in vitro culture (IVC) were cell-culture tested and were obtained from Sigma-Aldrich (St. Louis, MO). Synthetic oviductal fluid (SOF) was prepared according to the