*Table 1. Effect of different protein media supplement on the penetration rate of ram sperm into sheep oocytes matured in vitro*

| Groups | Protein in culture medium (%) | Number of oocytes | Penetration rate (%) | Monospermically fertilized oocytes (%) |
|---|---|---|---|---|
| 1. | (FCS) | 78 | 89.7 | 26.9 |
| 2 | (BSA) | 72 | 84.7 | 63.9 |
| 3 | (OF) | 156 | 77.6 | 55.8 |

*Table 2. In vivo survival of sheep embryos produced in vitro and transferred to recipients*

| Groups | No. of recipients | No. of recipients pregnant | | |
|---|---|---|---|---|
| | | at 33 days (%) | at 53 days (%) | at 80 days (%) |
| 1 (FCS) | 10 | 5 (50) | 2 (20) | 1 (10) |
| 2 (BSA) | 10 | 8 (80) | 4 (40) | 4 (40) |
| 3 (OF) | 10 | 7 (70) | 6 (60) | 6 (60) |

## Discussion

The production of a viable oocyte is the first step towards a successful *in vitro* embryo achievement. Because it is known that embryonic development can be readily compromised by deficiencies introduced during the process of oocyte maturation (Moor et al., 1998), in our experiment we tried to improve this process by introducing OF to the culture medium.

OF proteins have been found to associate with the zona pellucida in many species including sheep (Gandolfi et al., 1989). The oviduct provides a passive source of protein, derived from serum and other fluids as a transudate. The major components are serum proteins, but several biologically active substances are also synthesized in the oviduct. Protein composition of OF has been described repeatedly, but the latest studies have shown that the secreted proteins include an oestrogen-dependent oviduct-specific glycoprotein, which is significantly conserved across many species (Buhi et al., 2000). Total protein has been measured and in general appears to be lower in comparison with serum (Hunter, 1988), and that is why in our experiment we used 20% OF to increase the protein level to become comparable with media containing 10% of serum.

Embryo mortality is a very common and difficult problem of ovine embryo production *in vitro*. Acquisition of the developmental competence involves synthesis and storage of a wide range of molecules during oocyte maturation, fertilization and early embryogenesis. After finished maturation, oocytes are exposed to a very high concentration of spermatozoa during IVF, which may ultimately have a negative effect on embryonic development and implantation, since dying spermatozoa produce oxygen radicals (Aitken, 1995; Aitken et al., 1996). Based on the fact that oviduct most probably provides the optimal conditions for early reproductive events, embryonic development and implantation rate (Hunter, 1988), we paid great attention to oviductal secretion as the protein supplement in the conducted experiments.

The incidence of early embryonic loss in adult sheep after natural mating is generally 20-30% and occurs predominantly during the first 5 weeks of pregnancy (Edey, 1976). Death of cultured embryos following transfer occurs largely between approximately day 15 and day 60 of pregnancy, most likely around the time of implantation (Bolet, 1986) and for all postcompaction-stage embryos; regardless of the source. The ability to elongate and to begin the initial stages of organogenesis in conjunction with attachment to the endometrium is the most critical time for the developing of ovine conceptus (Thompson et al., 1994). Thompson (1997) has demonstrated that *in vitro* produced embryos have a low survival rate (40%), and embryo/foetal loss occurs at 30–35 days after transfer.

In addition to implantation and organogenesis, the embryo mortality depends on several factors such as *in vitro* culture system, stage and number of transferred embryos. Slavík and Fulka (1992) received a high embryo mortality rate following transfer of a large number of embryos produced *in vitro* from randomly chosen oocytes. This factor also affects the embryonic mortality in prolific breeds (Hanrahan, 1980). Furthermore, Kleeman et al. (1990) have demonstrated that the rate of embryonic loss increases with an increasing ovulation rate. In our study we used one defined sheep breed as donors and recipients. Survival of embryos following incubation of oocytes in OF exceeds 60% after the transfer. Supplementation of culture medium with FCS did not appear to be very successful. Only 1 out of 10 ewes was pregnant at 80$^{th}$ day of gestation. This high loss rate was probably due to an increased incidence of polyspermic fertilization followed by a reduced developmental capacity of embryos. There were no statistical differences between groups; that is why we claim that OF can be an alternative source of protein supplement to the culture media. Due to the presence of biologically active substances it may represent conditions more close to those *in vivo*. After transfer of embryos incubated in oviductal pro-

teins, the large offspring syndrome was not recorded, while in the group of embryos using a routine protocol, large offsprings were observed (our preliminary experiments). More extended experiments focused on the effect of oviductal proteins on the incidence of the large offspring syndrome are in run.

Perhaps, culture of embryos *in vitro* to the morula or blastocyst stage could increase the pregnancy rate and allow selection based on the embryo viability before transfer. Using this scheme, O'Brien et al. (1996) received 65.7% pregnant recipients diagnosed by ultrasound following transfer of embryos in the blastocyst stage. In our study we transplanted embryos in the two pronuclei stage, avoiding long-term *in vitro* culture of embryos. Our method did not allow selection based on the advanced cell stage; on the other hand, this system maximizes exposure of embryos to the oviductal environment. In summary, OF added to the culture medium increased the survival rate after embryo transfer into recipients.

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