

EFFECT OF PENTOXIFYLLINE ADDED TO FROZEN-THAWED SEMEN ON SHEEP FERTILITY

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INTRODUCTION

In our country, the sheep fertility obtained by intrauterine artificial insemination using frozen-thawed semen has been very inconsistent (30-60%). In several species (human, dog, equine, pig) pentoxifylline (PTX) has been used as an additive to sperm to improve sperm characteristics. The mechanism of action on spermatozoa is generally assumed to be the inhibition of sperm phosphodiesterase activity, resulting in elevation of complementary adenosine monophosphate levels in spermatozoa. PTX enhanced post-thaw motility and membrane integrity when added after thawing (1). On the other hand, GnRH administration at 35 hours after pessary removal improves pregnancy rates in progestagen-treated ewes (2). GnRH induces ovulation in all the ewes. The aims of this study were to compare the effects of pentoxifylline (PTX) on frozen-thawed semen and GnRH administration on sheep fecundity.

MATERIALS AND METHODS

Two hundred and forty multiparous Australian Merino ewes of 4 to 6 years old were separated at random in two groups: (T1) Progestagen-impregnated intravaginal pessaries containing 60 mg of medroxy progesterone acetate were inserted for 14 days to synchronize estrus. At pessary removal eCG (350 UI.) was administered; and (T2) the same treatment plus 10 µg GnRH administration at 35 hours after pessary removal. These treatments were later broken into three subgroups in order to add PTX to the thawed semen fifteen minutes before intrauterine insemination: These subgroups were: A) the addition of 2.5 mM PTX in 0.4 mL dose volume, B) the addition of 2.5 mM PTX in 0.25 mL dose volume, C) control without PTX and 0.25 mL of dose volume, containing the same number of spermatozoa (40×10^6). Thus, the experimental design was a 2 x 3 factorial. In a preliminary experiment, different PTX concentrations were evaluated (2.0, 2.5, 3.5, 5.0, 7.0 mM). The most beneficial effect (motility) was observed when 2.5 mM of PTX was added to semen in the process of thawing. Pregnancy was diagnosed and fetuses counted by ultrasonography 30 days after insemination (Aloka SSD 500; 5MHz,

linear-array transrectal probe). Lambing was determined by udder examination 150 days after insemination and verified by the number of lambs born per group 10 days later.

RESULTS AND DISCUSSION

No effect of GnRH administration on the pregnancy rate and lambing was detected (fertility 46.6 vs. 48.3; fecundity 56.6 vs. 54.2 %, T1 and T2 respectively). When PTX was used, the volume of the dose affected the lambing rate (46.3 vs. 62.3, $P < 0.10$, A vs. B). The PTX at 0.25 mL of dose (B) had a tendency to improve the lambing rate (62.3 vs. 51.8, B and C, respectively). There was an interaction between PTX addition and GnRH administration ($P < 0.05$) which determined the highest fertility and fecundity (41.5 vs 58.0; 53.7 vs. 73.7%, T1B vs. T2B fertility and fecundity, respectively). A significant improvement in fertilization rate was also demonstrated in human and other animal species. The improvement of sperm characteristics by PTX is most important when the female fertility is better (T2). In our case the fecundity increased because both male (PTX added to sperm) and female fertility (GnRH administration) were enhanced. These results indicate that pentoxifylline associated to GnRH administration 35 hours after pessary removal improves sheep fertility and fecundity.

REFERENCES

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SYNCHRONIZE ESTRUS TREATMENT	350 PMSG	350 PMSG + 10 µg GnRH	
FERTILITY	46,6	48,3	
FECUNDITY	56,6	54,2	
n	120	120	
PENTOXIFYLLINE ADDED (thawed semen)	PENTOXIFYLLINE A.I. DOSE VOLUME 0.25	PENTOXIFYLLINE A.I. DOSE VOLUME 0.4	CONTROL
FERTILITY	47,5	43,3	43,2
FECUNDITY	62,3 a	46,3 b	51,8 a b
n	79	80	81
P<0.10			
PENTOXIFYLLINE A.I. DOSE VOLUME 0.4	350 PMSG	350 PMSG + 10 µg GnRH	
FERTILITY	41,5	58,0	
FECUNDITY	53,7	73,7	
n	41	38	P<0.05