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Blocade of the β-adrenoceptors with carazolol improves fertility of artificial insemination in Awassi sheep

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Summary

This investigation aimed to study the effect on fertility parameters of one or two artificial inseminations (AI) and the administration (carazalol) before insemination of β-adrenoceptor blocker in Awassi sheep. A total of 144 Awassi sheep with their oestrous synchronized were divided into 4 groups. Group A_1 had a single artificial insemination applied at 52 hours after (the sponge was removed), group A_2 had a single artifical insemination applied while for preinsemination 0.01 mg/kg carazalol were given i.v. Group B_1 had artificial insemination appled twice at 52 and 60 hours. B_2 group had artificial insemination appled twice at 52 and 60 hours and for preinsemination 0.01 mg/kg carazalol were given i.v.

As a results, the ratios of not observed oestrus after 25 days were 55.6%, 61.1%, 63.9% and 69.4% in groups A_1, A_2, B_1 and B_2 respectively. The ratios of pregnancy were diagnosed ultrasonographically after the 30th day of artificial insemination as 47.2%, 50.0%, 52.8% and 61.1% in groups A_1, A_2, B_1 and B_2 respectively. The ratios of birth by means of artificial insemination were 41.7%, 47.2%, 47.2% and 55% in groups A_1, A_2, B_1 and B_2 respectively. The degree of fertility was found to be higher in the group in which the sheep applied artificial insemination twice with 8 hours intervals and administrated β-adrenoceptor blocker preinsemination. However, the difference between groups was not significant ($p>0.05$).

Keywords: insemination, beta-receptor blocker, sheep

Artifical insemination gained an important place in sheep breeding in our time, as a result of recent important genetical developments in breeding sheep using pedigree rams of high quality through artificial insemination techniques. Despite the high degree of conception with some of these techniques, they are not in frequent use, because of the difficulty in performing the surgical methods, their high cost, necessary experience and anesthesia (12, 25, 27, 28).

It is a fact that the ratio of pregnancy with artifical insemination in sheep with fresh sperms is greater while the ratio of pregnancy through single dose of artificial insemination with fresh sperms is over 60%. The ratio of success rarely exceeds 40% in artificial insemination with frozen or refrigerated sperms with added preservatives (9, 14, 19).

Sperms ejected to genital system through artificial insemination or mating reach the oviduct by the contraction of the uterus muscle. As a result of the diminishing or cessation of the uteral contractions, the arrival of spermatozoids in the fertilizations site is delayed. Boorish handling of sheep and occurrence of trauma in the pelvic tissues of the female animals during catheterization as in artificial insemination by a nonexpert individual give rise to stress which, in turn, causes adrenalin to be secreted from vegetative nerve endings. The secreted adrenalin stimulates β_2 adrenoceptors and thus can cease uterine contractions preventing their responding to oxytocin.

It has been observed that if fertilization occurs with aged spermatozoids as a result of the cessation of uterus contractions and remaining in the uterus long enough to delay their arrival in fertilization site and thus lose their fertility, the emerging zygot has been unable to survive (4, 11, 18, 22). In addition, it has been reporte that in animals subjected to stress the uterine contractions are inhibited for long and do not occur even when oxytocin is supplied exogenously (5).

β-adrenoceptors blockers are used widely in medical practice both in humans and animals. They interact with β-adrenoceptors, antogonizing the effect of adrenalin

secreted under stress. This interacting competitive antagonism can be reversible (3, 16, 23, 29, 30). β-adrenoceptors blockers have been used in veterinary medicine in enhancement of fertility, facilitation of birth and expulsion of the placenta, notably by antagonization of the effect of stress. They were administered intramuscularly or intravenously in usual doses of 0.01 mg/kg. The Maximum Residue Limit (MRL) of carazolol in bovine milk and meat has been reported to be 0.5 and 1.5 mg/kg respectively (2, 15).

This study aimed to improve fertility in Awassi sheep that, owing to their heavy tails, cannot be inseminated naturally with Merino rams. For this purpose, the sheep have been syncronized in the mating season, inseminated once or twice after receiving beta-receptor blocker.

Material and methods

Animals and experimental design. The experiment was carried out during the middle of breeding season using a total of 144 Awassi sheep in the same care and administration conditions in the farm of Veterinary Faculty, Dicle University.

All sheep were synchronized according to the schedule described previously. The sheep were treated with progestagen vaginal sponges (40 mg of fluorogestone acetate, Choronogest®, Intervet) for 13 days. After 13 days, all the sheep with complately the sponges removed, were administrated a single injection of 500 IU PMSG (9, 14, 24). The sheep in group A_1 were inseminated only once with 0.25 ml sperm 52 h after the sponges were removed (10, 31). Artificial insemination was conducted by administering sperms into the first half of the cervix (5-15 mm into the cervix) by means of a speculum (11, 26, 28). The sheep in group A_2 were inseminated once with 0.25 ml sperm 52 h following the removal of their sponge. They received 0.1 mg/kg IV of carazolol, (Suacron® 100 ml, Bayer, Spain) 5 min. before insemination (2, 18). The sheep in group B_1 were inseminated twice at 52 and 60 h with 0.25 ml sperm after their sponges were removed. The sheep in group B_2 were inseminated in the same way and with the same procedure as their counterparts in group B_1, but the whole process was repeted again in this group. The sheep received 0.01 mg/kg IV carazalol twice, 5 min. before insemination. In case pregnancy did not occur or embryonic deaths occurred, and hoping that those (displaying oestrus again) would have additional chance of conception an Awasi ram was included in the herd 18 days following the latest insemination (28).

Pregnancies were diagnosed at 30 day following the first insemination (Transrectal) using real time B-mode ultrasonography (Pie Medical, 410477 Mod, Netherlands; 5 MHZ, linear prob) (12, 17, 31).

Pregnancies were confirmed on average 150 (145-155) days following the latest/first insemination with udder structure of animals and the births realized. The tail structure and other phenotypical features of the lambs born averagely 168 days after the latest artifical insemination were examined to determine whether they were mated by the Merino ram (artifical insemination) or by the Awassi ram (natural mating).

Collection and preparation of sperms. The sperms used in insemination was obtained from 8 mature Merino rams, aged 2-6 year of proven fertility. Since the rams were not accustomed to mating with sheep whose tails are large, the sperms were collected with an artifical vagina fitted with a graduated test tube. The rams were used at random for groups during insemination. After their motility was determined sperms were drawn into 0.25 ml payets in a way to have approximately 200×10^6 motilite spermatozoids in the insemination doses (9, 11, 28, 31). The sperms taken from each ram was kept at 18-25°C room temperature and used within 1 h at the latest.

Statistical Analyses. In all qualitative observations, results of the different groups were compared with chi-square-test.

Results and discussion

All data of nonreturned, pregnancy, crossbred lambs and twin lambs are presented in tab 1.

No statistically significant difference has been found between the values in groups ($p > 0.05$).

There is a multitude of factor that affect the proportion of pregnancy achieved through artificial insemination, mainly folding system, environmental conditions, the individual health and physiological status of the sheep itself (1).

Cervically performed artificial insemination in sheep is easy and less costly than other methods of insemination (25). According to some researchers one of the reasons for low fertility in cervically performed artificial inseminations is that the cervix in the sheep, owing to its complex anatomical structure prevents the penetration of insemination catheter, functioning as an important barrier (9, 13, 31).

Ehling et al. (10) reported achievement rates of fertilization as high as 80% and pregnancy as high as 70-80 in artificial insemination using laparoscopically obtained fresh sperms. Wulster-Radcliffe et al. (31) obtained pregnancy rates as high as 67.5% diagnosed on the 30th day after servical insemination in which they themselves devised. They reported in the same study that 60% of those pregnancies resulted in births. That the ratios obtained by the researchers mentioned above were higher than those in our study may have resulted from the direct administration of the sperms to the uterus thanks to artificial insemination catheter that they used. It is a known fact that the place where sperms are left in the female genital organs affects fertility ratios in artificial insemination catheter considerably reduces the ratio of fertilization.

While the effect of stress on the reproduction of the sheep has not been studies adequately, one of a large number of effects of stress in cows is that it lowers fertility by increasing the secretion of endogenous adrenalin. Thus

Tab. 1. The ratios of nonreturned, pregnancy, crossbred and twin lambs

Groups	n	Rate of non-returned before 25 days		Rate of pregnancy with USG at 30. days		Rate of crossbred lambs		Rate of twin lambs	
		n	%	n	%	n	%	n	%
A_1	36	20	55.6	17	47.2	15	41.7	6	16.7
A_2	36	22	61.1	18	50.0	17	47.2	6	16.7
B_1	36	23	63.9	19	52.8	17	47.2	7	19.4
B_2	36	25	69.4	22	61.1	20	55.6	8	22.2

it has known that the interval between the birth and the next insemination in cows undergoing stress is 13-14 days longer cows and 0.5 more insemination is needed for pregnancy to occur (6, 7, 18).

In this study done on 182 cows to investigate the affect of stress Dobson (8) reports that the pregnancy rate in the first insemination of the cows suffering lameness stress is lower by 16% than in healthy cows suffering the stress of difficult labour are delayed when compared with those having normal delivery. In their study on cows displaying oestrus, Kırşan et al. (18) obtained pregnancy by 97% in the group treated with carazolol before insemination, and by 78% in the group without carazolol. It is reported that in a study by Paulenz (27) the ratio of the lambs born as a product of double insemination is greater by 5% than that of the lambs born as a product of single insemination and this ratio ranges between 5% and 10% in the literature. In our study the differences between the groups has been found to be 8.3%. In the light of this data it may be suggested that carazolol and double insemination can increase the ratio of pregnancy and birth which corroborates opinion.

Karen (17) reports that the diagnosis of pregnancy by means of 5 MHz-ultrasonography is as low as 12% on the 25th days after insemination. But it can rise up to 85% by the 32-34th days. Understandably, the ratio we obtained in our study is lower than those presented by the author, which can be ascribed to our lack of practice in the ultrasonographic diagnosis of pregnancy. Langford (20) estimates that the ratio of embryonic death within first 2 weeks of fertilization in his study has ranged 9% to 24%. The same author reports that he has found the ratio of embryonic death to be 13% in another of his studies (21) and that, based on the other findings from the same study, double insemination does not have any beneficial effect on the rate of pregnancy. It was observed in our study that the rate of pregnancy in the group inseminated twice was greater by 2.8% to 8.3% and that the rate of those which did not give birth to crossbred lambs despite the diagnosis of their pregnancy 30 days after the insemination. In other words, the rate of embryonic death was 5.6%.

Conclusion

It has been reported that β-receptor blockers have been used in sufficient quantities in order to enhance fertility in cows. Although an increase in pregnancy rate of with these practices have been reported the number of the studies with sheep is quite limited. Based on our study, we think that the administration of β-receptor blockers (carazolol), prior to double artificial insemination with 52 hours apart, can increase fertility. We believe that more additional studies are needed to verify our thought.

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