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Hiralal S Janagi
PG Student, Department of Gynecology and Obstetrics, Veterinary College, KVAFSU, Nandinagar, Bidar, Karnataka, India

Tandle MK
Professor and Head, Department of Gynecology and Obstetrics, Veterinary College, KVAFSU, Nandinagar, Bidar, Karnataka, India

Vinay PT
Assistant Professor, Department of Pharmacology and Toxicology, Veterinary College, KVAFSU, Nandinagar, Bidar, Karnataka, India

Usturge SM
Director of Research, KVAFSU, Nandinagar, Bidar, Karnataka, India

Jadhav NV
Retired Professor of Poultry Science, Veterinary College, KVAFSU, Bidar, Karnataka, India

Correspondence
Tandle MK
Professor and Head, Department of Gynecology and Obstetrics, Veterinary College, KVAFSU, Nandinagar, Bidar, Karnataka, India

Reproductive performance evaluation of Kenguri ewes by breeding with NARI-Suwarna frozen thawed semen

Hiralal S Janagi, Tandle MK, Vinay PT, Usturge SM and Jadhav NV

Abstract
The present study was conducted to evaluate reproductive performance of Kenguri ewes after breeding with NARI- Suwarna frozen thawed semen. Oestrous cycle of Kenguri ewes (n=10 + 10) was synchronized by injecting PGF2α. All the ewes were inseminated twice with gap of 12 hours using frozen thawed semen dose of NARI Suwarna (Deccani + Garole) strain. Pregnancy diagnosis was initiated on days 24, 34 and 44 of post artificial insemination using trans-abdominal ultrasonography. Based on the present findings it can be concluded that the estrous cycle may be synchronized by injecting PGF2α. on days 0 and 9 in or day 0 and day 11 with 100 % estrus induction rate in Kenguri ewes. The incidence of false positive pregnancy diagnosis was higher on days 24 and 34 by using transabdominal ultrasonography. The climate temperature, heat stress may be one of the reasons for the early embryonic mortality in Kenguri ewes. The lambing rate by inseminating the Kenguri ewes with frozen thawed semen was lowest (10%) in trans-cervical method. None of the Kenguri ewe from both the treatment and control groups showed any disease or accident during gestation and lambing after using NARI Suwarna frozen thawed semen. The sex of both the lambs was female with body weight ranging from 2.35 to 2.60 with gestation period range of 147 to 151 days.

Keywords: Kenguri ewes, Reproductive performance, NARI-Suwarna

Introduction
Artificial insemination (AI) was introduced as a supplement to natural mating in sheep and goats in Norway during 1960's. Artificial insemination of sheep has been applied most extensively in the former Soviet Union. Most ewes have been bred using 0.05 ml of undiluted semen within 20 min of taking the collection from the ram. In Eastern Europe, South America and Australia, AI is widely used in sheep-breeding programmes but its use is much less widespread in Western Europe and North America. Breeding by Artificial Insemination now being practiced in various sheep breeding farms in India. It has been tried with success on sheep breeding farm at Poona and Mathura on experimental basis [1, 2]. The Nimbkar Agricultural Research Institute, Phaltan, District Satara, Maharashtra state has developed NARI Suwarna and NARI composite breeds of sheep with more than 60% ewes having twin lambs, by introducing the FecB gene from the Garole breed of Sunderban, West Bengal. NARI Suwarna ewes have about 90% Deccani breed proportion or 60% Deccani and 30% Madgyal and only 10% Garole breed proportion and are capable of producing and raising twin lambs to a weaning weight of 13-15 kg each. Kenguri is a purely mutton purpose breed of sheep, also known as Tengury. Kenguri breed is known for its medium body size and longer legs. Their body colour is mostly dark brown along with fleece. The breed is known to thrive well under scarcity condition and sparse vegetation. It is well documented that, factors such as management of rams and ewes, seasonality, synchronization treatment or the technique of insemination itself may affect the reproductive success of ewes after cervical AI with cooled semen [3]. Climatic conditions have also been reported as cause of reproductive function impairment in sheep [4].
Real-time ultrasound imaging provides an easy and reliable means of pregnancy and litter size diagnosis in many species. Ewes are usually examined with external probes between 40 and 100 days gestation but earlier pregnancy makes it difficult to image with external probes. However, a probe inserted per rectum provides closer opposition to the uterus that improves imaging [5].

Materials and Methods

Experimental Design

The present study "reproductive performance evaluation of Kenguri ewes by breeding with NARI-SUWARNA frozen thawed semen" was carried out during the breeding season of sheep. The study was carried out on 30 Kenguri ewes that had previously lambed in the age group of 3-4 years belonging to sheep unit, ILFC, Veterinary College, Bidar. All the ewes were monitored under uniform management conditions and reared under the semi-intensive housing system.

Estrus synchronization: All these Kenguri ewes were divided equally into 3 groups then were weighed and dewormed with Albendazole liquid. The estrous cycle of the ewes was monitored. The estrous cycle was synchronized as per the following protocol.

GROUP 1: (n=10)	PGF2α	PGF2α	A. I. twice
	i----------i----------1		
	0 day	9 days	10 days
GROUP 2: (n=10)	PGF2α	PGF2α	A. I. twice
	1----------,----------[		
	0 day	11 days	12 days
GROUP 3: (n=10)	Detection of estrus and A. l		
Control	1----------1		
	Control as per estrus exhibition		

Hormonal preparations: Synthetic analogue of prostaglandin containing Cloprostenol sodium was used in the present experimentation. Trade Name Pragma™, each ml containing 263µg/ml Cloprostenol sodium, administered intramuscularly, with a luteolytic dose of 1 ml (263 µg) and is available as 2 ml vial.

Estrus detection and artificial insemination: The estrus was detected in all the ewes by parading vasectomized rams twice in a day early in the morning and evening hours. All the experimental ewes were inseminated twice with gap of 12 hours using frozen thawed semen of NARI Suwarna (Deccani + Garole) strain (Semen straws procured from Nimbkar Agriculture Research Institute, Phaltan, Dist. Satara, Maharashtra state). The day of the artificial insemination was recorded as day 0 for calculating the approximately gestational stage of the ewe that was later confirmed from the lambing date.

Pregnancy diagnosis: Pregnancy diagnosis was initiated on days 24, 34 and 44 of post insemination using trans-abdominal ultrasonography.

Trans-abdominal ultrasonography (Day 24, 34 and 44 after breeding): All the ewes were fasted by withholding food and water overnight for 12 hours before scanning. The ewes were scanned trans-abdominal on days 24, 34 and 44 by using scanner equipped with probe of 3.5 MHz sector probe (SAMSUNG Sync Master E 1720 Logic Book XP). The ventral abdominal wall was shaved closely and transducer was applied at inguinal regions of both sides after adding coupling gel. A ewe was designated as pregnant by imaging apparent conceptus (anechoic, elongated structures) within uterine fluid.

Birth weight, liter size and sex ratio: The gestation length, birth weight, litter size and sex ratio of lambs was recorded. The statistical data was analyzed as per standard procedure. The data obtained from various parameters was analyzed as per standard statistical procedure.

Results and Discussion

Estrus synchronization: Group-1 ($PGF_2\alpha$ on days 0 and 9): Out of 10 Kenguri ewes synchronized for estrus, 5 ewes (50.00%) shown estrus onset with mean period of 44.70 ± 0.12 hours after 1st dose of $PGF_2\alpha$ with estrus duration of 30.00 hours while remaining 5 ewes (50.00%) shown estrus onset with mean period of 68.00 ± 0.00 hours after 2nd dose of $PGF_2\alpha$ with estrus duration of 34.00 hours respectively. The overall estrus rate was 100.00% by injecting $PGF_2\alpha$ on days 0 and 9 (Table 1).

Group-2 (PGF2α on days 0 and 9): Out of 10 Kenguri ewes synchronized for estrus, 7 ewes (70.00%) shown estrus onset with mean period of 52.14 ± 2.42 hours after 1st dose of $PGF_2\alpha$ with estrus duration of 33.00 hours while remaining 3 ewes (30.00%) shown estrus onset with mean period of 44.00 ± 0.00 hours after 2nd dose of $PGF_2\alpha$ with estrus duration of 32.00 hours respectively. The overall estrus rate was 100.00% by injecting $PGF_2\alpha$ on days 0 and 11 (Table 1).

Table 1: Estrus synchronization in Kenguri ewes

Groups	Group – 1 (PGF2α on day 0 & 9)	Group – 2 (PGF2α on Day 0 & 11)
No. of ewes	10	10
Ewes in estrus after 1st PGF2α (n)	5	7
Estrus rate (%)	50	70
Onset of estrus (h)	44.70±0.12	52.14 • ± 2.42
Duration of estrus (h)	30±0.0	33±0.0
Ewes in estrus after 2nd PGF2α (n)	5	3
Estrus rate (%)	50	30
Onset of estrus (h)	68±0.0	44±0.0
Duration of estrus (h)	34±0.0	32±0.0
Overall estrus rate (%)	100	100

Control Group

Out of 10 Kenguri ewes, seven ewes (70.00%) shown estrus in control group with mean estrus duration of 36.00 ± 0.00 hours. Results presented in Table 1 show that, estrus rate after PGF2α injection was not affected by treatment being 50% and 70% after first PGF2α and 50% and 30% after 2nd PGF2α on day 9 and 11 respectively. This means that exhibiting estrus in ewes was highest (100%) in both the treatment groups. Onset of estrus after PGF2α injection was shorter in Group 1 (PGF2α on day 0 & 9) than Group 2 (PGF2α on day 0 & 11). Meanwhile, estrus duration was not affected by treatment ranged between 30 to 33 hours in both treatment groups (Table 1).

The earlier study compared the double injection system (125-µg cloprostenol 11 days apart) with a single injection and a combination of short-term *progestagen* treatment (MAP, 5 days) with cloprostenol injection at sponge removal in Clun Forest ewes. In agreement with the present findings, they found a 100% estrus response in the double injection and MAP-PGF combination treatment, whereas estrus response was reduced in the single injection group (52.9%; P<.05) [6]. The present findings of estrus synchronization are in line with the findings wherein although MAP + eCG protocol presented superior results, because it increased Progesterone serum concentrations, PGF2α protocol was as efficient in synchronizing estrus as the former [7]. Estrus synchronization programme in ewes commonly involve the synchronization of luteal regression using prostaglandin treatment. Therefore, improved synchronization of estrus could be achieved by the synchronization of the follicular waves in addition to the synchronization of luteolysis [8].

Pregnancy Diagnosis: Trans-abdominal ultrasonography (Days 24, 34 and 44 after breeding)

In-group 1, out of 10 Kenguri ewes 5 were diagnosed pregnant on days 24 and 34, but only 1 ewe was pregnant on day 44 by trans-abdominal ultrasonography. Out of 10 Kenguri ewes, only one ewe lambed with 10% lambing percentage. In-group 2, out of 10 Kenguri ewes 3 were diagnosed pregnant on days 24 and 34 but only 1 ewe was pregnant on day 44 by trans-abdominal ultrasonography. Out of 10 Kenguri ewes, only one ewe lambed with 10% lambing percentage. In control group, out of 10 Kenguri ewes 2 were diagnosed pregnant on day 24 while 1 was pregnant on day 34 but none of ewe was pregnant on day 44 by trans-abdominal ultrasonography. Out of 10 Kenguri ewes, none of ewe lambed with 0% lambing percentage (Table 3).

As per Table 2, 100% sensitivity was noted in Group 1 and Group 2 for ultrasonography for pregnancy diagnosis in Kenguri ewes on days 24 and 34 respectively. However, in control group, even up to day 44 of ultrasonography the sensitivity was 0% for pregnancy diagnosis in Kenguri ewes. The specificity was highest (77.78%) in Group 2 for the pregnancy diagnosis in Kenguri ewes by ultrasonography on day 34 followed by control group on day 44 (70%) and least in-group 1 for day 24 (55.56%) (Table 2). The predictive values were higher in Group 2 for day 34 (33.33%) followed by Group 1 on day 24 (20%) and then followed by control group on day 44 (0%) for pregnancy diagnosis by using ultrasonography in Kenguri ewes. The negative predicted values for pregnancy diagnosis in Kenguri ewes were 100% in all the three groups (Table 2)

In contrast to the present findings, the sensitivity and specificity of the technique were high after 29 days, reaching approximately 100% from days 46 to 106 of gestation [9, 10]. In one more study reported high accuracy for pregnancy diagnosis by trans-abdominal B-mode ultrasonography (94 % to 100%) and the determination of fetal numbers (92% to 99%) at days 29 to 106 of gestation. They concluded that, real time B-mode ultrasonography appears to be the most practical and accurate method for diagnosing pregnancy and determining fetal number in sheep [11]. In another study, the accurate and sensitive diagnosis of pregnancy by real-time trans-abdominal ultrasonography can successfully be made 100% accuracy between 61 – 81st days and 94.10% accuracy between 46 and 60th days of pregnancy in sheep [12].

Table 2: Sensitivity (Se), specificity (Sp) and predictive (+PV, - PV) values of using trans-abdominal (5 MHz) ultrasonography for pregnancy diagnosis in Kenguri ewes

Groups	Control			Group -1			Group -2		
Day of ultrasonography	24	34	44	24	34	44	24	34	44
Correct +ve (Pregnant)	0	0	0	1	1	1	1	1	1
Correct –ve (Nonpregnant)	8	9	0	5	5	9	7	7	9
False +ve (Nonpregnant)	2	1	0	4	4	0	2	2	0
False –ve (Pregnant)	0	0	0	0	0	0	0	0	0
Se %	0	0	0	100	100	100	100	100	100
Sp %	80.00	90.00	0	55.56	55.56	100	77.78	77.78	100
Predictive PV+ ve %	0	0	0	20	20	100	33.33	33.33	100
Predictive PV –ve %	100	100	0	100	100	100	100	100	100

Table 3: Comparative pregnancy detection by ultrasonography and lambing rate in Kenguri ewes

Groups	No. of ewes	Ultrasonography on days			Lambing	
		24	34	44	No. of ewes	%
Control	10	2	1	0	0	0
Group 1	10	5	5	1	1	10
Group 2	10	3	3	1	1	10

However, the pregnancy rate achieved in this study was clearly lower than that achieved in Norway, despite the fact that Norwegian semen processing and insemination techniques were employed. The lower pregnancy rate is also unlikely to be due to insemination techniques as levels of fertility obtained with fresh semen were acceptable [13]. Differences in pregnancy rate may be due to factors such as timing of ovulation or the structure of the cervix, which in turn may be breed dependent. Asynchrony of AI and ovulation is probably the commonest cause of failure of AI [14]. The effect of asynchrony is likely to be exacerbated when frozen-thawed semen is used. The time ovulation varies with

many factors, including type of synchronization, breed and season and varies both within and between flocks [15].

Conclusion

The estrous cycle was synchronized by injecting $PGF_2\alpha$. on days 0 and 9 in Group 1 and day 0 and day 11 in Group 2n with 100 % estrus induction rate in Kenguri ewes. The incidence of false positive pregnancy diagnosis was higher on days 24 and 34 by using trans-abdominal ultrasonography. The climate temperature, heat stress may be one of the reasons for the early embryonic mortality in Kenguri ewes. The lambing rate by inseminating the Kenguri ewes with frozen thawed semen was lower (10%) in trans-cervical method.

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