The Pharma Innovation Journal 2021; SP-10(11): 920-923

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(11): 920-923
© 2021 TPI
www.thepharmajournal.com
Received: 01-09-2021
Accepted: 03-10-2021

Nischitha BM
Department of Veterinary Gynaecology and Obstetrics, Karnataka Veterinary Animal Fisheries Science, Bidar, Karnataka, India

G Sudha
Department of Veterinary Gynaecology and Obstetrics, Karnataka Veterinary Animal Fisheries Science, Bidar, Karnataka, India

KS Gunaranjan
Department of Veterinary Gynaecology and Obstetrics, Karnataka Veterinary Animal Fisheries Science, Bidar, Karnataka, India

GP Kalmath
Department of Veterinary Animal Physiology and Department of Animal Extension, Karnataka Veterinary Animal Fisheries Science, Bidar, Karnataka, India

V Jagadeeshwary
Department of Veterinary Animal Physiology and Department of Animal Extension, Karnataka Veterinary Animal Fisheries Science, Bidar, Karnataka, India

Corresponding Author
G Sudha
Department of Veterinary Gynaecology and Obstetrics, Karnataka Veterinary Animal Fisheries Science, Bidar, Karnataka, India

Relationship of *in vivo* and *in vitro* post thaw seminal parameters of Bandur Ram semen

Nischitha BM, G Sudha, KS Gunaranjan, GP Kalmath and V Jagadeeshwary

Abstract
The present study was conducted to evaluate relationship of post thaw laboratory seminal parameters to *in vivo* fertilization rate in Bandur rams. Five adult Bandur rams were selected for the study with four collections per ram, total 20 collections were used for *in vitro* evaluation and 100 multiparous ewes were selected for *in vivo* evaluation. Total of 140 semen straws were used from five rams. From each ram with four ejaculates, 28 semen straws were used and for each collection seven straws of which five straws were used for *in vivo* evaluation and two straws for *in vitro* evaluation. The mean per cent obtained was post thaw motility of 35.40 ± 2.25, 44.53 ± 2.71 of viability, 33.80 ± 3.01 of plasma membrane integrity, 96.01 ± 0.25 % of acrosome integrity and 8.55 ± 0.79 per cent of total morphological defects (head defect 2.50 ± 0.44 %, mid piece defect 2.33 ± 0.28 % and tail defect was 3.33 ± 0.28 %). The mean conception rate obtained at field level was 43.00 ± 3.64 %. No significant relation was established in between laboratory parameters and *in vivo* conception rate. From the field study it is concluded that the results of artificial insemination vary between the flocks and largely depends on the nutritional status of the ewes and conception rate was affected by detection of estrus, depth of penetration of the cervix and skill of the inseminator.

Highlights
- *In vitro* evaluation of post thaw semen quality in Bandur rams.
- Relationship of *in vitro* evaluation to *in vivo* fertilization rate in Bandur rams.
- No significant relation was found in between *in vitro* and *in vivo* fertilization.

Keywords: Bandur ram semen, cryopreservation, *In vitro*, *In vivo*, conception rates

Introduction
Artificial insemination (AI) plays a major role in sheep production for genetic improvement, conservation of the ovine breeds and prevention of spread of the contagious diseases (Donovan *et al*., 2001; Halbert *et al*., 1990) [8]. To improve the efficiency of AI, laboratory evaluation of semen characters like ejaculate volume, mass sperm motility, progressive motility, viable sperm concentration and percentage abnormal sperms prior to insemination is a major indicator for the assessment of the conception rates (Amman and Hammerstedt, 1993; Al-Ghalban *et al*., 2004) [5, 3]. Hence there is a need to develop an effective *in vitro* laboratory measure of sperm function that correlate reliably with *in vivo* fertility (O'meara *et al*., 2008) [11]. The correlation between laboratory tests and *in vivo* fertility will vary depending on how *in vitro* fertility was defined and how field fertility data were obtained and presented (Larsson and Rodriguez- Martinez, 2000) [10]. Several authors have opined that few techniques have been designed to correlate *in vivo* relatively with *in vitro* fertility. The present study was conducted to evaluate the relationship between the laboratory test and *in vivo* conception rate of the frozen Bandur ram semen.

Material and Methods
Selection of rams, semen collection and extension
The study was carried out in five apparently healthy adult Bandur rams aged 2- 5 years with body condition score of 3-4 housed at Small Ruminant Semen Station (SRSS) located at Veterinary College, Hebbal, Bangalore (12.9716 °N, 77.5946 °E). Semen was collected from all the five rams using artificial vagina maintained at 42 ^{0}C under adequate pressure during collection. Good quality semen with sperm motility greater than 90 per cent was used for cryopreservation. The semen was extended using Tris-egg yolk- citrate glycerol extender (pH 6.8-7.0).

Following cryopreservation, semen straws of different collections from each ram was thawed at 37 ^{0}C for 30 sec and evaluated for motility. The collections showing ≥ 30 percent post thaw motility were used for *in vitro* and *in vivo* study. Total of 140 semen straws were used from five rams. From each ram with four ejaculates, 28 semen straws were used and for each collection seven straws of which five straws were used for *in vivo* evaluation and two straws for *in vitro* evaluation.

Post thaw evaluation

Post thaw motility, viability, plasma membrane integrity and acrososme integrity were evaluated in the laboratory. Post thaw motility was carried out using phase contrast microscope (OLYMPUS™, CX41). Under 200X magnification motility was assessed based on progressive forward movement of the sperm at different fields. Post thaw viability was assessed using eosin and nigrosin stain as per standard procedure (Agarwal *et al.*, 2016) [1]. Minimum of 200 sperms were counted from different microscopic field under 400X magnification using phase contrast microscope and viability was determined. Hypo Osmotic Swelling Test (HOST) was used for the assessment of the functional plasma membrane integrity (Jeyendran *et al.,* 1984) [9]. Minimum of 200 sperm cells were analyzed randomly. The spermatozoa with varying degree of coiled tails were considered as HOST positive and percentage was assessed under 400X magnification using phase contrast microscope. Acrosome integrity was performed using Giemsa stain. The stained smear was examined for minimum of 200 sperm cells using phase contrast microscope under 400X magnification. Acrosome integrity was graded by visual examination of the stained spermatozoa. Intact acrosome partially lost or damaged acrosome was recorded as per the procedure. Morphology defects of the spermatozoa was evaluated using rose Bengal stain. Two hundred stained spermatozoa were observed in different fields under 400X using phase contrast microscope. Morphological abnormalities were analyzed as head, mid-piece and tail abnormalities.

***In vivo* evaluation**

Selection of ewes

The laboratory evaluated semen samples were used for artificial insemination at field level for fertility evaluation in 100 ewes aged between 2-4 years. Two hundred and forty ewes belonging to farmers around Bangalore (12.9716 °N, 77.5946 °E) were screened by ultrasonography (EASI-SCAN™, BCF Technology Limited, UK) for pregnancy status and animals were managed under grazing system. The selected ewes were synchronized with short term progesterone protocol (Wildeus, 2000). Using 350 mg progestagen intravaginal sponge (Avikasil-S, CSWRI, Avikanagar, Rajasthan, India) left in situ vaginally for seven days. On seventh day with the removal of sponge 125 mcg cloprostenol sodium (ESTRUMATE®, MSD Animal health, Pune, India). Cervical insemination was done with frozen thawed semen after 48-55 hrs of sponge removal and 4 mcg of Buserelin acetate (RECEPTAL® VET, MSD Animal health, Pune, India) administered intramuscularly. After insemination the ewes were segregated and fenced separately for seven days from rams. Pregnancy diagnosis was done using trans-abdominal ultrasonography (EASI-SCAN™, BCF Technology Limited, UK) using linear probe 5/7.5 Mhz after 30-35 days of insemination. Conception rate was correlated with the *in vitro* evaluation of semen parameters.

The collected data was subjected for Pearson's correlation; the coefficients were determined to provide a linear association between the seminal parameters and *in vivo* fertility. Regression analysis was used to establish the prognostic values of semen quality parameters for in vivo fertility as a dependent variable. *In vitro* data was subjected for ANOVA in between the rams (table 1). The significance level was $p < 0.05$.

Results

Relationship between post thaw seminal parameters with conception rates.

The conception rates obtained in our study was 43.00 ± 3.64 per cent with cervical insemination with mean post thaw motility between rams was 35.40 ± 2.23 percent. There was no significant relationship of post thaw motility with conception rate between the rams. The overall mean percentage of viability of all the rams was 44.53 ± 2.71 per cent. The mean percent of viability in between rams with conception rates did not vary significantly and there was negative correlation found in between the viability and post thaw motility (p= 0.016, r= -0.186). The mean percentage of plasma membrane integrity of all the rams was 33.80 ± 3.01 per cent. The mean percentage of plasma membrane integrity in between rams with conception rates did not vary significantly (p <0.05). The mean percentage of the values of acrosome integrity of all the rams is 96.08 ± 0.25 per cent. The mean percentage of the values of acrosome integrity in between rams and with correspondence to the conception rates did not vary significantly and there was negative correlation between acrosomal integrity and plasma membrane integrity (p=0.040, r= -0.125). The mean percentage of tail morphological defects was 3.33 ± 0.57, mid piece defects 2.33 ± 0.28, Head defects 2.50 ± 0.44 and total morphological defects 8.15 ± 0.79 per cent The total morphological defects in correspondence to the conception rates did not vary significantly in between the rams and total morphological defects was negatively correlated with the conception rates (p=0.056, r= -0.367).

Table 1: *In vitro* post thaw seminal parameters and *in vivo* conception rates in Bandur ram semen (Mean ± SE).

RAM	PTM	VIA	PMI (HOST)	ACI	MD Head defects	MD Mid piece defects	MD Tail defetcs	MD Total	CR
R1	33.75±1.25	32.38±2.51	28.62±5.14	96.37±0.23	4.50±0.94^a	3.62±0.46^a	3.25±0.42	11.37±1.35	45.00±2.50
R2	43.75±2.36	46.37±2.30	35.00±4.33	95.00±0.17	2.25±0.23^{ab}	2.25±0.23^{ab}	2.62±0.18	7.12±0.32	50.00±6.45
R3	33.75±2.13	55.50±1.61	37.00±3.15	96.00±0.33	2.50±0.10^{ab}	2.50±0.10^{ab}	2.87±0.21	7.87±0.25	40.00±4.08
R4	30.75±4.37	45.00±3.63	29.12±2.75	96.37±0.31	2.00±0.14^{ab}	2.00±0.14^{ab}	3.00±0.32	7.00±0.59	35.00±4.78
R5	35.40±2.04	47.37±1.75	36.75±2.59	96.62±0.27	1.25±0.23^b	1.25±0.23^b	4.87±1.39	7.37±1.20	45.00±2.50
Total	35.40±2.23	44.53±2.71	33.80±3.01	96.08±0.25	2.50±0.44	2.33±0.28	3.33±0.57	8.15±0.79	43.00±3.63

Note: Values within the columns with different superscript indicates significance. Mean ± SE, (P<0.05).

PTM (Post thaw motility), VIA (Viability), PMI (plasma membrane integrity), ACI (Acrosome integrity), MD Head (Morphological defects of head), MD Mid piece (Morphological defects of mid piece), MD Tail (Morphological defects of Tail), MD total (Total morphological defects), CR (Conception rates)

Discussion

In the comparison between the *in vitro* and *in vivo* fertility in bandur ram semen cryopreserved using tris egg yolk cryprotectant. The mean conception rate recorded in the present study was 43.00 ± 3.63 per cent with the post thaw motility of 35.40 ± 2.23 per cent, viability of 44.53 ± 2.71 per cent, HOST 33.80 ± 3.01 per cent, acrosome integrity of 96.08 ± 0.25 per cent and total morphological defects of 8.15 ± 0.79 per cent respectively. The lambing rates obtained by Aisen *et al*. (2002) [2] in merino ewes was 18.50 per cent using cervical insemination of Pampinta sheep breed semen with 45.00 per cent of post thaw motility, 70.00 per cent acrosome integrity and 40.00 per cent of HOST. The obtained conception rate was higher than compared to Byrne *et al.* (2000) [6] with 49.0 ± 4.14 per cent intact acrosome with fertilization of 39.30 per cent which was inseminated cervically, where as conception rate obtained by Silva *et al.* (2011) [15] was 66.67 % with post thaw motility of 69.01 ± 1.97, plasma membrane integrity of 35.06 ± 3.87 and 29.50 ± 7.42 per cent acrosome integrity respectively. As stated by Allai *et al.* (2018) [4] the conception rates will not only depend on the seminal parameters and successful fertilization but also, the anatomical structure of the cervical canal which hinders the penetration of the insemination catheter. There is no consistency between the assessment of seminal parameters *in vitro* and field fertility after AI (Paulenz *et al*., 2005) [12]. Despite the achievement of high (88.00-93.00 %) egg fertilization rates with frozen thawed semen after uterine insemination, the rates of non-return after 23 days and of lambing were poor (34.00 %, 23/68; and 25.00 %, 17/68) due to excessive embryonic mortality (Salamon and Lightfoot, 1970) [3]. They found that embryonic mortality was low after cervical insemination and a substantial loss occurred after uterine deposition of semen. However, they reported higher embryonic mortality for frozen-thawed than for fresh semen. It seems, therefore, the embryonic mortality is an additional factor contributing to low fertility after cervical insemination with frozen semen apart from transport and viability of spermatozoa. However, this could vary depending on the method and time of insemination, inadequate transport and reduced viability of spermatozoa in the ewe's tract. There are also claims of faster transport of frozen-thawed spermatozoa after cervical insemination into the oviducts and 4- 6 hrs of survival. Apart from the viability of spermatozoa in the female tract, fertility depends on the probability of penetration of spermatozoa to the site of fertilization. Fertility after cervical insemination seems to be linked with transport and survival of spermatozoa *in vivo*, and has also been examined by comparing the egg fertilization rates after cervical and surgically performed tubal or uterine inseminations (by laparotomy) (Salamon and Maxwell, 1995) [14].

Conclusion

Although laboratory seminal parameters assessed were in acceptable range they were not significantly related to conception rates. It may be concluded from the field study that the results of artificial insemination vary between the flocks of the same village, and it largely depends on the nutritional status of the ewes. As ewes in the selected villages were on zero maintenance (grazing only) and BCS of 1.5-2 it affected the expression of the heat and conception rate. Conception rate was also affected by depth of penetration of the cervix and skill of the inseminator.

In order to obtain better conception rates using artificial insemination, at field level it is recommended to conduct insemination at detected estrous, choose apparently healthy ewes with better body condition score; exclude maiden ewes and lactating ewes, reduce stress to ewes by proper restraining during insemination, follow proper thawing of frozen semen, reduce time of deposition and improve penetration.

References

1. Agarwal A, Gupta S, Sharma R. Eosin-nigrosin staining procedure. In: andrological evaluation of male infertility. Edt. Cham., springer international publishing, 2016, 73-77.
2. Aisen EG, Medina VH, Venturino A. Cryopreservation and post-thawed fertility of ram semen frozen in different trehalose concentrations. Theriogenology 2002;57(7):1801-1808.
3. Al-Ghalban AM, Tabbaa MJ, Kridli RT. Factors affecting semen characteristics and scrotal circumference in Damascus bucks. Small rumin. Res 2004;53(1-2):141-149.
4. Allai L, Benmoula A, Da Silva MM, Nasser B, El Amiri B. Supplementation of ram semen extender to improve seminal quality and fertility rate. Anim. Reprod. sci 2018;192:6-17.
5. Amman RP, Hammerstedt RH. *In vitro* evaluation of sperm quality: an opinion. J Androl 1993;14(6):397-406.
6. Byrne GP, Lonergan P, Wade M, Duffy P, Donovan A, Hanrahan JP *et al*., Effect of freezing rate of ram spermatozoa on subsequent fertility *in vivo and in vitro.* Anim. Reprod. Sci 2000;62(4):265-275.
7. Donovan A, Hanrahan JP, Lilly T, Boland MP, Byrne GP, Duffy P. AI for sheep using frozen-thawed semen. End of project report: sheep series no. 11 teagasc. Agriculture and food development authority. 2001.
8. Halbert GW, Dobson H, Walton JS, Buckrell BC. The structure of the cervical canal of the ewe. Theriogenology 1990;33:977-992.
9. Jeyendran RS, Van Der Ven HH, Perezpelaez M. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fertil 1984;70:219-228.
10. Larsson B, Rodriguez-Martinez H. Can we use *in vitro* fertilization tests to predict semen fertility? Anim. Reprod. Sci 2000;60:327-336.
11. O'meara CM, Hanrahan JP, Prathalingam NS, Owen JS, Donovan A, Fair S *et al*. Relationship between *in vitro* sperm functional tests and *in vivo* fertility of rams following cervical artificial insemination of ewes with frozen-thawed semen. Theriogenology 2008;69(4):513-522.
12. Paulenz H, Söderquist L, Ådnøy T, Nordstoga AB, Berg KA. Effect of vaginal and cervical deposition of semen on the fertility of sheep inseminated with frozen-thawed semen. Vet. Rec 2005;156(12):372-375.
13. Salamon S, Lightfoot RJ. Fertility of ram spermatozoa

frozen by the pellet method. Iii. The effects of insemination technique, oxytocin and relaxin on lambing. J. Reprod. Fertil 1970;22:409-42.
14. Salamon S, Maxwell WMC. Frozen storage of ram semen ii. Causes of low fertility after cervical insemination and methods of improvement. Anim. Reprod. Sci 1995;38(1-2):1-36.
15. Silva SV, Soares AT, Batista AM, Almeida FC, Nunes JF, Peixoto CA. *In vitro* and *in vivo* evaluation of ram sperm frozen in tris egg-yolk and supplemented with superoxide dismutase and reduced glutathione. Reprod. Domest. Anim 2011;46(5):874-881.