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Effect of post-thaw supplementation of seminal plasma to the cryopreserved spermatozoa of Malabari bucks on *in vivo* fertility#

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Abstract

The study was conducted to assess the effect of post-thaw supplementation of seminal plasma to the cryopreserved spermatozoa of Malabari bucks on in vivo fertility. The ejaculates collected from Malabari bucks of good (n=3) and poor (n=3) semen freezability were pooled separately and cryopreserved. After thawing, seminal plasma from good semen freezability bucks was supplemented in such a way that group I (GFSP) included thawed semen of good freezability with supplementation, group II (GF) included thawed semen of good freezability without supplementation, group III (PFSP) included thawed semen of poor freezability with supplementation and group IV (PF) included thawed semen of poor freezability without supplementation. After a period of incubation for 10 min. at 37°C, the semen from each group was used for transcervical insemination of Malabari does of proven fertility. The results showed that there was a significant improvement in the conception rate with post-thaw supplementation. Percent of animals kidded also differed significantly in the four groups. But, prolificacy did not differ in GF and GFSP.

Keywords: *Post-thaw supplementation, seminal plasma, cryopreserved, Malabari bucks, conception rate*

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Malabari, the native goat breed of Kerala has good milk and meat production ability, high prolificacy and adaptability. As dual-purpose animals, Malabari goats play a substantial role in the livestock economy of Kerala. Hence, it is essential to establish a good population of this breed with high genetic merit. Artificial Insemination (AI) is advantageous in this aspect for the rapid and widespread distribution of selected germplasm of superior sires. AI using cryopreserved semen is being used in goats but, the results are not up to that of bovines (Behera, 2012).

Even though cryopreservation is beneficial for the long-term preservation of germplasm and their widespread use over distance, the process of freezing and thawing inflicts irreversible damage to the spermatozoa which negatively affects their fertilisation potential. Cryopreservation also results in sublethal damages which are not fatal to the spermatozoa but, affect the fertilisation. Cryoelution or loss of proteins from the spermatozoan membrane during cryopreservation is such a sublethal damage associated with the post-thaw fertility of spermatozoa (Pini *et al.,* 2018).

As a mode of compensation, the beneficial effect of post-thaw supplementation of seminal plasma to the cryopreserved spermatozoa has been recorded in ram (Maxwell *et al*., 1999) and boar (Okazaki *et al*., 2009; Garcia *et al*., 2010) and such studies are lacking in buck semen. Hence, the study was conducted to evaluate the effect of post-thaw supplementation of seminal plasma to the cryopreserved spermatozoa of Malabari bucks on *in vivo* fertility.

Materials and methods

The entire study was carried out during the period from January 2021 to December 2022 with 108 semen ejaculates collected from Malabari bucks of good and poor semen freezability (three each) aged two to three years and weighed 42-46 kg, maintained under uniform feeding, housing and other managemental conditions at the Artificial Insemination Centre, Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy. The six animals selected for the study were of previous records regarding semen freezability and it was confirmed with a minimum of six cryopreservation followed by their post-thaw evaluation for each buck. Thus, three animals with more than 35 per cent progressively motile spermatozoa were considered as of good semen freezability (Group A) and those with less than 30 per cent progressively motile spermatozoa were considered as of poor semen freezability (Group B).

Semen ejaculates were collected from the selected bucks using Danish-type artificial vagina (12×3.8 cm) twice weekly with an interval of two to three days (Behera *et al.,* 2015; Urmila *et al*., 2023). After the initial evaluation, those ejaculates of Group A with more than 80 per cent progressively motile spermatozoa were pooled and the same procedure was adopted for Group B too. The pooled ejaculates were extended to 400 million progressively motile spermatozoa per mL with a tris egg yolk-based extender (Bhai and Joseph, 2015) and packed in French medium straws (IMV, France). Cryopreservation of the straws was carried out in the conventional method of static vapour freezing.

For the post-thaw supplementation, seminal plasma was separated (John, 2016) from Malabari bucks of group A (GFSP) and stored at -80ºC in aliquots. After 24h of cryostorage, the French medium straws containing semen of each group were thawed in a water bath of 37°C for 60s. Following supplementation of thawed seminal plasma (10 %), the semen was incubated for 10 min and used for artificial insemination.

For the *in vivo* fertility studies, healthy Malabari does of proven fertility presented to the AI centre, CVAS, Mannuthy and from the KVASU Goat and Sheep farm, Mannuthy were used (n=75). Cervical insemination of the animals was carried out at 12h and 36h of the observed oestrus as follows:

Pregnancy was confirmed by transrectal ultrasonography using a B mode real-time ultrasound scanner (MyLab, Gamma, Esaote SpA, Italy) with a linear array transrectal

Group	No. of goats inseminated	Semen used for AI
Group I	23	Cryopreserved semen of good freezability + GFSP
Group II	25	Cryopreserved semen of good freezability + GFSP
Group III	16	Cryopreserved semen of poor freezability + GFSP
Group IV	11	Cryopreserved semen of poor freezability + GFSP

transducer (5-10 MHz) by the 60th day of insemination. Conception rate (per cent of does conceived), per cent of kidded and prolificacy (average number of kids per doe conceived) were calculated.

The data obtained were statistically analysed using SPSS (Statistical Package for Social Studies) software version 24.0 in which the Chi-square test was used to compare the conception rate and prolificacy was analysed with ANOVA.

Results and discussion

In the present study, out of the 25 does inseminated with cryopreserved spermatozoa of Malabari bucks having good semen freezability, 13 conceived and the conception rate was 52 per cent. The cryopreserved spermatozoa of Malabari bucks having good semen freezability supplemented with seminal plasma were used to inseminate 23 does of which 17 conceived (73.9 %). In the case of the 11 does inseminated with cryopreserved spermatozoa of Malabari bucks having poor semen freezability, none conceived whereas with the cryopreserved spermatozoa of Malabari bucks having poor semen freezability supplemented with seminal plasma, one doe was conceived out of 16 inseminated (6.25 %) (Table 1).

Table 1. Effect of post-thaw supplementation of seminal plasma to the cryopreserved semen of Malabari bucks on conception rate

Group	No. of goats inseminated	No. of goats conceived	Conception rate (%)
Group I	23	17	73.90[a]
Group II	25	13	52.00[b]
Group III	16	1	6.25[c]
Group IV	11	0	0
Values with different superscripts in column differ at 1 % level			

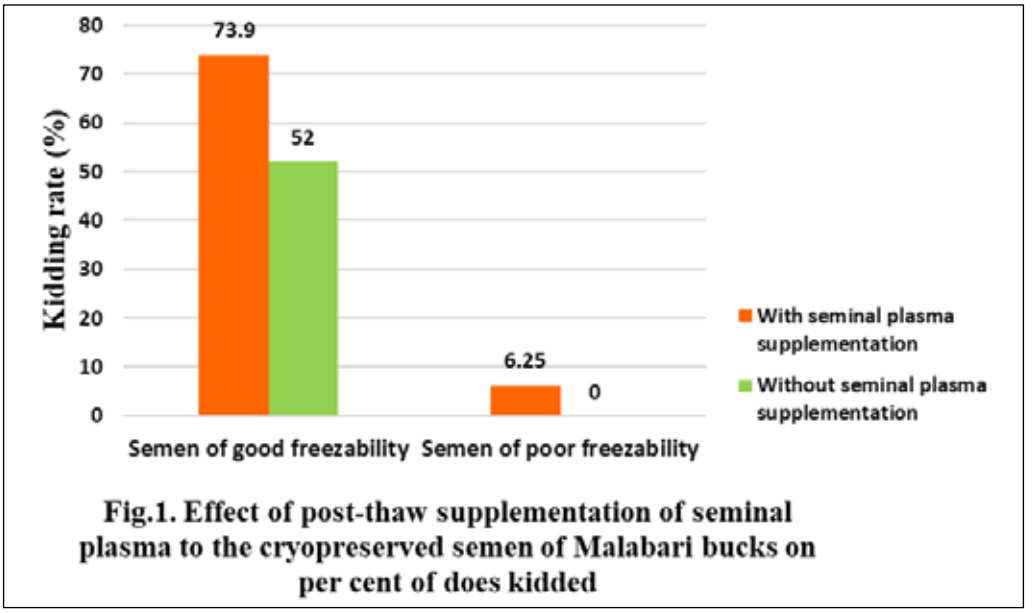

Fig.1. Effect of post-thaw supplementation of seminal plasma to the cryopreserved semen of Malabari bucks on per cent of does kidded

Regarding the effect of post-thaw supplementation of seminal plasma on *in vivo* fertility, there are no published reports for buck semen. However, improvement in conception rate was observed in sheep (Maxwell *et al.*, 1999) and swine (Okazaki *et al.*, 2009; Garcia *et al.*, 2010) when the cryopreserved spermatozoa were supplemented with seminal plasma. These results are consistent with the present results. Yet, contradictory results were reported with the cryopreserved semen of ram (O'Meara *et al.*, 2007) and boar (Ghaoui *et al.*, 2007) in which there was no increase in pregnancy rate with post-thaw supplementation of seminal plasma.

The beneficial effect of seminal plasma supplementation can be attributed to the protective proteins, exosomes containing high concentrations of cholesterol and sphingomyelin as the major phospholipid (Sostaric *et al.*, 2008), metabolic support with energy substrates like fructose, reduction of tyrosine phosphorylation of spermatozoan proteins (Andrade *et al.*, 2012; Okazaki *et al.*, 2012) and antioxidants (Li *et al.*, 2017). Post-thaw supplementation of GFSP could maintain progressive motility, viability and membrane integrity of cryopreserved spermatozoa up to six hours after thawing. Post-thaw oxidative stress was also found to be reduced with supplementation of GFSP (Unpublished data). Such quality maintenance of spermatozoa might have supported the *in vivo* fertility in a better manner compared to the spermatozoa without supplementation of seminal plasma.

Considering the values, a conception rate of 72.22 per cent was obtained in Kacang does (Susilowati *et al.*, 2020) which is similar to the result obtained in Group I. The conception rate reported by Ritar and Salamon (1983) as 53.3 per cent is similar to the result of Group II whereas a lower value of 26.38 per cent (Memon *et al*., 2012) was observed in Boer does. The conception rate of does is influenced by the parity of the does, managemental conditions, source and type of the semen used and the site of insemination. Differences in these factors might have contributed to the varying results compared to other studies (Arrebola *et al.,* 2012).

All the conceived does kidded. Hence, the percentage of animals kidded (Fig 1) was also significantly different in the four groups ($p<0.01$). Corresponding prolificacy were 1.69 ± 0.13, 1.71 ± 0.11, 1 and zero in the respective groups. Prolificacy did not differ significantly with the supplementation of seminal plasma in the good semen freezability group. Results are presented in Fig 2.

Following artificial insemination with cryopreserved semen, a kidding percentage of 38.10 per cent, 43.6 per cent and 66.67 per cent

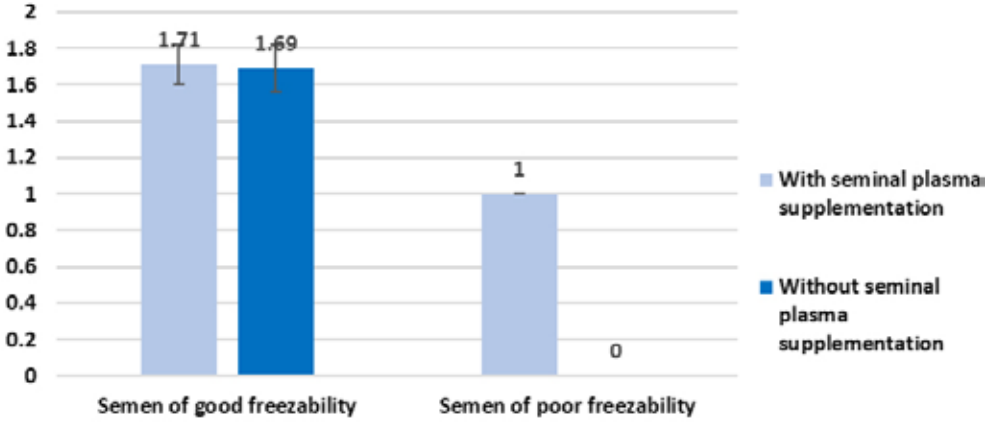

Fig.2. Effect of post-thaw supplementation of seminal plasma to the cryopreserved semen of Malabari bucks on prolificacy

was reported in Florida (Dorado *et al*., 2007), Majorera (Batista *et al*., 2009) and Kacang (Susilowati *et al.*, 2020) breeds, respectively. Further, it was also documented by Arrebola *et al.,* (2012) that kidding could also be affected by the health status and managemental conditions of does too.

Conclusion

Post-thaw supplementation of seminal plasma to the cryopreserved spermatozoa of Malabari bucks resulted in significant improvement in *in-vivo* fertility. However, no significant variation was noticed with respect to prolificacy.

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Conflict of interest

The authors declare that they have no conflict of interest.

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