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Research Article

EFFICACY OF EGG YOLK AND SOYBEAN LECITHIN BASED SEMEN EXTENDERS FOR CRYOPRESERVATION OF KANKREJ BULL SEMEN

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Abstract: Total of 60 ejaculates from 6 Kankrej bulls were collected, split and processed using soybean lecithin based (AndroMed®) and egg yolk based (TFYG) extenders. The various semen parameters and pregnancy rate of cryopreserved semen were assessed. The mean values of individual motility, live sperm, abnormal sperm and acrosomal integrity were significantly ($P < 0.05$) higher in AndroMed® extended semen than the TFYG extended semen. The pregnancy rate was didn't differ significantly in TFYG and AndroMed® extended semen. In conclusion, the animal protein-free extender based on soybean lecithin is a viable alternative to the traditional egg yolk-based extenders for cryopreservation of kankrej bull semen.

Keywords: *Kankrej bull, Cryopreserved semen, Pregnancy rate, Soybean lecithin*

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Introduction

The semen extenders are ionic or non-ionic substances that maintain osmolarity and provide buffering capacity; a source of lipoprotein or high molecular weight material to prevent cold shock such as egg yolk, milk, or soy lecithin; glucose or fructose as an energy source apart from additives in the form of enzymes and antibiotics [1], which as such play an important role in yielding optimum post-thaw recovery of spermatozoa [2]. The egg yolk-based Tris-fructose-egg yolk-glycerol extender has been employed world-wide and recommended because of its excellent protection of sperm cell [3]. But, the addition of egg yolk in extender reduces the respiration and motility of the spermatozoa [4]. Besides, the extender containing additive of animal origin such as egg yolk also poses a potential risk of microbial contamination [5,6]. Therefore, a well-defined and pathogen-free substitute for egg yolk is suitable for extenders used for semen [7]. Researchers have replaced egg yolk and milk in semen extenders by adding soy-lecithin [8]. The unique composition of soy-lecithin and its effect on structural configuration of spermatozoa provided a pave to the researchers for its use as primary source of lipoproteins in semen extenders for cattle [9,10], buffalo [11], ovine [12] and equine [13]. The present study was conducted to compare a cryoprotective extender based on egg yolk with extender free from animal protein.

Materials and Methods

Semen collection and processing

A total of sixty ejaculates were collected within 10 weeks from six healthy mature Kankrej bulls (10 ejaculates per bull; bulls were 5-6 years old) with the aid of an artificial vagina (inside temperature 43°C). High quality semen with more than 80 per cent initial motility was employed. Semen processing was performed by incubating the ejaculate in a water bath at 34°C. Each ejaculate was divided into two aliquots and diluted at room temperature with the cryopreservation extender (final concentration of 20×106 spermatozoa per straw).

Either an egg yolk-free extender (SL) or an egg yolk- containing extender (TFYG) was used. For the former, we used AndroMed® (Minitube GmbH, Tiefenbach, Germany), a novel soybean lecithin based extender. After dilution, semen was processed at room temperature as split samples (0.25 ml (0.25 ml capacity, ministraws-TBS™, IMV, L'Aigle, France) followed by a 3-h equilibration of the straws at 4°C. Finally, semen was cryopreserved in a nitrogen atmosphere under appropriate conditions using a freezing processor (Micro-Digitcool, IMV, L'Aigle, France). Thawing was performed into a water bath at 37°C for 30s.

Post thawed motility

The post thawed motility analysis of cryopreserved semen was carried out on pre-warmed glass slide at 37°C after covering the semen drop with cover glass, under high magnification (45x). The motility was recorded as percentage of progressive motile spermatozoa. For this purpose, a minimum of 4 to 5 microscopic fields were observed and the values were recorded as percentage.

Sperm viability and Abnormality

Differential staining technique was used for counting the live and dead spermatozoa [14]. The morphological abnormalities of spermatozoa were counted from the same slide. The eosin - nigrosin stain was used for staining [15]. For staining, proportion of stain and semen was kept as 6:1. The uniform thin smears were prepared immediately on clean grease free slides. A total of 200 spermatozoa were counted from stained slides using 100X (oil immersion) objective lens of the microscope and values were expressed as percentage

Acrosomal integrity

The acrosome integrity in the spermatozoa was evaluated by simplified Nigrosine-Eosin-Giemsa staining technique [16].

The Eosin-Nigrosine stained smear was washed gently in slow running tape water till the Eosin-Nigrosine stain was completely washed off and was air dried (Before and after washing, few slides at random were counted and compared for the average number of sperms per microscopic field to ensure that sperms are not washed away from the smear). The smear was covered with Geimsa working solution for 3 min, washed in tape water and dried in air and examined under 100X (oil immersion) objective lens of the microscope and values were expressed as percentage. Total 200 spermatozoa were counted in different fields.

Fertility trial

The semen of the Kankrej bulls, cryopreserved either in TFYG or AndroMed® with post-thaw motility more than 50 per cent, was used to inseminate the Kankrej cows at the Livestock Research Station (LRS), Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, as well as in the field, in 9 Data Recording (DR) Units (villages) of LRS distributed in the Banaskantha district.

Artificial insemination was performed once in estrus cows by recto-vaginal method after thawing the frozen semen at 37°C for 30 seconds by the artificial insemination workers in 497 Kankrej cows (216 cows with TFYG and 273 AndroMed cryopreserved sperm). Following artificial insemination, cows non-return to estrus, were examined for pregnancy after 2 months through per-rectal palpation. The pregnancy of each cow was recorded on the basis of positive signs of pregnancy.

Statistics 1

The various semen parameters were compared using unpaired t- test. The data were as Mean ± S.E. The level of significance was set at 5%. The data were analyzed using SPSS statistical software [17].

Results

The various semen parameters such as post thawed motility, viability, abnormality and acrosome integrity were given in [Table-1]. The post thawed motility, percentage of live sperms and intact acrosome were significantly ($P<0.05$) higher in spermatozoa cryopreserved in AndroMed® than the TFYG. The abnormality was significantly reduced in spermatozoa cryopreserved in AndroMed® than the TFYG. The pregnancy rate didn't differ significantly between the spermatozoa cryopreserved in AndroMed® and TFYG [Table-2]. The pregnancy rate was at par in both AndroMed and TFYG cryopreserved semen.

Table-1 *Mean (±SE) post-thaw of seminal characteristics Kankrej bull semen cryopreserved in TFYG and AndroMed® extenders*

Parameter	TFYG	AndroMed®
Individual Motility (%)	53.25 ± 0.71^{x}	60.83 ± 0.85^{y}
Live Sperm (%)	61.59 ± 0.68^{x}	68.85 ± 0.58^{y}
Abnormal Sperm (%)	12.31 ± 0.29^{x}	9.83 ± 0.18^{y}
Acrosomal Integrity (%)	74.68 ± 0.32^{x}	78.60 ± 0.29^{y}

Discussion

The difference in motility between the two diluents (AndroMed® and TFYG) was statistically significant ($P < 0.05$). It has been observed that egg yolk in extender can reduce respiration and motility of the spermatozoa]. High density lipoproteins (HDLs) in egg yolk are one factor that decreases the quality of semen by causing efflux of cholesterol from the sperm plasma membrane and resulted in fluidity that increases the sensitivity to cold shock [18]. Soy-lecithin (SL) extender could play a protective role for sperm cryopreservation due to its low viscosity and less debris. So, this decreased motility could be due to the observation that TFYG extender is more viscous than SL extender. Similar results have found by various researcher [19-22] that SL extender gives better post thaw motility than egg yolk-based diluent.

The semen diluted in soy-lecithin based (AndroMed®) extender showed significantly higher live sperm count and acrosomal integrity and lower sperm abnormalities than TFYG extender in post thawed semen. It is believed that egg yolk can change the sperm chromatin structure which results in poor post thaw viability [23,24]. Researchers have suggested that inconsistent egg yolk composition is detrimental to sperm viability [25]. Soy-lecithin is thought to be a better emulsifier [26], which, in freezing extender might promote cryoprotectants to distribute uniformly and reduce its local concentration, which leads to relieve the toxicity of cryoprotectants on spermatozoa during freezing thawing process [27].

The lower number of spermatozoa with intact acrosome in egg yolk based extender might be due to acrosomal destructions resulting from intrusion of Ca^{2+}, which is present in high concentrations in egg yolk and rapidly enters the cells when the temperature is below 30°C. Moreover, egg yolk was reported to have some factors which can destabilize the sperm plasma membrane and cause capacitation [28]. Researchers believed that steroid hormones and its precursors present in egg yolk were associated with poor fertilizing potential of spermatozoa.

Table-2 *Pregnancy rates (PR %) of TFYG and AndroMed® extended Kankrej bull semen*

	TFYG			AndroMed®		
	N	Pregnant	PR %	N	Pregnant	PR%
Field	207	116	56.04	245	137	55.92
LRS	17	9	52.94	28	15	53.57

The pregnancy rates of AndroMed® extended semen were similar with those obtained with TFYG extended semen. Similarly, non-significant difference in fertility using semen diluted with soylecithin based and egg yolk based extenders has been found in HF bulls and in Brown Swiss and Simmental bulls.

Conclusion

The post thawed motility, viability, and acrosome integrity was significantly improved in cryopreserved semen using Andromed diluent in Kankrej bull. The pregnancy rate of semen cryopreserved using AndroMed® and TFYG was at par. The animal protein-free extender based on soybean lecithin is a viable alternative to the traditional egg yolk-based extenders for cryopreservation of kankrej bull semen.

Application of research: To circumvent problems associated with traditional extenders.

Research Category: Animal Husbandry

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Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: College of Veterinary science & Animal Husbandry, Sardarkrushinagar, 385506

Breed name: Kankrej bulls

Conflict of Interest: None declared

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