JOURNAL OF POULTRY SCIENCE AND TECHNOLOGY
Journal homepage: www.jakraya.com/journal/jpst

ORIGINAL ARTICLE

Pattern of Fertile Period in Kadaknath Using Fresh vs Preserved Semen

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Received: 06/11/2014

Revised: 26/11/2014

Accepted: 27/11/2014

Abstract

The attempts have been made to examine fertility pattern in fertile period in Kadaknath chicken using fresh vs preserved semen. For this study 60 female and 30 male birds from each breed were taken. Standard procedures were used for semen collection, dilution, artificial insemination and fertility examination. The fertility pattern in fertile period of fresh 0, 6 and 24 hr stored semen (at 3-5^{0}C) in all diluents (normal saline, CARI semen diluent, Beltsville Poultry Semen Extender (BPSE), and Lake's semen diluents) was determined in 60 healthy hens from same breed using Artificial insemination (A. I.) technique. A. I. with freshly ejaculated semen (0 hr) indicated that fertilizing ability of spermatozoa in fertile period was reduced gradually and significantly ($P<0.05$) with the increase of duration after artificial insemination (AI) (from 2-12 days) irrespective of the diluents. Similarly a gradual reduction in fertility along with enhancement of fertile period was examined at 6 and 24 hr stored Kadaknath chicken semen. Fertility in fertile period was found very poor in the semen stored and diluted with normal saline for 6 hrs, whereas in the same diluent, no fertility after 24 hrs storage of semen. At all the semen storage period (0, 6 and 24 hrs), CARI semen diluent showed numerically higher fertility in fertile period than other diluent. From this study it may be concluded that CARI diluents is better than other diluents for short term storage of Kadaknath semen at refrigerated temperature.

Keywords: Fertile period, Kadaknath, Semen, Diluents.

Introduction

A significant feature of the reproductive physiology of the hen is her ability to prolong the survival of sperm in oviduct after a single natural mating or A.I. Sperm are able to survive in the oviduct for long period of time because there are specialized regions which receive the sperm, protect and nourish them. These regions are called the sperm storage tubules (SST). Sperm stored in the SST are able to fertile the eggs for a specific period of consecutive days. This period is called the fertile period. The length of fertile period varies according to species such as domestic fowl (White Leghorn WL) - 12 days, duck - 7 days, Japanese quail- 6 days, domestic turkey- 28 days, guinea fowl - 7 days.

Stored spermatozoa from SST tubules are slowly released over time to ensure an adequate population of spermatozoa at the site of fertilization (Bakst, 1993). These tubules are located at the junction of uterus and vagina called utero-vaginal junction. The precise mechanisms supporting prolonged sperm storage in the SST are unknown but are thought to include reversible suppression of respiration and motility of spermatozoa as well as stabilization of the plasma membrane and maintenance of the acrosome (Bakst, 1993). For the maximum fertility, it is important that an optimum number of sperm must enter the SST that needs good eversion of vagina and a deep insemination as close as possible to the SST.

Over the course of egg production the efficiency of the SST decreases, therefore, even late season declines in fertility and hatch are not uncommon with fresh inseminations. When semen is stored 24 h or longer in vitro, fertility problems are magnified due to short fertile period. Avian spermatozoa are normally inseminated into the lower vagina from where, only 1-2% is able to reach and enter the SST at the utero-vaginal junction, where they are subsequently stored for days or weeks before fertilization. Sperm nearly take more than 24 h to reach the upper part of the oviduct (Bakst, 1994). A fertility curve can be drawn for a population of hens based on the daily number of fertile eggs produced after one insemination to each female. Such a curve is an indication of spermatozoa

survival in the oviduct (Lake and Stewart, 1978). The knowledge of fertile period is very helpful in achieving maximum fertility and hatchability in poultry (CARI, 1982).

There are many extenders available for poultry semen but all are complex, expensive, time consuming in nature and difficult to prepare under the field conditions. Besides, these extenders were prepared targeting to exotic (WL) breeds (Lake, 1960; Sexton, 1977). No specific or universal extender is available for Kadaknath (native). Keeping this in view the effect of various diluents (extenders) such as CARI semen diluent, BPSE (Sexton, 1977) and Lake's semen diluents (Lake, 1960) at different storage period (0, 6 and 24 hr) on fertile period was investigated from 2-12 days after A.I. in Kadaknath chicken. Normal saline was also used as it is routinely employed for diluting the avian semen.

Materials and Methods

Thirty healthy and adult males from the same hatch Kadaknath (desi fowl) were maintained in individual cages under uniform husbandry conditions. For the fertile period study, sixty healthy and adult hens from the same hatch of same breed were selected and maintained under uniform management. Semen samples were collected every alternate day during study period by abdominal massage method (Burrows and Quinn, 1937). Pooled semen of Kadaknath chicken was diluted in CARI diluent ($K_2HPO_4.3H_2O$, KH_2PO_4, Sodium glutamate, Sodium acetate and N-TRIS, Tri Potassium Citrate, Magnesium chloride ($MgCl_2$) and Fructose in distilled water with pH and osmolarity, 7.2 and 330 mOsmol/kg H_2O respectively), and other semen dilutor such as normal saline (0.89 %(w/v) NaCl), BPSE (Sexton, 1977) and Lake's diluent (Lake, 1960). One part (1 ml) of good quality of semen was taken in 5 ml round bottom glass tube (length=7cm, diameter=1 cm) and mixed with one parts (1ml) of the respective diluent.

In this way 3 set of each diluent were prepared for A.I. in sixty hens of each breed, which were equally divided in to 4 groups with 15 birds in each at 3 different time intervals (0, 6 and 24 hr after semen collection). Diluted semen samples targeted for AI at 6 and 24 hr were stored at 4- 6^0C. For artificial insemination, vaginal eversion was made by gentle pressure on left side of the abdomen to cause eversion of oviduct (vagina) through the cloaca (Quinn and Burrows, 1936). Subsequently, with the help of tuberculin syringe which contain the doses (60-100 million spermatozoa) of diluted semen, inseminated (0.1 ml) into the well everted oviduct (to the depth 3-4 cm or as close as possible to the sperm host gland) and concurrently with the release of pressure on the abdomen so that oviduct revert to its normal position.

Fertility in fertile period of birds was assessed by incubating the eggs (99.5^0F temperature and 55-60% relative humidity) laid by hens 2 to 12 days after single intra-vaginal insemination. To determine the fertile pattern, fertilization of egg were examined by candling at 9^{th} day of incubation. Some eggs were broken for fertility assessment in which fertility was not clear by candling. The percent fertility was determined by the ratios of number of fertile eggs to the number of total egg set in the incubator. Fertile period was determined for each day separately. Statistical analysis was done using statistical software package (SPSS-16) for ANOVA (Snedecor and Cochran, 1989) and Duncan's multiple range tests (Duncan, 1955) by comparing means for significant differences.

Results and Discussion

Data and fertile period curve on the effect of various semen diluents (extenders) on fertile period (2-12 days) of Kadaknath spermatozoa at different time of interval (i.e. 0, 6 and 24 hr) is presented in Table 1-3. A. I. with freshly ejaculated semen (0 hr store) indicated that fertilizing ability of spermatozoa reduced gradually and significantly ($P<0.05$) with the increase of duration after A.I. (from 2-12 days) irrespective of the diluent (Table 1). The gradual and significant reduction of fertility from 2-12 day after A.I. may be due to the poor retention of sperm in consecutive days by the sperm host gland located at the junction of uterus and vagina (Sturkie, 1986). Similarly a gradual reduction in fertility in fertile period along with enhancement of fertile period was examined at 6 and 24 hr stored Kadaknath semen. Fertility was found very poor in the semen stored for 6 hrs and diluted with normal saline (Table 2), whereas in the same diluent, no fertility after 24 hrs storage of semen at low temperature (Table 3). When semen is stored 24 hrs or longer in-vitro, fertility problems are magnified due to short fertile period (Donoghue and Wishart, 2000). It is of interest to note that the range of fertility in the semen diluted in normal saline and stored for 24 hrs, showed zero fertility. This may be due to the fact that normal saline is lack of energy source (sugar) and sodium glutamate responsible for maintaining osmotic pressure of avian semen. At all the semen storage period, Kadaknath semen exhibited numerically superior fertility in fertile period in CARI semen diluents as compared to others (Table 1-3). This suggests that the ingredient of CARI diluent is comparatively more suitable to the survival of spermatozoa of this breed of chicken.

Table 1: Effect of different semen diluents on fertilising ability of spermatozoa in 0 hr stored semen in Kadaknath (mean ± SEM, n=6)

Stages of fertile period (Days)	Normal Saline	CARI Diluent	BPSE Diluent	Lake's Diluent
2	80.00^{4}±1.83	83.33^{8}±1.12	81.33^{7}±1.28	70.00^{4}±1.83
3	79.33^{4}±1.87	83.33^{8}±1.12	79.00^{67}±1.32	76.67^{45}±2.11
4	78.33^{4}±2.79	82.33^{78}±1.65	80.00^{67}±1.83	79.33^{5}±1.87
5	75.00^{4}±1.83	78.00^{7}±0.97	75.67^{6}±1.87	75.00^{45}±1.83
6	75.00^{4}±1.83	79.00^{78}±0.63	75.00^{6}±1.83	70.00^{4}±1.83
7	60.00^{3}±3.65	70.00^{6}±1.83	65.00^{5}±1.83	70.00^{4}±1.83
8	48.33^{2}±2.79	65.00^{5}±1.83	60.33^{5}±1.65	61.67^{3}±3.80
9	45.00^{2}±1.83	55.00^{4}±1.83	50.00^{4}±1.83	45.00^{2}±1.83
10	31.67^{2}±3.80	49.33^{3}±1.48	40.00^{3}±1.83	30.00^{1}±3.65
11	28.33^{2}±1.05	35.67^{2}±1.48	30.00^{2}±1.83	28.33^{1}±3.80
12	20.00^{1}±1.83	32.00^{2}±1.83	28.00^{2}±1.83	25.00^{1}±1.83

Means values bearing different superscript (1, 2...8) in column differs significantly (p<0.05).

Table 2: Effect of different semen diluents on fertilising ability of spermatozoa in 6 hr stored semen (4-5^{0}C) in Kadaknath (mean ± SEM, n=6)

Stages of fertile period (Days)	Normal Saline	CARI Diluent	BPSE Diluent	Lake's Diluent
2	20.00^{2}±6.58	77.00^{5}±1.32	73.33^{4}±1.05	72.00^{6}±0.73
3	16.67^{2}±4.22	76.67^{5}±0.92	70.33^{4}±1.65	66.67^{56}±4.02
4	20.00^{2}±1.59	74.00^{5}±1.32	74.33^{4}±1.87	58.33^{5}±3.80
5	16.67^{2}±2.79	73.00^{5}±1.32	79.00^{4}±3.48	58.33^{5}±4.59
6	11.67^{12}±1.05	69.67^{5}±3.39	60.33^{3}±3.84	71.67^{6}±2.79
7	11.67^{12}±1.05	19.00^{1}±4.83	14.67^{1}±1.84	11.67^{1}±3.80
8	13.33^{12}±1.05	58.67^{4}±4.40	55.33^{3}±1.84	30.67^{34}±1.48
9	13.33^{12}±1.05	55.00^{34}±1.83	53.33^{3}±5.59	33.67^{4}±2.84
10	6.67^{1}±2.11	40.33^{3}±1.65	43.33^{2}±2.11	30.00^{34}±3.65
11	6.67^{1}±2.11	28.33^{2}±1.05	23.33^{1}±5.58	21.67^{23}±1.05
12	6.67^{1}±1.05	21.67^{12}±1.05	18.33^{1}±1.05	18.33^{12}±3.80

Means values bearing different superscript (1, 2...7) in column differs significantly (p<0.05)

Table 3: Effect of different semen diluents on fertilising ability of spermatozoa in 24 hr stored semen (4-5^{0}C) in Kadaknath (mean ± SEM, n=6)

Stages of fertile period (Days)	Normal Saline	CARI Diluent	BPSE Diluent	Lake's Diluent
2	0.00	73.00^{5}±3.48	68.33^{78}±1.05	53.33^{5}±5.58
3	0.00	74.00^{5}±0.37	71.67^{8}±2.11	43.33^{45}±5.58
4	0.00	68.33^{5}±1.05	61.67^{67}±1.05	46.67^{45}±2.11
5	0.00	67.67^{5}±0.92	60.00^{6}±3.65	50.00^{45}±3.65
6	0.00	68.67^{5}±3.68	61.67^{67}±3.80	46.67^{45}±7.60
7	0.00	43.33^{4}±2.11	45.00^{5}±1.83	36.67^{34}±5.58
8	0.00	38.33^{3}±2.11	36.67^{4}±2.11	30.00^{23}±3.65
9	0.00	25.00^{2}±1.83	25.00^{3}±1.83	28.33^{123}±3.80
10	0.00	18.33^{12}±2.79	21.67^{23}±1.05	23.33^{123}±2.11
11	0.00	16.67^{1}±2.79	16.67^{12}±2.11	15.00^{1}±1.83
12	0.00	18.33^{12}±1.05	11.67^{1}±2.79	18.33^{12}±2.79

Means values bearing different superscript (1, 2...8) in column differs significantly (p<0.05).

Conclusion

It is concluded that CARI diluent is better than other diluents for short term storage of Kadaknath semen.

Acknowledgement

The authors sincerely thank Directors of IVRI and CARI, Izatnagar for providing necessary facilities for research work.

References

Bakst MR (1993). Oviductal sperm storage in poultry: a review: *Reproduction, Fertility* and *Development*, 5: 595-599.

Bakst MR (1994). Oviducal sperm storage in poultry. In: *Proc. 9th European Poultry Conference, Glasgow.*

Burrow WH and Quinn JP (1937). The collection of spermatozoa from the domestic fowl and turkey. *Poultry Science*, 16: 19-24.

CARI (1982). *Proceedings of First National Workshop on AI.* Central Avian Research Institute, Izatnagar.

Donoghue AM and Wishart GJ (2000). Storage of poultry semen. *Animal Reproduction Science*, 62: 213-232.

Duncan B (1955). Multiple range and multiple F tests. *Biometrics*, 11: 1-12.

Lake PE (1960). Studies on the dilution and storage of fowl semen. *Journal of Reproduction and Fertility*, 1: 30-35.

Lake PE and Stewart JM (1978). *Artificial insemination in poultry. Bulletin 213. Her Majesty's Stationary office, Ministry of Agriculture, Fisheries and Food, London.*

Quinn JP and Burrows WH (1936). Artificial insemination in fowls. *Journal of Heredity*, 27: 31-37.

Sexton TJ (1977). A new poultry semen extender. *International Poultry Science*, 56: 1443-1446.

Snedecor GW and Cochran WG (1989). *Statistical Methods.* Eighth ed. Iowa: Blackwell Publishing.

Sturkie PD (1986). *Avian Physiology* (3rd Edn.), Springer-Verlag, New York, USA.