EFFECT OF OSMOTIC PRESSURE AND pH ON THE SHORT-TERM STORAGE AND FERTILITY OF BROILER BREEDER SPERM

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ABSTRACT

Effect of osmotic pressure and pH on the short-term storage of cockerel semen using modified Van Wambeke milk based extender 'Experimental Extender' was studied. Six broiler breeder cockerels were used and 7 ejaculates were collected from each cockerel every second day. In experiment 1, semen was diluted in the Experimental Extender with osmotic pressures 350, 375 or 400 mOsm and pH 7.0 and stored at 5ºC. In experiment 2, semen was diluted in the Experimental Extender with osmotic pressure 375 mOsm and pH 6.4, 7.0 or 7.4 and stored at 5ºC. The effect of osmotic pressure and pH on percentage motility, sperm speed and clumping was evaluated at 4, 24 and 48 hr. There was a significant difference ($P<0.05$) among the osmotic pressures evaluated in terms of percentage motility, speed of sperm cells and extent of clumping during 48 hr of semen storage. The 375 mOsm osmotic pressure was found to be best ($P<0.05$) for the short-term storage of cockerel semen. There was a significant difference ($P<0.05$) among different pH values evaluated in terms of percentage motility after 24 and 48 hr of semen storage. pH 7.0 and/or 7.4 were found to be optimum ($P<0.05$) for the short-term storage of cockerel semen. Fertility/hatchability was higher (88%, $P<0.05$) when sperm were stored in the Experimental Extender with osmotic pressure 375mOsm and pH 7.0, and in Fecondil (86%) as compared to normal saline (79%). In conclusion, the Experimental Extender with 375 mOsm osmotic pressure and pH 7.0 is suitable for the short-term storage of poultry semen and it improves fertility of the spermatozoa as well.

Key words: Broiler, sperm, osmotic pressure, pH, storage.

INTRODUCTION

Artificial insemination (AI) is an imitation system of female birds mating. The method of semen preservation makes it possible to increase the number of hens inseminated by a particular male. Prolonged storage of semen at sub-zero temperatures enables the creation of sperm banks from selected sires to be used after their death. In poultry, AI improves the egg hatchability and thus decreases expenses (Omparakash *et al*., 1992, Figueiuredo *et al*., 1999). Moreover, proportion of males kept for breeding can be reduced. Although labour costs have increased through the use of AI, more chicks are being produced at lower costs on an over all basis (Figueiuredo *et al*., 1999).

The quality of undiluted semen of cockerels deteriorates within 30 minutes or sooner depending upon its initial quality (Lake, 1971). Development of diluents for short or long term preservation of semen will therefore have wider use of AI in poultry and/or pet birds. Compared to other species, the number of spermatozoa per ml of fowl semen is relatively high. Several physiological saline solutions and other fluid media have been used for dilution of cockerel and turkey stag semen to enable its usage for a larger number of females (Sarkar *et al.*, 1995).

In poultry, the use of freshly diluted semen (within 20 minutes) proved to be a means of higher fertility (Figueiuredo *et al.*, 1999)**.** It seems impossible to store undiluted cockerel and turkey semen *in vitro* for more than half an hour without losing its fertilizing capacity. Diluents and semen storage techniques are designed to minimize the rate of loss of viability of spermatozoa *in vitro*. Keeping in view the importance of AI, this study was designed to investigate the effect of different osmotic pressures and pH values on the short-term preservation of cockerel semen.

MATERIALS AND METHODS

Experimental birds

Six sexually mature Hubbard broiler breeder males, 36 weeks of age, were used as semen donors. These males had been raised with females from 22 to 35 weeks of age as per usual farm practice. The males were housed individually in deep littered, single floor pens of 3 x 4 ft. Each bird was kept in isolation and screened-out from the others and was given 125 gm commercial non-medicated broiler breeder feed once daily. The birds were subjected to 16 hours photoperiod (16L: 8D). Room temperature was controlled between 18-21ºC using gas heaters. Proper ventilation and fresh clean water was provided *ad libitum*.

Semen collection and evaluation

After 10 days of sexual rest, the males were trained for semen collection by abdominal massage technique every second day. Fourteen days training of the males led to better response. Drinking water was withdrawn 4 to 6 hr before semen collection. Feathers around the cloacae were removed at regular intervals for clear semen collections.

At abdominal massage, semen that was free of transparent fluid was expelled forcibly and directly from the bulbous ends of the vas differentia**.** Seven ejaculates were collected from each cockerel every second day for each experiment. Semen of each bird was collected individually into a separate plastic tube. Before dilution in the storage medium, the semen of individual birds was evaluated. Ejaculates of >0.1 ml volume showing milky, pearly white to milky white and thick creamy consistencies and more than 60% motile sperms were used for further study.

Processing of semen

Storage medium referred to as the Experimental Extender in the text was the modification of the milk based extender used earlier (VanWambeke, 1972). The composition of the Experimental Extenders of different osmotic pressures is given in Table 1.

Table1: Composition of the Experimental Extender at different osmotic pressures

Ingredients	Osmotic pressure (mOsm)		
	350	375	400
Sodium citrate $2H_2O$ (gm)	1.3298	1.3298	3.2899
Citric acid (gm)	0.3398	0.3398	0.5799
Sodium glutamate (gm)	20.1000	20.1000	20.1000
Glucose (gm)	15.5997	20.1000	20.1000
Skimmed milk (ml)	200	200	200
Homogenized milk (ml)	150	150	150
Total volume (ml)	1000	1000	1000

In experiment 1, after evaluation the semen was diluted two-fold in the Experimental Extender with osmotic pressures 350, 375 or 400 mOsm with pH 7.0 and stored at 5ºC, and effect of osmotic pressure on percentage motility, sperm speed and clumping was evaluated at 4, 24 and 48 hr. To estimate the rate of motility, a drop of diluted semen was placed on the clean pre-warmed slide at 37ºC, covered with a clean cover slip and examined under the microscope (400X). Speed of motility was calibrated on the following criteria, which were developed after examining several semen samples:

0 = zero
2 = sluggish, pendular or just to and fro movement of sperm cells.
4 = moderate, morphology was visible in motile spermatozoa.
6 = high, slight morphology was visible in motile spermatozoa.
8 = very high, morphology was not visible in motile spermatozoa.

Extent of clumping or agglutination was calibrated on the following basis:

Clumping (0) = agglutination present in all microscopic fields.
Mild clumping (5) = agglutination present in 1-2 fields.
No clumping (10) = all microscopic fields clear.

In experiment 2, semen was diluted two-fold in the Experimental Extender with osmotic pressure 375 mOsm and pH 6.4, 7.0 or 7.4 and stored at 5ºC and effect of pH on percentage motility was evaluated at 4, 24 and 48 hrs, as described earlier.

Determination of fertility/hatchability

The Experimental Extender with osmotic pressure 350 mOsm and pH 7.0 was used for hatchability trials. A total of 17,800 hens were inseminated with 0.04 ml dose of semen for each female using this Experimental Extender at 2-fold dilution. For control purpose, the standardized imported extender, the Fecondil (IMF, Nouzlly, France), and normal saline were used on 17000 hens each at the same farm. The hatchability rate was determined by candling the eggs at 10 days after the start of incubation.

Statistical analysis

The data regarding percentage motility, speed of sperm cells and the presence and/or absence of agglutination of diluted semen were subjected to analysis of variance using the computer programme, Statistical Package for Social Sciences (SPSS) 10.0 version. Multiple comparisons of means were made using Least Significant Difference (Steel and Torrie, 1982).

RESULTS

Motility was higher ($P<0.05$) at 4, 24 and 48 hr when sperm were stored in the Experimental Extender with 375 mOsm osmotic pressure as compared to other osmotic pressures (Table 2) Sperm speed was higher ($P<0.05$) only up till 4 hr when sperm were stored in the Experimental Extender with 350 mOsm osmotic pressure as compared to other osmotic pressures. However, the situation changed later on and at 24 and 48 hr, sperm speed was higher ($P<0.05$) when sperm were stored in the Experimental Extender with 375 mOsm osmotic pressure as compared to other osmotic pressures (Table 2)

Regarding clamping, after 4 hr of semen storage, clumping appeared in the Experimental Extender with 400 mOsm osmotic pressure more extensively as compared with other osmotic pressures (Table 2) However, after 24 hr of semen storage, the 375 mOsm osmotic pressure was better ($P<0.05$) because least

clumping was present as compared with 350 and 400 mOsm osmotic pressures.

Table 2: Effect of osmotic pressure on the motility, speed and clumping of cockerel spermatozoa stored for 48 hr at 5ºC in the Experimental Extenders

		Osmotic pressure (mOsm)		
	Time (hr)	350	375	400
Motility (%)				
	0	94.28 ± 0.71[a]	95.00 ± 0.00[a]	87.85 ± 2.14[b]
	4	85.00 ± 2.44[a]	92.85 ± 1.01[b]	82.85 ± 2.85[a]
	24	58.57 ± 3.40[a]	75.71 ± 2.02[b]	50.00 ± 5.34[a]
	48	3.57 ± 2.83[a]	32.85 ± 4.21[b]	2.14 ± 1.48[a]
Speed				
	0	8.00 ± 0.00[a]	6.66 ± 0.42[b]	4.66 ± 0.42[c]
	4	8.00 ± 0.00[a]	6.00 ± 0.00[b]	4.00 ± 0.00[c]
	24	4.66 ± 0.42[a]	6.00 ± 0.00[b]	3.33 ± 0.42[c]
	28	1.33 ± 0.42[a]	4.00 ± 0.00[b]	0.00 ± 0.00[c]
Clumping				
	0	10.00 ± 0.00[a]	10.00 ± 0.00[a]	10.00 ± 0.00[a]
	4	6.66 ± 1.05[a]	10.00 ± 0.00[b]	3.33 ± 1.05[c]
	24	2.50 ± 1.12[a]	6.66 ± 1.05[b]	0.83 ± 0.83[c]
	48	0.00 ± 0.00[a]	1.66 ± 1.05[b]	0.00 ± 0.00[a]

*Data are mean ± SE (n=7).
[a,b,c] Denote significant difference (P<0.05) in rows.

Effect of pH on sperm motility, while keeping the osmotic pressure constant at 375 mOsm, is presented in Table 3. After 4, 24 and 48 hr of semen storage, sperm motility was higher (P<0.05) at pH 7.0 and 7.4 as compared with pH 6.4. However, at 4, 24 and 48 hr, no difference (P>0.05) in sperm motility was observed between pH 7.0 and 7.4.

For hatchability trials, the osmotic pressure and pH of the Experimental Extender were 375 mOsm and 7.0, respectively. Hatchability was higher (P<0.05) when sperm were stored in the Experimental Extender (88.2%) and Fecondil (86.4%) as compared to normal saline (79.0%).

Table 3: Effect of pH on the motility (%) of cockerel spermatozoa stored for 48 hr at 5ºC in the Experimental Extender with osmotic pressure 375 mOsm*

Time (hr)	pH		
	6.4	7.0	7.4
0	93.33 ±1.05[a]	92.14 ± 2.14[a]	95.0 ± 0.00[a]
4	75.80 ± 4.90[a]	88.57 ± 1.43[b]	91.0 ± 2.92[b]
24	13.33 ± 6.15[a]	68.57 ± 4.04[b]	74.0 ± 5.09[b]
48	0.00 ± 0.00[a]	29.28 ± 6.21[b]	30.0 ± 5.47[b]

*Data are mean ± SE (n=7).
[a,b]Denote significant difference (P<0.05) in rows.

DISCUSSION

Spermatozoa maintain maximum metabolic activity when semen is diluted with an isotonic extender. However, both hypotonic and hypertonic extenders reduce the metabolic activity and disrupt the membrane integrity that leads to clumping and finally death of the spermatozoa. The results of the present study show that 375 mOsm osmotic pressure is optimum for the short-term storage of semen and is isotonic for the cockerel spermatozoa. Huyghebaert *et al*. (1983) reported that 366-460 mOsm was the best range of osmotic pressure for semen diluents. Similarly, Graham and Li (1986) reported that 447 mOsm was an optimum osmotic pressure for the short-term preservation of cockerel semen. However, in the present study, there was no difference in the motility of spermatozoa when semen was stored for 4 hr in diluents with 350, 375 and 400 mOsm. Duration of storage has not been mentioned in either of the fore mentioned studies.

The present study showed that osmotic pressure (both hyper and hypo) affected motility of spermatozoa after 24 hr of semen storage. Ashizawa and Wishart (1987) stated that the difference in the percentage motility at different osmotic pressures may be due to changes in the ionic concentrations of storage media that lead to irreversible losses to the plasma membrane which, in turn, lead to clumping or death of the cells.

The results of the present study also showed that clumping was high at 350 and 400 mOsm osmotic pressures. Whereas least clumping was observed at 375 mOsm osmotic pressure, as it was isotonic with the cells. Higher clumping rates over a period of 4 hr storage at osmotic pressures 350 and 400 mOsm could be due to hypo and hyper tonicity, while the least clumping at 375 mOsm indicates the iso-tonicity of the medium. Also, higher clumping at 350 and 400 mOsm osmotic pressures may be due to change in integrity of the plasma membrane, that results in the exposure of anions and cations of the protein molecules of sperm plasma membrane, which in turn, results in the clumping of spermatozoa. Similarly, contamination of poultry semen with urine and bacteria is a common problem which not only leads to attraction between positively and negatively charged spermatozoa that result in clumping but also leads to increase in the osmotic pressure (Boersma and Stoall, 1997).

In the present study, optimum pH for the short-term (48 hr) storage of cockerel semen was between 7.0 and 7.4, while pH 6.4 was not suitable for sperm preservation because this acidic pH can cause

irreversible damage to plasma membrane permeability, leading to death of the sperm cell. Huyghebaert *et al*. (1983) reported that during short-term storage of semen the optimum pH ranged from 6.3 to 7.4. According to Barna and Boldizsar (1994), pH 7.25 was considered to be optimum for the maintenance of motility and fertility during storage. Our results are in agreement with the findings of these studies and support the conclusion that in acidic pH the motility of spermatozoa is affected probably due to change in the metabolic activity and disturbance in the cellular respiration.

The hatchability results showed that the highest fertility (88.1%) was observed with the Experimental Extender, whereas the hatchability with Fecondil and normal saline was 80.4 and 79.0%, respectively. Higher hatchability (90%) using glucose-citrate extender with 367 mOsm osmotic pressure and pH 7.2 has also been reported by Cheng *et al.* (1987). Schamm and Lohle (1985) reported that extender containing glucose-glutamate ingredient had relatively better fertility rates.

In conclusion, the Experimental Extender with 375 mOsm osmotic pressure (isotonic with cockerel semen) and pH 7.0 not only is suitable for the short-term storage of poultry semen but also improves fertilizing capacity of the spermatozoa.

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