MILK WHEY AS A DILUENT FOR BUFFALO SEMEN*

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Summary. Citric acid whey was observed to be a suitable diluent for preserving buffalo semen both at room and at refrigerated temperature, as revealed by motility studies and conception rate. A 35 to 40% recovery rate was obtained with buffalo spermatozoa frozen in citric acid whey with glycerol.

The buffalo is a milch animal of considerable economic importance in some Asian countries, and efforts are being made to improve the characteristics of the breed. To hasten this process, it is of paramount importance to make full use of the best buffalo bulls and an effective method for the preservation of semen is vital if this is to be achieved. The conventional egg-yolk citrate (EYC) diluent used for bull semen (Phillips & Lardy, 1940; Salisbury, Fuller & Willet, 1941; Maule, 1962) is less effective for buffalo semen (Prabhu, 1956; Roy, Srivastava & Pundey, 1956; Bhattacharya, 1962; Pavithran, Vasanth, Bhima Sena Rao & Anantakrishnan, 1972), the buffalo spermatozoa survived for a shorter time than bull spermatozoa (Gokhale, 1958; Rao, 1958; Bhattacharya, 1962). Compositional differences between bull and buffalo semen were also reported earlier (Banerjee & Ganguli, 1971, 1973). The present communication reports the results of experiments on the use of milk and its fraction (mainly whey) on the motility and fertility rates of buffalo spermatozoa. Some preliminary results on this aspect were reported earlier (Bhosrekar, Ganguli & Stephen, 1972; Ganguli, Bhosrekar & Stephen, 1973).

Ejaculated semen was collected from ten buffalo (Murrah) bulls with an artificial vagina (Herman & Madden, 1953). Those samples with a mass activity of 3 to 4 or greater (motility scale, Herman & Madden, 1953) were pooled, and 0.5 ml of the pooled semen was diluted slowly with 4 ml of diluent. Half of each mixture was stored at 30° C and the other half at 5° to 7° C for 1 hr.

The following diluents were used (all contained 1000 i.u. sodium pencillin G/ml + 3 mg streptomycin sulphate/ml): (i) Egg yolk citrate (EYC); (ii) Soda bicarb–glucose–fructose egg yolk (SGFE) (Tomar, Pande & Desai, 1964); [These two served as controls for the preparations made from skim milk of buffalo, cow and goat, the skim milk was prepared in an Alfa-Laval cream separator and all milk preparations were heated to 92° C for 10 min before use];

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(iii) Skim milk, (iv) Skim milk egg yolk (SMEY) (Tomar & Desai, 1961); (v) Ultracentrifugal whey (UCW) obtained by centrifuging skim milk at 105,000 *g* for 30 min; (vi) UCW+7% glycerol (UCWG); (vii) Micellar casein, the residue after the removal of UCW; (viii) Citric acid whey (CAW) obtained by curdling skim milk with citric acid at pH 4.5; the casein was filtered off through a cotton plug and the pH of CAW was adjusted to 6.8 with 10% NaOH; (ix) CAW+7% glycerol (CAWG); (x) As for CAW, but using HCl instead of

TEXT-FIG. 1. Motility rate of buffalo spermatozoa in different diluents: ●, EYC; ○, CAW; ×, HCl-whey. For abbreviations, see text.

citric acid; (xi) As for CAW, but with acetic acid; (xii) CAW from colostrum; (xiii) CAW with fructose; (xiv) CAW with lactose; (xv) Fraction of skim milk retained by dialysis membrane (dialysed skim milk); (xvi) Fraction of skim milk which passed through a dialysis membrane.

The dialysis fluid was citrate buffer.

In addition to being held at 30° C and 5° C, semen diluted in glycerol was deep frozen over liquid nitrogen (Settergren, 1966).

The efficacy of the diluents was tested by examining the motility of spermatozoa at 37° C under the microscope (at 200 times magnification) after dilution and subsequently every hour. Limited fertility trials with semen extended with CAW were also conducted by artificial insemination (Herman & Madden, 1953). A single observer estimated the sperm motility in a single sample from each diluent every hour for 8 hr and then every 24 hr. For room temperature studies, motility was recorded every hour up to 18 hr. The experiment was repeated eight times.

Milk preparations from buffalo and goat did not preserve sperm motility as well as did cow's milk preparations. Micellar casein, dialysed skim milk and CAW from colostrum were all ineffective.

At 30° C, more than 50% of the spermatozoa were motile after 16 hr in CAW. The addition of lactose or fructose did not affect this. Citric acid whey was superior to HCl whey which was better than EYC (Text-fig. 1). Acetic acid whey was not effective.

Table 1. Motility of buffalo spermatozoa in different diluents at 4° C

Diluent used	% motility		
	0 hr	96 hr	144 hr
Skim milk†	98.0 ± 0.80	—	—
Skim milk egg-yolk	80.0 ± 0.82	60.0 ± 1.82	—
Citric acid whey	73.0 ± 2.11	52.0 ± 4.90	30.0 ± 14.20
CAW + glycerol (7%)	82.0 ± 15.55	62.0 ± 17.11	50.0 ± 3.07
Ultracentrifugal whey (UCW)	80.0 ± 0.0	44.0 ± 2.45	—
UCW + glycerol (7%)	83.0 ± 1.11	62.0 ± 3.07	52.0 ± 3.74
Egg-yolk-citrate	76.0 ± 1.83	15.0 ± 1.83	—
Soda bicarb- glucose-fructose-egg yolk	77.0 ± 1.64	17.0 ± 3.34	—

* The data are expressed as mean ± standard error.

† Skim milk and whey were preparations from cow's milk only. In all cases, at least eight experiments were carried out.

The results obtained with cow's milk preparations at 5° to 7° C are presented in Table 1. As a result of the prolonged survival obtained with CAW, 297 inseminations were performed using spermatozoa in CAW after storage at 5° to 7° C for up to 72 hr. A conception rate of 50% was achieved. Citric acid whey was preferred to UCW because it was easier to prepare. A 35 to 40% recovery rate was obtained with buffalo spermatozoa frozen in CAWG in polyvinyl straws.

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