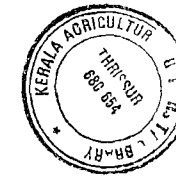
STUDIES ON

THE PRESERVATION OF BOAR SEMEN IN VARIOUS EXTENDERS



By

V. VIJAYA KUMARAN

THESIS

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DECLARATION

I hereby declare that the thesis entitled "STUDIES ON THE PRESERVATION OF BOAR SEMEN IN VARIOUS EXTENDERS" is a bona fide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title of any other University or Society.

V. VIJAYAKUMARAN.

Mannuthy,

12-8-1977.

CERTIFICATE

Certified that the thesis entitled "STUDIES ON THE PRESERVATION OF BOAR SEMEN IN VARIOUS EXTENDERS" is a record of research work done independently by Shri V. Vijayakumaran, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

Dr. C.P. Neelakanta Iyer,
Associate Professor
(Chairman, Advisory Committee).

Mannuthy,
12-8-1977.

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C O N T E N T S

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INTRODUCTION

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The rural economy and welfare of a country to a large extent depend upon the maintenance, improvement and utilisation of livestock which when properly planned and conducted will undoubtedly contribute towards the enhancement of national health and wealth. It is well recognised that artificial insemination is the best and probably the only tool in the hands of husbandrymen to achieve a rapid qualitative and quantitative improvement of any species of livestock in a short span of time.

There is accumulation of evidence to show that pig, by virtue of its biological characteristics like rapid growth rate, economical feed conversion and high reproductive ability could play an important role in bridging the protein gap of any country. Knowing these facts well, many of the European countries have shown keen interest for the development of the swine industry. Consequent to this, about 10 to 20 per cent of swine population of these countries are now being bred artificially. It has been taken up with great enthusiasm by many of the developing countries like Sri Lanka, Thailand, China, Burma, Philipines etc., from the tropical area. But India is yet to make a beginning on artificial insemination programme of swine.

The common village pig in India is a scrub animal with no definite breed characteristics and endowed with genetic

limitations of slow growth, small size, low quality pork and small litter size. The above factors coupled with primitive conditions of management clearly indicate the necessity of a scientific breeding policy in India to improve the native stock. In addition to the genetic improvement of the animals, artificial insemination can bring about a regular contact between technicians and farmers and thereby opening the way for regular and continuing provision of advice on management, rearing and feeding. Thus it forms a spear head in animal production extension work, the failure of which is evident in India.

The development of artificial insemination in any species depends to a large extent on developing semen preservation techniques *in vitro* for several days without causing a serious reduction in the fertilizing capacity of the sperms. The phenomenal progress of artificial insemination in cattle was largely possible due to the availability of suitable extenders for preservation of bull semen. Relatively few experiments have been carried out on storage of boar semen. The conventional diluents containing egg yolk-citrate or egg yolk-phosphate which proved very useful for the preservation of bull semen did not give promising results with boar semen. But in general, it has proved more refractory than that of many other animals in maintaining fertilizing capacity *in vitro*. Literature does not suggest a wholely acceptable extender for boar semen.

The report of Murthy and Rao (1975) appears to be the only published work available from India on preservation of boar semen. Perusal of available literature does not throw evidence of any other work in this country. Since the literature available on this subject from other countries are conflicting in nature, it is imperative to have more sustained studies and trials before practicing artificial insemination of swine in India.

The present investigation has, therefore, been taken up to study the efficacy of various extenders for preservation of boar semen with the ultimate object of evolving a suitable extender for boar semen.

REVIEW OF LITERATURE

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The study of artificial insemination in pigs was started as early as in 1927 (Rommele, 1927). Before the advent of egg yolk by Lardy and Phillips (1939) as a medium for diluting semen, various biological fluids like blood, serum and physiological solutions such as Ringers' and Lockes' were in use as diluents which served mainly to increase the volume of semen than to preserve the sperms. Since then varied and various semen extenders have been used to increase the volume of semen and to prolong the viability of sperms in swine.

Iasley and Bogart (1944) reported, that the addition of yolk-phosphate buffer used for bull semen generally increased the resistance of boar sperm to thermal shock. A solution of 5 per cent glucose with addition of small quantities of sodium sulphate or sodium-potassium tartrate and peptone were initially used as the boar semen diluents (Anderson, 1945). Rimoldi (1947) found that sperms stored better in these diluents if they were concentrated by centrifugation. Ito et al. (1948) recommended 0.7 per cent sodium chloride or 5 to 6 per cent glucose or 10 per cent saccharose as a diluent for boar semen. They recommended a storage temperature of 15-20°C for whole semen and 3-10°C for fractionated semen.

Noll (1950) opined that egg yolk-citrate and egg yolk-citrate-sulphanilamide diluents gave poor results with boar semen. He also observed that egg yolk alone and egg yolk-

sulphanilamide proved to be much better than egg yolk-citrate and egg yolk-citrate-sulphanilamide diluents at all temperatures. Egg yolk diluent in the ratio of 1:2 kept majority of sperms alive for nine days. In a diluent containing egg yolk, pasteurized milk and streptomycin the spermatozoa retained 70 to 100 per cent motility for 72 hours (Sindelic, 1956). According to a U.S.D.A. release (1957) 46 per cent of sows conceived on insemination with semen stored for 30 to 40 hours at 59-68°F with an egg yolk-citrate diluent. On a comparative study of four different diluents viz., phosphate diluent, skim milk or milk powder, egg yolk-glycine and egg yolk-citrate, best storage was observed with egg yolk-citrate diluent (Aamdal and Hogset, 1957). Further, the semen stored for 24 hours at 15-20°C in a diluent consisting of 3 per cent sodium citrate and 30 per cent egg yolk showed motility as good as fresh semen.

Dauzier and Du Buisson (1958) obtained highest conception rate (43.3%) when sows were inseminated with semen stored whole and diluted at the time of insemination with a solution of 2 g sodium bicarbonate and 40 g glucose in 100 ml water. Dziuk and Henshaw (1958) obtained a conception rate of 55, 5 and 32 per cent respectively with semen stored for 1, 2 and 3 day in an egg yolk-glucose-sodium bicarbonate diluent. According to Dziuk (1958) motility was optimum when semen was extended (1 part to 2 part diluent) and stored at 7°C in a diluent containing 3 g glucose, 0.15 g sodium bicarbonate, 30 ml egg yolk and 70 ml distilled water with 1000 units of penicillin and 1 mg streptomycin per ml

diluent. Niwa (1958) observed that semen diluted in an egg yolk-citrate diluent containing antibacterial agents and stored at 15°C showed 70 per cent or more motile spermatozoa after 5 to 7 days storage. Solis (1958) recorded that there was total cessation of motility of spermatozoa by 24 hours when stored in egg yolk-citrate extender at 5°C. He further noticed that motility was 18.4 to 22.8 per cent with two levels of glycerol and egg yolk and three levels of glycine after 72 hours. The percentage of motile sperms in homogenised milk after 12 hours storage at 9°C was 47.2 per cent in contrast to 72.2 per cent in fresh semen stored at the same temperature(Stratman, 1958).

Arhipovec (1959) observed spermatozoal survival for 5 to 6 days in boar semen diluted with yolk-glucose with or without citrate at 0°C storage temperature. Holt (1959) carried out fertility trials using semen diluted in egg yolk-citrate, egg yolk-glycine and heat treated skim milk. Though, he did not observe any difference between diluents when semen was used on the first day, there was very low conception rate when 24 hour old semen was used, especially with milk diluent. From preliminary tests with large number of semen diluents, Williems (1959) reported that pasteurized skim milk with 10 per cent egg yoak was the most satisfactory one. In a slowly cooled semen at 5°C, the percentage of living spermatozoa after four days storage was about 60 per cent, but it showed a rapid reduction after 24 hours in quickly cooled semen.

According to DunVei (1960 boar semen diluted in glucose-yolk-citrate and kept in the dark for four days at 15-23°C, spermatozoal motility decreased only slightly on the first day and then rapidly. Based on the results of motility of semen in various diluents at 5, 12 and 20°C, Irwin (1960) reported that a yolk-milk combination at 5 and 20°C was the most favourable diluent. Semen diluted in yolk-glucose-glycocol retained fertility for two days and the percentage of conception was higher in females inseminated with freshly diluted semen than those inseminated with 24 hours old semen (Feredean and Slavescu, 1961). Jokinen (1961) observed no difference in conception rate when inseminations were carried out with one to eight hour old semen, diluted 1:5 or 1:6 with sodium citrate-glucose to which 10 per cent egg yolk and a small quantity of streptomycin were added. In a series of experiments, Subin (1961) recorded greater sperm motility in semen samples diluted in glucose-egg yolk-bicarbonate diluent than in glucose-egg yolk-citrate or in milk after 3 to 4 days preservation. At room temperature it was 10 to 15 per cent greater. Niwa (1961) recorded that boar semen can be stored well in egg yolk-citrate diluent enriched with glucose or fructose and in milk diluent containing glucose.

Akatov (1962) carried out fertility trials with fresh semen, semen diluted in glucose and fresh semen saturated with carbon dioxide and obtained conception rates of 81.2, 94.3 and

86.7 per cent respectively for semen stored upto 12 to 24 hours. Arhipovec (1962) reported that addition of 0.025 to 0.030 ml of one per cent solution of ascorbic acid or salicylic acid to fresh undiluted or to diluted (glucose-salt-yolk) semen maintained a high percentage of spermatozoal motility for 3 to 4 days at 18 to 26°C. On a comparative trial with pure coconut embryo water (TNI), coconut embryo water + one per cent egg yolk (TNI-1), coconut embryo water + 5 per cent egg yolk (TNI-2) and heated or fresh embryo water + 45 per cent egg yolk (TNI-3) Clamohoy et al. (1962) observed a similar trend in motility in all diluents which decreased from about 50 to 20 per cent from day-1 to day-4. Feredean et al. (1962) claimed to have obtained favourable results with a diluent containing 5 to 7 per cent egg yolk, 4 to 5 per cent glucose and 0.6 per cent glycerol. Pasztor and Toth (1962) obtained good results with semen stored in heated skim milk-egg yolk diluent but addition of glucose did not increase the sperm motility at 12 to 16°C. Subin (1962) tested diluents containing various combinations of yolk, citrate, glucose, bicarbonate and milk at storage temperatures of 15 to 20°C, 5 to 7°C or 0°C and motility was retained for 3 to 4 days when the diluent contained bicarbonate, citrate or milk except at 0°C. During storage at 13 to 19°C the average duration of sperm motility over 0.6 was 80.7, 80.2 and 82.0 hours for semen diluted with glucose-egg yolk, milk-glucose-egg yolk and milk powder-glucose-egg yolk diluents respectively (Tung et al. 1962). But on storage at 3 to 5°C, the average duration of sperm motility over 0.6 was maintained upto

96.8, 108.3 and 106.6 hours respectively. Further, these diluents were found to be superior to glucose-yolk-citrate and glucose-yolk-carbonate diluents. According to Wettke and Hugh (1962) semen diluted 1:6 with a skim milk (Melico)-glucose or citrate-glycine diluent and stored at temperatures of 4 and 12°C, best results were obtained with skim milk at 12°C, in which 60 per cent motility was maintained upto 72 hours and 50 per cent upto 96 hours. The effect of adding gelatin on maintenance of forward motility in boar spermatozoa stored at 12°C in a skim milk diluent and a centrifuged egg yolk-citrate diluent were studied by Wettke et al. (1962). During storage upto 120 hours, sperm motility was reduced in samples containing gelatin. But in centrifuged egg yolk-citrate diluent, spermatozoal motility was as well maintained as in skim milk diluent. Stratman and Self (1962) observed no difference in conception rate between natural mating and artificial insemination done within one hour after collection using semen diluted with milk.

When boar semen diluted in glucose-salt with one per cent ascorbic acid or salicylic acid, the percentage of viable spermatozoa decreased from 80 to 90 per cent after storage for one day to 35 per cent after storage for four days (Arhipovec, 1963). Ikoev (1963) studied the spermatozoal motility in bicarbonate-citrate, glucose-yolk-citrate, milk-glucose-salt and glucose-yolk-salt diluents at 15-25°C for three days. The motility was optimum in bicarbonate and glucose-yolk-citrate diluents. In general, motility was greater in the 3-constituent diluents than

in other diluents. Carbondioxide saturation improved preservability of all the diluents. Among 13 diluents used honey, glycocol + milk and milk + honey were found to be best for semen preservation at 0-3°C and milk or glycocol aerated with carbondioxide was best at 18-22°C (Kalev and Zagorski, 1963). According to Liess and Grove (1963) antibiotics treated semen on saturation with carbondioxide retained 70 to 80 per cent motility even after 72 hours storage. Dilution of semen with egg yolk-citrate diluent after reactivation resulted in a significant increase in motility. Mizuho et al. (1963) observed that when milk or egg yolk was found to be good for maintaining sperm motility, Krebs' phosphate, Ringers' solution, saline solution etc. were found suitable for increasing semen volume. Prokopeev (1963) reported that boar semen diluted with glucose-sodium bicarbonate-boric acid-egg yolk and stored at 5-7°C, 75 and 46 per cent of the spermatozoa showed progressive motility after 3 and 10 days respectively. Spremberg (1963) obtained good results upto two days with a skim milk diluent containing 10 per cent egg yolk and antibiotics particularly when carbondioxide was introduced.

Boar semen was diluted 1:3 with either a skim milk diluent or IVT diluent both containing egg yolk and saturated with carbondioxide (Heidrich et al. 1964). On sixth day the forward motility was 51.5 per cent in IVT and 48.3 per cent in skim milk. Motility was still better when sperm-rich fraction was used instead of whole ejaculate. Kalev and Zagorski (1964) compared spermatozoal survival in semen samples diluted with 10 diluents

at the rate of 1:1, 1:4 and 1:9 and stored at 0-3°C. Spermatozoal survival was longest in the honey-glycocol (166 hours) and fresh skim milk (164 hours) diluents and for shorter period in honey or honey + milk or glycerol, glucose-yolk-citrate, tartrate and sugar diluents. Prokopeev (1964) observed a reduction in conception with semen stored for three days in glucose-sodium bicarbonate-yolk diluents. But the conception rate on the first day of storage was equal to that of natural mating. In a comparative study of several diluents, Smidt (1964) obtained best results with IVT diluent. Tung et al. (1964) observed a significant reduction in conception rate on the third day of storage of semen in milk-citrate-glucose-yolk diluent which on subsequent trial was found to be better than glucose-citrate-yolk diluent. Wettke and Rohloff (1964) tested the following diluents with semen from four boars: (1) IVT without egg yolk (2) IVT without egg yolk + 25 g skim milk powder (3) IVT without egg yolk + 50 g skim milk powder and (4) skim milk (100 g powder per litre distilled water). Semen from one boar stored well in diluent-1 while for the other three boars best results were obtained with diluents containing skim milk.

Kohring (1965) diluted sperm-rich fraction of semen with (a) IVT + 40 per cent egg yolk (b) 10 per cent skim milk + 40 per cent egg yolk (c) Hoffmann's glucose + 40 per cent egg yolk and (d) 10 per cent skim milk + 10 per cent glycerol + 20 per cent egg yolk, all the diluents were saturated with carbondioxide both after dilution and before use. After storage for 1 to 6 day

the samples were reactivated using a 1:4 dilution with 3.5 per cent glucose solution or IVT diluent. Glucose was found to be superior to IVT as a reactivator. Diluent (d) had the best preservation properties after 24 hours storage and reactivation with glucose. Plisko (1965) proved that semen diluted with glucose-yolk-citrate and stored for seven days at 18-22°C maintained good sperm motility without marked reduction in fertility. Boar semen was diluted 1:1 to 1:3 with (a) modified IVT diluent (b) 10 per cent skim milk (Melico) (c) original IVT + 10 per cent egg yolk or (d) 10 per cent Melico + 10 per cent egg yolk and containing antibiotics and saturated with carbondioxide before use (Pusch, 1965). Measurement of forward motility over 60 hours showed that the presence of egg yolk gave better preservation of spermatozoa. Diluent (c) gave the best result (62.3%) and was 10.5 percentage units better than (d). Based on forward motility upto 120 hours Rohloff and Kohring (1965) reported best results with a diluent saturated with carbondioxide containing 10 per cent skim milk + 20 per cent egg yolk and 10 per cent glycerol. Semen diluted in a diluent containing citric acid and carbonate which produced carbondioxide, on reactivation with a glucose-saline solution yielded average motilities of 64.5, 55, 40.9 and 24.1 per cent respectively after 1, 2, 3 and 4 days storage (Schmalfeldt, 1965). Subin and Subina (1965) revealed that sperm-rich fraction of the ejaculate diluted with bicarbonate-glucose-yolk diluent and stored at 15°C maintained good fertility for 3 to 4 days. Tung *et al.* (1965) diluted boar semen

with six diluents and stored at 13-18°C for four days. Motility was highest in milk-citrate-yolk-glucose diluent followed by milk-citrate yolk diluent. For a comparative study Vera Cruz (1965) diluted semen with (1) 3 per cent egg yolk-citrate (2) glycine (2,3,4,5 or 6,) egg yolk (25%) (3) diluent-2 + 15 per cent glycerol or (4) 4 per cent glycine solution. The reduction in motility was 72 and 77 per cent respectively in diluent-1 and diluent-2. Motility above 50 per cent was maintained in (2) with 4 per cent glycine-egg yolk for three days. Wang et al. (1965) obtained better results with fresh milk-citrate-glucose diluent than milk powder-citrate-glucose diluent on a preservation study. On a trial Lingam and Campbell (1965) used 10 diluents with and without antibiotics, a simple glucose-saline buffer and a skim milk buffer each gave good result, the former preserving 60 per cent motility after 96 hours and 40 per cent after five days storage at an optimum temperature of 15°C.

According to Arhipovec (1966) more than 50 per cent sperm motility was maintained for four days in semen diluted with honey, glucose or fructose-trilon B (EDTA)-citric acid and stored at 14-18°C and for 4 to 5 days in semen diluted with glucose and glucose-citrate. Addition of egg yolk or EDTA to glucose-citrate diluent did not increase sperm motility at 0-2°C. Aslanjan et al. (1966) diluted semen with a bicarbonate-citrate-glycocol diluent and stored at 15-16°C for two days with good results. Ikoev (1966) diluted second fraction of the

ejaculate with glucose-yolk-citrate alone or with added carbon-dioxide and glucose-salt-yolk respectively at 1:1 ratio and observed that spermatozoal motility was greater in the two glucose-yolk-citrate diluents than in glucose-salt-yolk diluent. Feredean et al. (1966a) obtained satisfactory conception rate on insemination with semen diluted in glucose-glycocol-egg yolk + antibacterial agents. Again, Feredean et al. (1966b) diluted semen at a ratio of 1:4 to 1:6 with various diluents and stored upto 72 hours at 10-18°C and the optimum spermatozoal motility and survival at 48 and 72 hours were obtained with a diluent containing 50 per cent isotonic glucose solution, 45 per cent skim milk and 5 per cent egg yolk. They, further, reported (1966c) that beneficial results were obtained when semen was diluted with glucose-glycocol-egg yolk to which streptomycin, penicillin and sulphanilamide were added separately or in various combinations. According to Im and Lee (1966) semen diluted with fat free dried milk or egg yolk-glucose-citrate, sperm motility after storage for 24 hours averaged 71.0 and 76.6 per cent respectively and after 48 hours 41.3 and 59.3 per cent respectively. Kalev and Zagorski (1966) reported that spermatozoal survival was longer at 15-18°C when helaton-glucose-citrate or a sodium citrate-potassium citrate-glucose-glutamic acid-yolk diluent was used than when glucose-citrate-yolk, glucose-bicarbonate, helatone-yolk or any of four honey diluents were used and at 1-3°C they survived longest when a honey-glycocol-yolk or a helatone-yolk diluent was used. Meding and Rasbech (1966) used an IVT diluent and a milk-yolk diluent for preservation of boar semen and found

no significant difference in conception rate between the two. Oivadis and Resetnikova (1966) diluted boar semen with glucose-yolk-citrate, glucose-helaton citrate + EDTA and glucose-bicarbonate-citric acid + EDTA diluents. In all the diluents the percentage live spermatozoa decreased while acrosomal defects of spermatozoa increased after storage for three days. Plisko (1966) studied a diluent having the following composition: glucose 60 g, chelate (EDTA, Trilon B) 3.7 g, 4 per cent sodium hydroxide solution 8 ml, 35.7 per cent solution of sodium citrate 10 ml and distilled water to 1000 ml. The addition of chelate + sodium citrate temporarily depressed the activity of various enzymes (DNA-ase, Protease etc.), that prolonged preservation of spermatozoa in semen stored at 16-22°C. Boar spermatozoa survived for 8 to 9 days and retained good motility for 5 to 6 days in semen diluted with glucose-citrate-sodium bicarbonate-EDTA diluent (Sadovnikova, 1966).

On the basis of semen diluted with 22 varients of glucose-citrate, glucose-bicarbonate or IVT diluent and stored at temperature between 2 and 26°C, Balasov and Silaeva (1967) opined that the addition of egg yolk is beneficial only for storage at temperatures less than 16°C, whereas EDTA significantly increased sperm survival during storage at 16-20°C. Foley et al. (1967) claimed better motility maintenance with glucose rather than fructose. Havinzon (1967) in a study of semen diluted in various diluents such as glucose-citrate-egg yolk (Milovanov), glucose-chelate-citrate, glucose-salt, glucose-tartrate-egg yolk, glucose

bicarbonate-egg yolk and glucose-citrate-egg yolk (Musenko), observed that Milovanovs' diluent preserved 60 per cent motility for five days, whereas in other diluents it decreased to 35 to 40 per cent by the third day. Satisfactory conception rate was obtained with semen diluted in egg yolk-citrate-glucose and IVT diluents upto 96 hours of storage (Jacobsson, 1967). Wermath (1967) obtained satisfactory conception rate with semen stored upto three days in an IVT diluent with or without carbonic acid addition. Clamohoy et al. (1967) compared boar semen diluted 1:1 with IVT, CUE and TNIA extenders and recorded conception rates of 65, 59 and 74 per cent respectively for gilts and 77, 25 and 70 per cent for sows. Storage at 15°C gave better results at 1-4 hours and at 1.5 to 2 days than storage at 5°C. Unsweetened milk and saline at 1:1 ratio were also used as a diluent.

The addition of trilon B with fructose, glucose or honey was tested by Arhipovec (1968) for preservation of semen. After storage at 10-20°C for 2 to 5 days, over 50 per cent motility was obtained from spermatozoa in semen to which any of the varient was added. Johnson et al. (1968) conducted a series of three trials using boar semen collected, centrifuged and diluted with Norman-Johnson (NJ-2) diluent and stored at 20-30°C for upto 96 hours. The decrease in conception rate to first insemination was not significant for semen stored upto 48 hours and the variation in the conception rate for that stored upto 72 hours was small. Plisko (1968) reported good conception rate

with semen stored for 1 to 3 days in a glucose-chelate (Trilon B)-citrate diluent. Rohloff and Kirschfeld (1968) undertaken a research trial with six diluents viz., skim milk (Melico), glucose, tris, laiciphos, glycerin and IVT. These standard diluents were tested out with egg yolk additives (10 or 20%) and with glycerol and dimethyl sulphoxide in concentration of 7.5 and 8 per cent. Best preservation results, judged by forøward motility of spermatozoa upto 96 hours were obtained with a glucose diluent containing glycerol. Vysockii et al. (1968) obtained 69 per cent conception rate on insemination with semen stored for 48 hours in glucose-bicarbonate-citrate diluent at 12-22°C and oxygenated just before use.

On a comparative study with semen diluted in glucose-yolk-citrate, IVT and bicarbonate diluent, Hasimov and Borisov (1969) observed that IVT diluent maintained good motility for four days at 15-25°C and the bicarbonate diluent for three days. According to Kurilo (1969) survival of spermatozoa stored for 96 hours in a diluent containing EDTA was 53 per cent versus 40 per cent in a simple sodium citrate diluent. A medium proposed by Serdjuk (1969) comprised of distilled water 100 ml, glucose 5 g, sodium citrate 0.3 g, trilon B 0.1 g, egg yolk 4-5 ml and tetracycline hydrochloride 5000-10000 units. Semen diluted in this medium maintained more than 50 per cent live spermatozoa for 4 to 6 days at 6-10°C. Vasiljev and Sergeev (1969) conducted a study with glucose-citrate-polyvinyl alcohol and found that the conception rate and litter size were reduced by the addition of

polyvinyl alchhol to the diluent except at the lowest dilution rate with greatest number of spermatozoa being inseminated. On a comparative study of semen diluted with glucose-trilon B-citrate and glycine-citrate-bicarbonate diluents, it was revealed that trilon diluent preserved better motility than other diluent (Kovriznyh and Ignatenko, 1969).

Burdeinaja (1970) observed no difference in motility between semen samples diluted 1:1, 1:3 or 1:5 in glucose-chelate-yolk-citrate diluent and stored for 24 hours at 6-10°C. Feredean (1970) found that heating and aeration of the semen before insemination improved the motility to a large extent. According to Kurilo (1970) glucose-chelate-citrate diluent has a strong inhibiting effect on the activity of acid and alkaline phosphotase enzymes resulting in 70 per cent sperm survival after 48 hours storage. Ooi (1970) noticed a reduction in semen motility from 56 to 33 per cent in a modified IVT diluent on storage from day-1 to day-4. He also obtained satisfactory results with semen stored in glucose-yolk-citrate diluent for one day at 15°C. Silaeva (1970) obtained 98 per cent conception rate with semen stored upto three days in a glucose-chelate-citrate-sulphate diluent at 16-20°C.

In a comparative study Babicheva (1971) diluted semen 1:2, 1:3, 1:4 or 1:8 with (a) glucose-EDTA-sodium hydroxide (b) dextrose-bicarbonate-citrate or (c) glucose-citrate-egg yolk-EDTA diluents and stored at 15-20°C, 15-20°C and 6-10°C respectively.

Sperm survival was highest in (c) at all dilution rates. Basic et al. (1971) obtained satisfactory conception rate on insemination with semen diluted in milk-yolk, glucose-citrate and glucose-yolk-glutamic-citrate diluent.

Pursel et al. (1972a) compared the efficiency of different diluents in preventing or reducing the detrimental effect caused to spermatozoa during cold shock. Spermatozoa diluted with tris-lactose, citrate or saline solution developed cold shock resistance during 3 and 5 hours incubation at 30°C and in glucose-bicarbonate and IVT diluents the spermatozoa did not develop resistance to cold shock. Pursel et al. (1972b) observed good motility with semen stored upto 72 hours in either Beltsville L1 (BL1) or Tris-fructose (TF) diluents at 15°C. Senegacnik and Bajt (1972) believes that the addition of EDTA into diluted boar semen exerts a favourable effect on spermatozoa by inhibiting their metabolism and at the same time protecting their fertilizing power. In a comparative study, Smidt (1972) recorded best results with semen diluted in trilon diluent than skim milk-egg yolk-glucose diluent, Plisko diluent and IVT diluent.

The effect of different degrees of dilution and different storage temperatures on boar semen was investigated by Balasghov and Silaeva (1973) and observed that sperm survival was highest in diluents containing chelating agents. Haeger and Mackle (1973) tried a two phase diluent, in which the main

diluent was based on EDTA and glucose and reported that semen could be stored in this diluent at 20-22°C for at least 3 to 4 days. Ian Watt (1973) reported good livability upto 75 hours for semen diluted in an extender consists of glucose and egg yolk. Based on DNA and lipid content of stored spermatozoa Kononov (1973) recommended a diluent containing dextrose, EDTA, sodium bicarbonate, polyvinyl alcohol, penicillin, streptomycin and egg yolk. Semen extended in this diluent gave good conception rate on insemination. In a comparative study Meding (1973) obtained higher conception rate with semen diluted in glucose-EDTA diluent than with IVT diluent. Pursel et al. (1973a) claimed that semen extended in BL1 diluent maintained boar sperm fertilizing capacity for 102 hours on in vitro storage. He also reported the ability of tris-lactose extender containing 2 to 4 per cent casein in protecting the acrosome against cold shock. The egg yolk-glucose-bicarbonate extender maintained acrosome integrity and sperm motility more effectively than Beltsville L1 or Beltsville L2 extenders (Pursel et al. 1973b). Silaeva (1973) stored boar semen at 10-20°C with a glucose-sodium-citrate-trilon B-ammonium sulphate-sodium carbonate diluent upto four days. Kourizhnykh and Ignatenko (1974) noticed highest sperm survival at 18°C in semen diluted with glucose-chelate-citrate diluent.

According to Arthur (1975) semen diluted in IVT extender with carbon dioxide saturation retained good motility upto three days at 15-20°C but without carbon dioxide saturation the

effective storage life will last only for about 36 hours. Kozumplik and Davidona (1975) reported on the basis of a comparative study between Beltsville-3 and chelate containing ejadyl diluents that the highest sperm motility was obtained with Beltsville type diluent. Murthy and Rao (1975) diluted boar semen in egg yolk-glycine, egg yolk-glucose-sodium bicarbonate, egg yolk-glucose-glycine-citrate, egg yolk-citrate, glucose-saline and whole milk extenders and stored at 5 and 15°C. They recorded best results with egg yolk-glucose-sodium bicarbonate and egg yolk-glucose-glycine-citrate diluents. Prokop'eev and Gurevich (1975) recommended a diluent containing glucose, boric acid, sodium citrate, potassium citrate, sodium bicarbonate and dimercaptopropxine sulphonate for preserving boar semen. Boar spermatozoa survived for an average of 79 hours when semen was diluted with Plisko diluent containing EDTA (Rzennik Kareta et al. 1975).

Ooi (1976) reported a reduction in motility from 59 to 35 per cent with IVT diluent when semen stored for three days in this diluent. Prokop'eev and Gurevich (1976) diluted semen using a medium containing glucose, boric acid, dimercaprol sodium sulphonate and sodium citrate. They have observed satisfactory motility upto four days in this diluent at 16-18°C. According to Cazart (1976) homogenised milk + antibiotics can effectively be used for preserving boar semen for eight hours and an egg yolk-glucose-calcium carbonate diluent + antibiotics for preserving upto 72 hours at 37°F.

MATERIALS AND METHODS

MATERIALS AND METHODS

Four pure Large White Yorkshire and two cross-bred boars (Large White Yorkshire x Landrace) belonging to the Pig Breeding Farm, Kerala Agricultural University, Mannuthy, were selected at random for the study. The age of the animals ranged from 9-12 months. All the pigs were maintained under identical conditions of feeding and management.

Semen samples were collected from each boar at weekly intervals using an artificial vagina of the Norwegian type. A total of 72 ejaculates were collected for the present study. Immediately after collection, the gelatinous fraction was removed by filtering the semen through two layers of muslin cloth. Using split sample technique, the gel-free semen was then diluted with each of the 13 different extenders at a proportion of 1:2. The composition of the various extenders used for the study are as follows:

(1) Whole milk extender (WME)

Fresh goat milk obtained from the University Goat Farm was heated at 93°C for 10 minutes and then cooled to room temperature. The top scum layer was removed immediately before use and the resulting milk was used as extender.

(2) Citric acid whey diluent (CAW)

The diluent was prepared as per Ganguli *et al.* (1973).

(3) Egg yolk-citrate diluent (EYC)

Egg yolk	25 ml
Sodium citrate dihydrate	1.96 g
Glass distilled water to	100 ml

(4) Egg yolk-glycine diluent (EYG)

Glycine	2 g
Egg yolk	30 ml
Glass distilled water to	100 ml

(5) Egg yolk-glucose-citrate diluent (EYGC)

Glucose	5 g
Egg yolk	10 ml
Sodium citrate dihydrate	1.96 g
Glass distilled water to	100 ml

(6) Egg yolk-glucose-glycine-citrate diluent (EYGGC)

Glucose	1 g
Glycine	0.33 g
Sodium citrate dihydrate	0.33 g
Egg yolk	20 ml
Glass distilled water to	100 ml

(7) Egg yolk-glucose-sodium bicarbonate diluent (EYGB)

Glucose	3 g
Sodium bicarbonate	0.15 g
Egg yolk	30 ml
Glass distilled water to	100 ml

(8) Egg yolk-glucose-sodium bicarbonate-milk diluent (EYGBM)

Glucose	2 g
Sodium bicarbonate	20 mg
Egg yolk	20 ml
Goat milk	40 ml
Glass distilled water to	100 ml

Goat milk was prepared in the same way as described for the diluent (1).

(9) Illini Variable Temperature diluent (IVT)

Glucose	3 g
Sodium citrate dihydrate	2 g
Sulphanilamide	300 mg
Sodium bicarbonate	210 mg
Potassium chloride	40 mg
Egg yolk	10 ml
Glass distilled water to	100 ml

(Not saturated with carbon dioxide)

(10) Coconut milk extender (CME)

Coconut water	17 ml
Egg yolk	7 ml
Sodium citrate dihydrate	2.2 g
Sulphanilamide	0.3 g
Dihydro streptomycin	0.135 g
Crystalline penicillin	0.060 g
Polymyxin B sulphate	0.010 g
Mycostatin (10 mg in 50 ml)	1 ml
Catalase	15,000 units
Glass distilled water to	100 ml

(Sodium hydroxide 10% solution few drops to adjust pH to 7.4).

The coconut milk extender was prepared according to the technique described by Norman (1964).

(11) Kiew or Varohm diluent-1 (Kiew-I)

Glucose	6 g
Disodium ethylene diamine tetra acetate (EDTA)	370 mg
Sodium bicarbonate	120 mg
Sodium citrate dihydrate	375 mg
Glass distilled water to	100 ml

(12) Kiew or Varohm diluent-2

Potassium chloride	40 mg
Sodium bicarbonate	210 mg
Glucose	600 mg
Sodium citrate dihydrate	2 g
Glass distilled water to	100 ml

Kiew or Varohm diluent-1 and 2 were mixed at a 2:1 ratio and the resulting mixture (Kiew-II) was used for dilution of semen.

(13) Glucose-glycine-EDTA-sodium bicarbonate-citrate diluent (GGEBC)

Glucose	4.5 g
Glycine	350 g
Disodium ethylene diamine tetra acetate (EDTA)	250 mg
Sodium bicarbonate	150 mg
Sodium citrate	800 mg
Glass distilled water to	100 ml

All the extenders except coconut milk extender were fortified with 1000 units of crystalline penicillin and 1000 micro grams of dihydro streptomycin per ml of diluent.

The semen samples were extended in the various extenders immediately after collection and the percentage of motile sperms

assessed. Each of the extended samples was then divided into two parts and stored at 5°C and 15°C respectively. The motility of the semen samples was assessed at 12 hour intervals upto 96 hours or until total cessation of motility.

Based on the results obtained from the preliminary observations seven extenders viz., WTE, CAW, EYC, EYG, EYGGC, EYGB and EYGBM were eliminated from further studies due to poor preservability. The data obtained from the remaining extenders were analysed as per Snedecor and Cochran (1967).

R E S U L T S

R E S U L T S

With the ultimate object of evolving a suitable extender for boar semen, 72 semen samples were extended in six diluents viz., Egg yolk-glucose-citrate (EYGC), Illini variable temperature (IVT), Coconut milk extender (CME), Kiew-I, Kiew-II and Glucose-EDTA-bicarbonate-citrate (GGEBC) diluent. Each of the extended semen sample was preserved at 15°C and 5°C and the livability of the sperms was assessed every 12 hours. The results obtained are presented in Tables 1 to 8 and Figs. 1 to 6.

Preservation at 15°C

The data on the preservation of boar semen in six extenders at 15°C are presented in Tables 1 to 4 and Figs. 1 to 6. It was observed from the Table 1 that initial motility of semen diluted with EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC was 59.17, 63.68, 55.76, 76.53, 76.81 and 76.88 per cent respectively. On statistical analysis no significant difference was observed in the sperm motility between Kiew-I and Kiew-II, Kiew-I and GGEBC and Kiew-II and GGEBC diluents.

At 12 hours of storage the percentage of progressively motile sperms was 53.89, 58.47, 50.28, 72.64, 71.94 and 71.94 respectively with EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC diluents (Table 1). The difference in percentage of motility was significant between all the diluents except between Kiew-I, Kiew-II and GGEBC diluents (Table 2). The percentage reduction of motility of sperms on 12 hours of preservation was 5.28, 5.21,

Kiew-II and GGEBC diluents (Table 3).

The percentage of progressive motility observed at 24 hours of storage in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC diluents was 49.03, 52.50, 44.38, 68.06, 66.6 and 67.15 respectively (Table 1). The difference in the percentage of motility between the diluents was statistically significant except between Kiew-I, Kiew-II and GGEBC diluents (Table 2). The percentage of reduction during storage from 12 to 24 hours period was 4.86, 5.97, 5.90, 4.58, 5.34 and 4.79 respectively in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC diluents (Table 3).

On 36 hours of storage the percentage of progressive motility observed was 43.75, 46.60, 38.61, 62.08, 60.90 and 61.25 with EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC diluents respectively and the reduction of sperm motility during 24 to 36 hours period was 5.28, 5.77, 5.77, 5.98, 5.70 and 5.90 per cent respectively in these diluents (Table 1 & 3). No significant difference in sperm motility was observed between Kiew-I, Kiew-II and GGEBC diluents (Table 2).

At 48 hours of storage the percentage of progressive motility in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC diluents respectively was 37.43, 40.97, 32.43, 55.69, 55.63 and 55.42 and the percentage of decline in motility was 6.32, 5.63, 6.18, 6.39, 5.27 and 5.83 respectively in these diluents (Tables 1 & 3). On statistical analysis no significant difference was observed between Kiew-I, Kiew-II and GGEBC diluents (Table 2).

The percentage of progressive motility of sperms at 60 hours preservation with EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC diluents respectively was 30.63, 33.33, 25.63, 49.58, 50.28 and 50.07 (Table 1). The difference in the percentage of progressive motility between Kiew-I, Kiew-II and GGEBC diluents was not statistically different (Table 2). The percentage of decrease in sperm motility during 48 to 60 hours period was 6.80, 7.64, 6.80, 6.11, 5.35 and 5.35 respectively with EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC diluents (Table 3)

Semen preserved for 72 hours in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC diluents respectively retained 22.50, 25.49, 18.26, 44.03, 43.75 and 44.65 per cent of progressive motility and the reduction of sperm motility during the preceding 12 hours storage was respectively 8.13, 7.84, 7.37, 5.55, 6.53 and 5.42 per cent in these diluents (Table 1 & 3). Motility differed significantly in all except between Kiew-I, Kiew-II and GGEBC diluents (Table 2).

The percentage of motility of semen preserved for 84 hours in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC diluents respectively was 15.49, 18.54, 12.50, 34.86, 35.90 and 36.18 and the reduction in motility of sperms during 72 to 84 hours storage period was observed to be 7.01, 6.95, 5.76, 9.17, 7.85 and 8.47 per cent respectively in these diluents (Table 1 & 3). No significant difference in sperm motility was seen between Kiew-I, Kiew-II and GGEBC diluents (Table 2).

observed in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC diluents was respectively 10.00, 11.70, 11.10, 16.00, 15.20 and 14.50 (Table 5). Analysis of the data revealed that significant difference in sperm motility was found only between EYGC and Kiew-I and EYGC and Kiew-II diluents (Table 6). The percentage of decrease in motility during 24 to 36 hours of storage was 10.20, 11.70, 13.30, 16.00, 21.50 and 15.50 respectively in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC diluents (Table 7).

After a period of 48 hours the percentage of motility observed in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC diluents was 3.80, 4.50, 4.60, 7.90, 7.70 and 6.60 respectively (Table 5). The difference between any two of all these diluents, with respect to percentage of sperm motility was not significant (Table 6 The reduction of sperm motility from 36 to 48 hours of storage was 6.20, 7.20, 6.50, 8.10, 7.50 and 7.90 per cent respectively in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders (Table 7).

The percentage of motile sperms at 16 hours preservation was 0.40, 0.40, 0.90, 2.20, 2.40 and 2.00 respectively in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC diluents (Table 5). The difference between the motility of sperms in these diluents was not significant (Table 6). The decrease of sperm motility during 48 to 60 hours of preservation was 3.40, 4.10, 3.70, 5.70, 5.30 and 4.60 per cent respectively with EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders (Table 7).

On analysis of the data it was observed that motility of sperms in various extenders at different periods of storage varied significantly ($P < 0.01$) (Table 8). Motility above 40 per cent was maintained only upto 12 hours in all the extenders. The decline in percentage of motility was very sharp in all the extenders during 12 to 24 hours of storage (Table 7). From Table 5 it could be seen that motility of sperms in the three extenders viz., Kiew-I, Kiew-II and GGEBC was significantly higher than that in EYGC, IVT and CME upto 36 hours of storage. However, after 36 hours of storage the motility of semen extended with various diluents was not significantly different (Table 6

Table 1. Percentage* of motility in different extenders during varying periods of storage at 15°C.

Extenders	Hours of storage								
	0	12	24	36	48	60	72	84	
EYGC	59.17	53.89	49.03	43.75	37.43	30.63	22.50	15.49	7
IVT	63.68	58.47	52.50	46.60	40.97	33.33	25.49	18.54	11
CME	55.76	50.28	44.38	38.61	32.43	25.63	18.26	12.50	6
Kiew-I	76.53	72.64	68.06	62.08	55.69	49.58	44.03	34.86	26
Kiew-II	76.81	71.94	66.60	60.90	55.63	50.28	43.75	35.90	28
GGEBC	76.88	71.94	67.15	61.25	55.42	50.07	44.65	36.18	28

* Mean of 72 observations.

Table 2. Comparison of motility in different extenders during varying periods of storage at 15°C.

Extenders	Hours of storage								
	0	12	24	36	48	60	72	84	76
EYGC	a1	b1	c1	d1	e1	f1	g1	h1	i1
IVI	a2	b2	c2	d2	e2	f2	g2	h2	i2
CME	a3	b3	c3	d3	e3	f3	g3	h3	i3
Kiew-I	a4	b4	c4	d4	e4	f4	g4	h4	i4
Kiew-II	a4	b4	c4	d4	e4	f4	g4	h4	i5
GGEBC	a4	b4	c4	d4	e4	f4	g4	h4	i5

Note: Means having the same suffix and letter are not significantly different.

Table 3. Rate of decline of sperm motility (in percentage) in different extenders during varying periods of storage at 15°C.

Extenders	Storage intervals (hours)							
	0-12	12-24	24-36	36-48	48-60	60-72	72-84	84-96
EYGC	5.28	4.86	5.28	6.32	6.80	8.13	7.01	7.50
IVT	5.21	5.97	5.77	5.63	7.64	7.84	6.95	7.08
CME	5.48	5.90	5.77	6.18	6.80	7.37	5.76	6.25
Kiew-I	3.89	4.58	5.98	6.39	6.11	5.55	9.17	8.12
Kiew-II	4.87	5.34	5.70	5.27	5.35	6.53	7.85	7.43
GGEBC	4.94	4.79	5.90	5.83	5.35	5.42	8.47	7.36

Table 4. Analysis of variance. Percentage of motile sperms at 15°C.

Source	df	SS	MSS	F
Between boars	5	3517	703.4	28.08**
Between diluents	5	375532	75106.4	2998.26**
Between time of storage	8	1013194	126649.3	5055.86**
Error	3869	96926	25.05	
Total	3887	1489169		

** Highly significant (P < 0.01).

Table 5. Percentage*of motility in different extenders during varying periods of storage at 5°C.

Extenders	Hours of storage					
	0	12	24	36	48	60
EYGC	59.10	45.20	20.20	10.00	3.80	0.40
IVT	63.60	45.90	23.40	11.70	4.50	0.40
CME	55.70	40.60	24.40	11.10	4.60	0.90
Kiew-I	76.50	59.30	32.00	16.00	7.90	2.20
Kiew-II	76.80	62.30	36.70	15.20	7.70	2.40
GGEBC	76.80	58.50	30.00	14.50	6.60	2.00

* Mean of 72 observations.

Table 6. Comparison of motility in different extenders during varying periods of storage at 5°C.

Extenders	Hours of storage					
	0	12	24	36	48	60
EYGC	ab	de	g	j	l	m
IVT	a	d	g	jk	l	m
CME	b	e	g	jk	l	m
Kiew-I	c	f	hi	k	l	m
Kiew-II	c	f	h	k	l	m
GGEBC	c	f	i	jk	l	m

Note: Means of treatments having at least one letter in common are not significantly different.

Table 7. Rate of decline of sperm motility (in percentage) in different extenders during varying periods of storage at 5°C.

Extenders	Storage intervals (hours)				
	0-12	12-24	24-36	36-48	48-60
EYGC	13.90	25.00	10.20	6.20	3.40
IVT	17.70	22.50	11.70	7.20	4.10
CME	15.10	16.20	13.30	6.50	3.70
Kiew-I	17.20	27.30	16.00	8.10	5.70
Kiew-II	14.50	25.60	21.50	7.50	5.30
GGEBC	18.30	28.50	15.50	7.90	4.60

Table 8. Analysis of variance. Percentage of motile sperms at 5°C.

Source	df	SS	MSS	F
Between boars	5	442.1	88.4	0.36
Between diluents	5	51077.8	10215.5	41.65**
Between time of storage	5	1554778.6	310995.7	1267.87**
Error	2576	631862.7	245.29	
Total	2591	2238161.2		

** Highly significant ($P < 0.01$)

Fig 1 Percentage motility of sperms at different hours of storage in glucose-yolk-citrate extender at 15° and 5°C

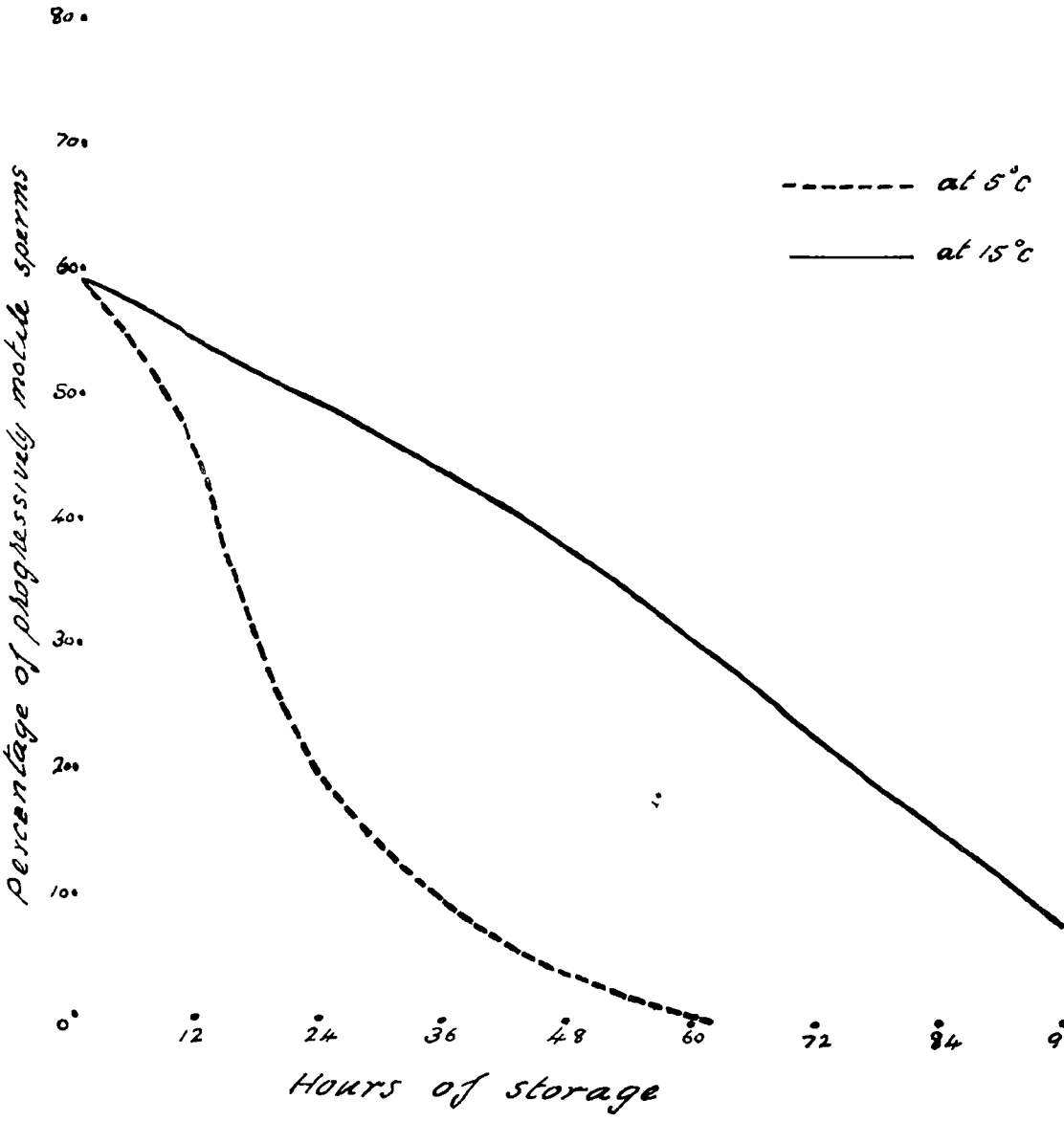


Fig 2 Percentage motility of sperms at different hours of storage in Illini Variable Temperature extender at 15° and 5°C

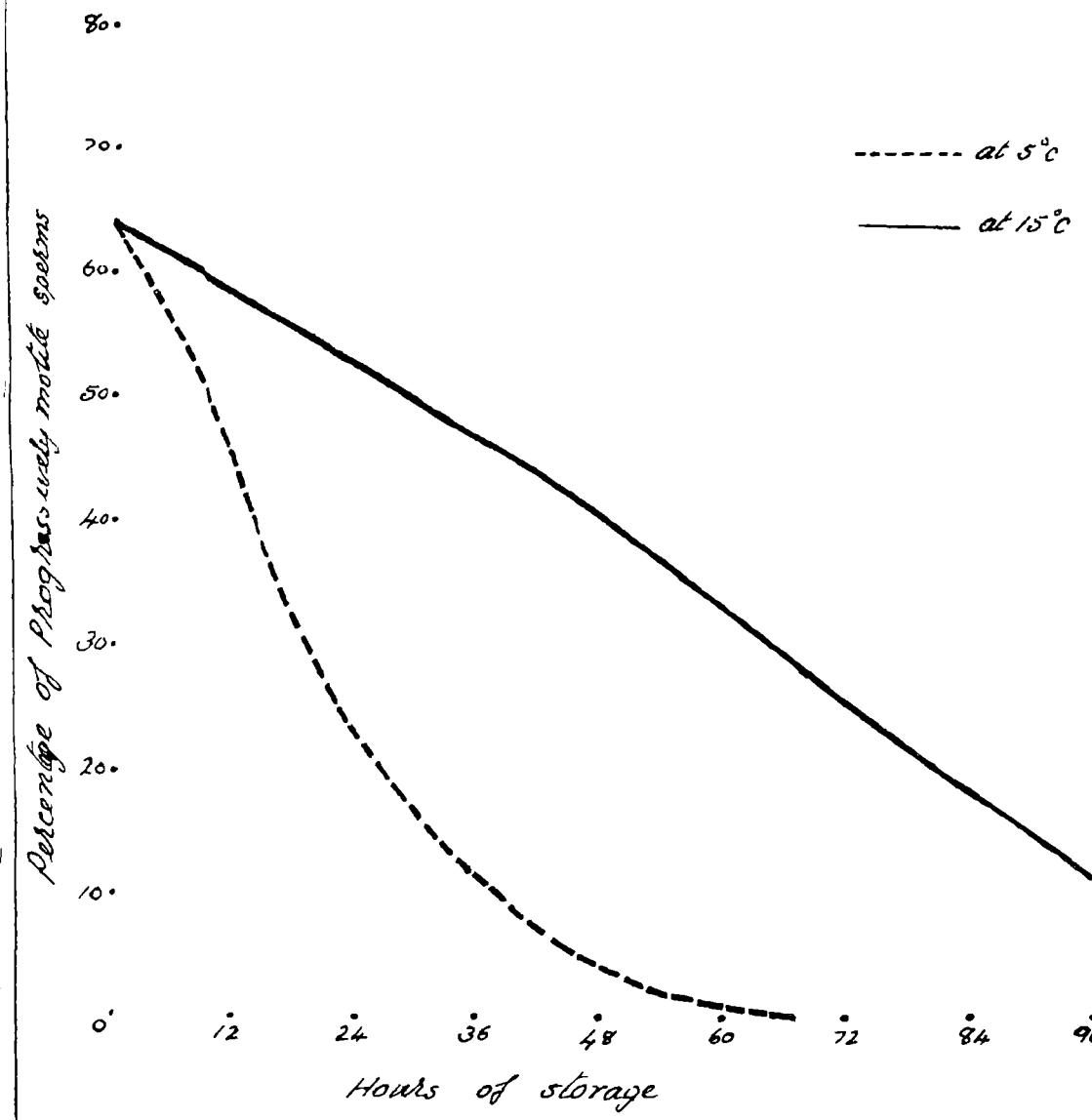


Fig 3 Percentage motility of sperms at different hours of storage in coconut milk extender at 15° and 5°C

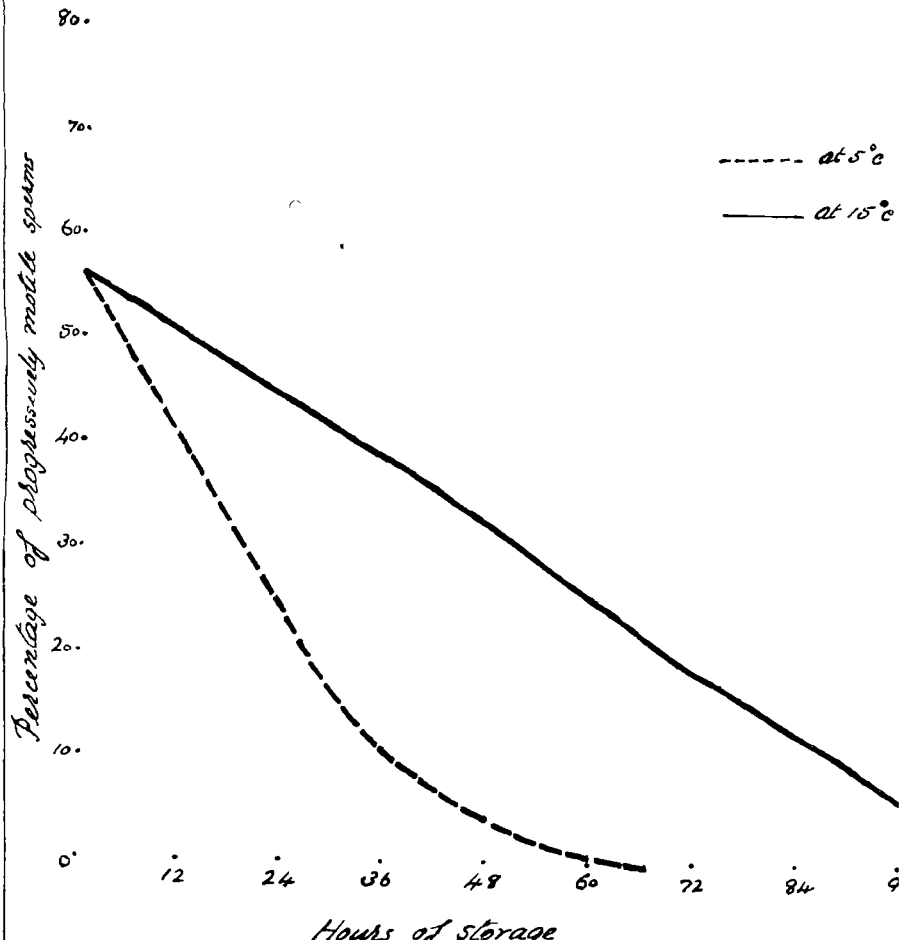


Fig 4 Percentage motility of sperms at different hours of storage in Kew-1 extender at 15° and 5°C

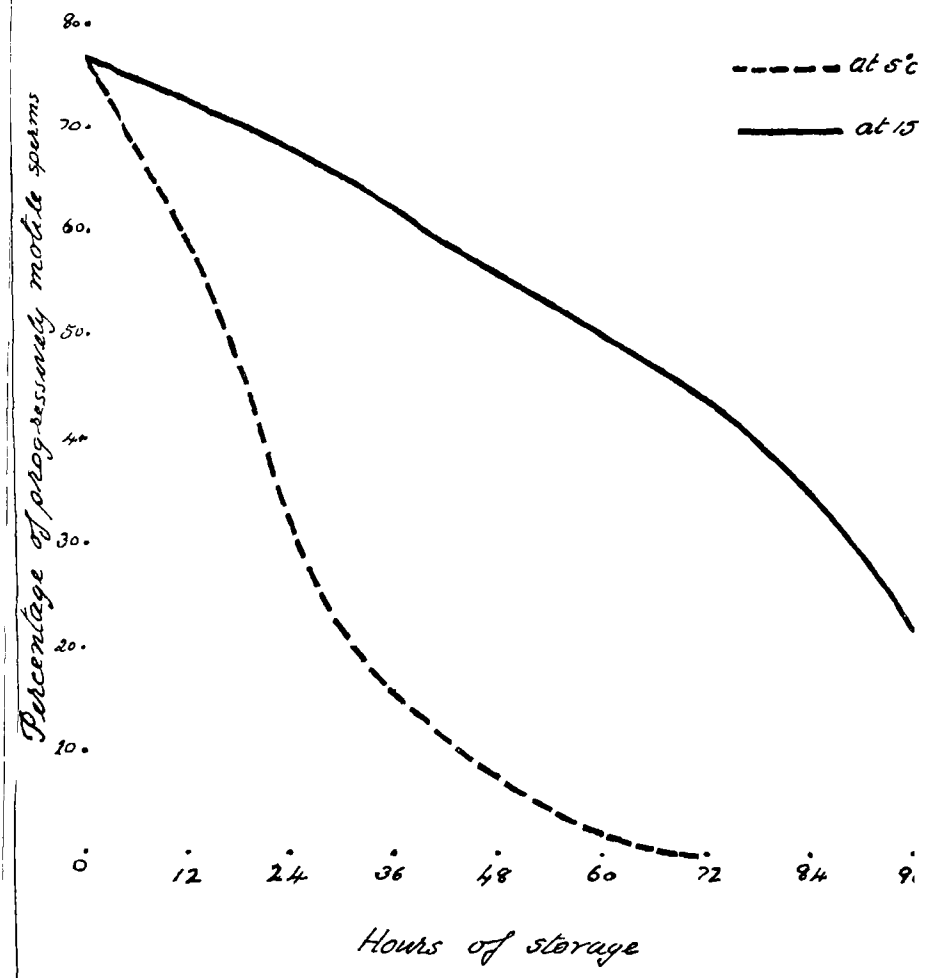


Fig 5 Percentage motility of sperms at different hours of storage in Krew-11 extender at 15° and 5°c

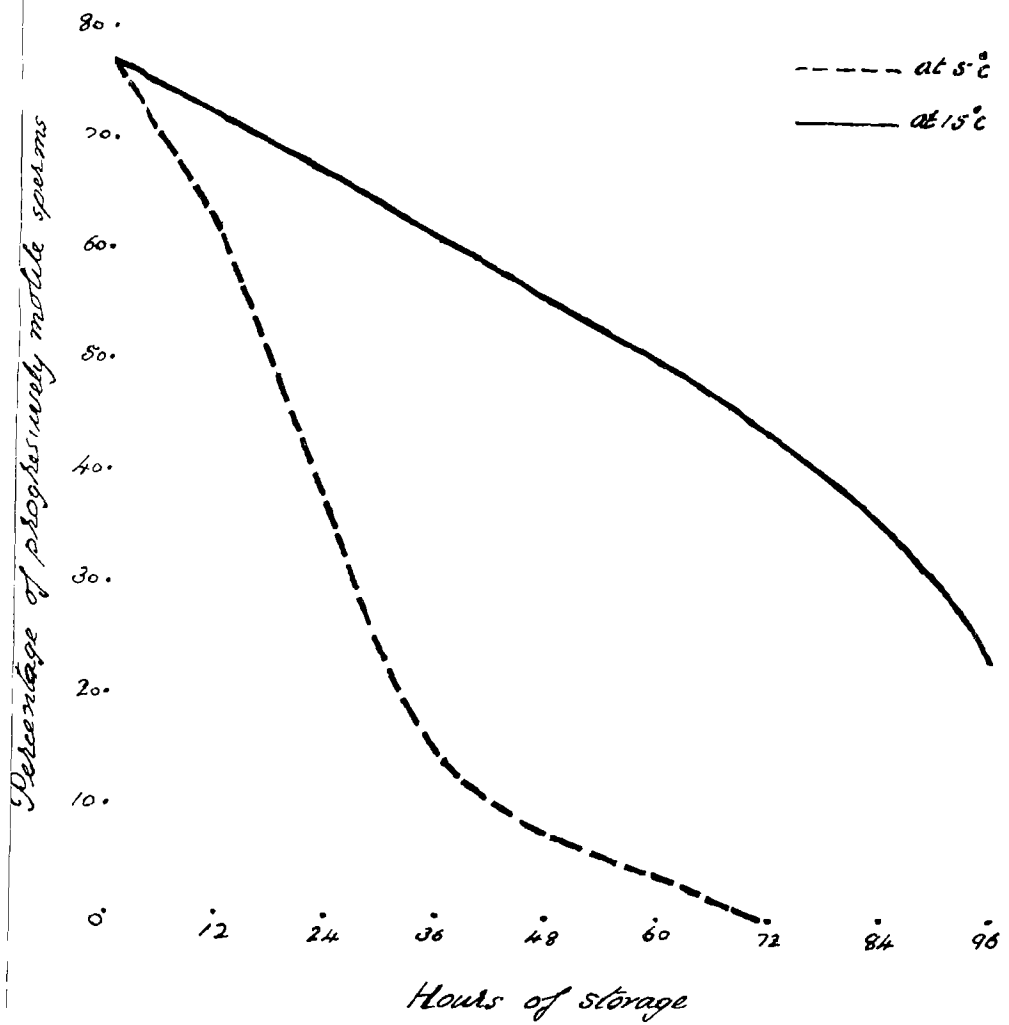
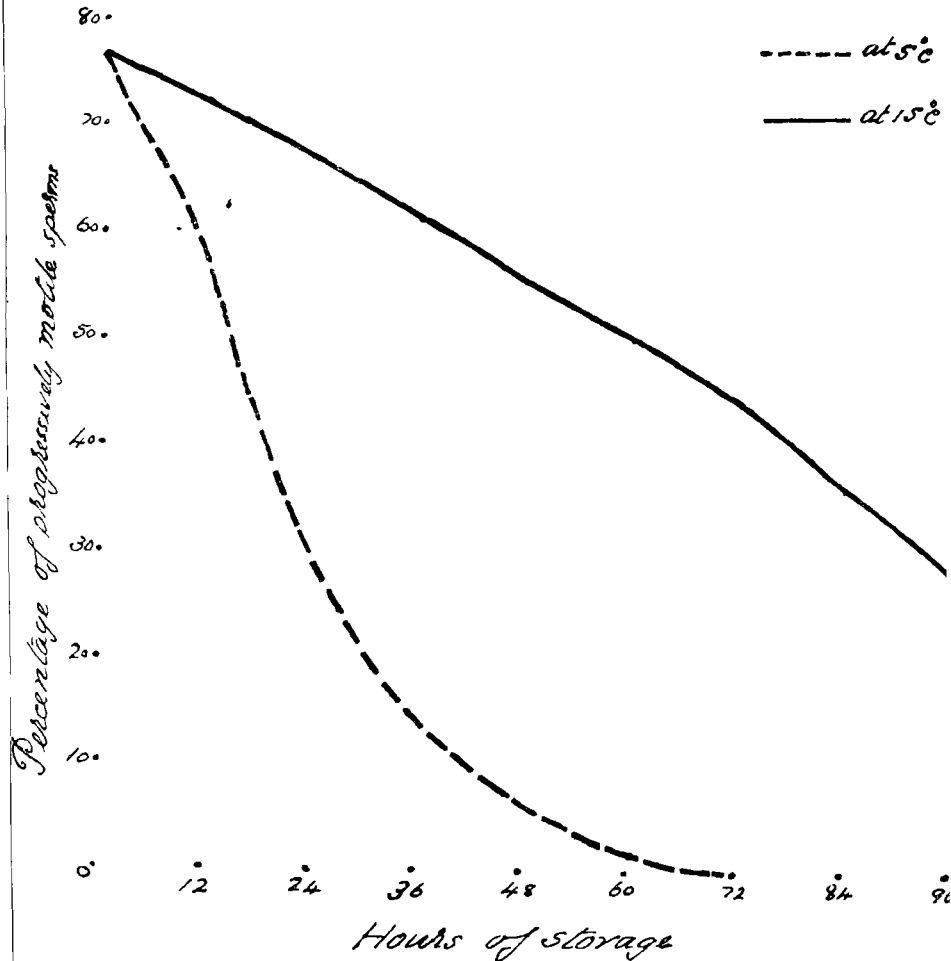


Fig 6 Percentage motility of sperms at diffe
ent hours of storage in glucose-glyce
EDTA-citrate-bicarbonate extender a
15° and 5°C



DISCUSSION

DISCUSSION

The present study was undertaken with the object of evolving a suitable extender for preservation of boar semen. Ejaculates from six healthy boars were extended in 13 diluents by split sample technique. Based on preliminary observations seven extenders were eliminated from further studies due to poor preservability. The data on the preservation of boar semen at 5°C and 15°C in the remaining six extenders viz., Egg yolk-glucose-citrate (EYGC), Illini Variable Temperature (IVT), Coconut milk extender (CME), Kiew-I, Kiew-II and Glucose-glycine-EDTA-bicarbonate-citrate (GGEBC) diluent were collected and statistically analysed (Tables 1 to 8 and Figs. 1 to 6).

It could be seen from the Table 1 that about 50 per cent motility was maintained upto 60 hours of storage at 15°C in Kiew-I, Kiew-II and GGEBC extenders while motility dropped below 45 per cent earlier to even 48 hours in the other three extenders. This clearly denotes that Kiew-I, Kiew-II and GGEBC are superior to the remaining three extenders. Analysis of the data revealed that there is no significant difference between Kiew-I, Kiew-II and GGEBC extenders at all stages of storage intervals except at 96 hours (Table 2). At this period Kiew-II and GGEBC extenders were found to be superior to Kiew-I ($P < 0.01$). However, there was no significant difference in the keeping quality of semen between Kiew-II and GGEBC extenders at 96 hours of storage.

In EYGC, IVT and CME extenders about 35 to 45 per cent motility was maintained upto 36 hours at 15°C (Table 1). Analysis of data revealed that there is significant difference in the motility of semen in these three diluents at 36 hours of storage. However, based on the comparative merits in the preservation of boar semen IVT, EYGC and CME extenders can be ranked serially.

It is evident from the Table 5 that uniformly poor motility was recorded in all the six extenders at 24 hours of storage and beyond, at 5°C. However, in Kiew-I, Kiew-II and GGEBC extenders nearly 60 per cent motility was maintained upto 12 hours of storage. Analysis of data revealed that there is no significant difference in motility between these diluents at 12 hours storage at 5°C (Table 6).

The result presently observed with Kiew-I extender agrees with the findings of Heydorn and Paufler (1976), who have also reported good preservability at 16°C to 18°C for three days with Kiew-I extender. Kovrizhniyh and Ignatenko (1974) reported good motility for 24 hours of storage in Kiew-I diluent, whereas motility upto 5 to 6 days have been reported by Sadovnikova (1966) in a similar diluent. Haeger and Mackle (1973) were able to preserve semen upto three to four days at 20-22°C in Kiew-II extender. This is comparable with the present observation to a certain extent.

In the present investigation it is noted that Kiew-I, Kiew-II and GGEBC extenders containing EDTA have been found to be superior to other diluents. The beneficial effects of EDTA in the boar semen extenders have been reported earlier (Balasov and Silaeva, 1967; Kurilo, 1969; Balasov and Silaeva, 1973 and Rzennik et al. 1975). The beneficial effect of EDTA has been reported to be due to its ability to prevent the decrease of aldolase enzyme activity (Kurilo, 1968) or due to its ability of depressing the activity of various enzymes such as DNA-ase, Protease etc. (Plisko, 1966). Addition of EDTA has been reported to result in a marked reduction in the concentration of sodium and potassium and slightly lower the concentration of calcium and phosphorus in spermatozoa (Semakov, 1976). Meding (1973) reported that the reduction in the fertilizing capacity of 1 to 2 day old semen can be prevented to an extent by using EDTA containing diluent. The present study also confirms that the diluents containing EDTA maintained better preservability than other diluents both at 15°C and 5°C.

The present study revealed that sperm motility could be maintained at 40 per cent level only upto 48 hours at 15°C with IVT diluent (Table 1). This is in keeping with the findings of some of the earlier workers (Ooi, 1970; Smidt, 1972; Arthur, 1975 and Ooi, 1976). On the contrary more favourable results for IVT diluent at 15°C have also been reported (Heidrich et al. 1964; Smidt, 1964 and Hasimov and Borisov, 1969).

In the present study unfavourable results were obtained from second day of storage onwards with EYGC at 15°C, which agrees with the findings of earlier workers (DunVei, 1960; Subin, 1961; Ikoev, 1966 and Im and Lee, 1966 and Murthy and Rao, 1975). However, there are several reports to indicate that EYGC has better preservability as a boar semen extender (Arhipovec, 1959; Kozumplik, 1961; Tung et al. 1962; Plisko, 1966; Oivadis and Resetnikova, 1966; Havinzon, 1967 and Riddell-Swan, 1960). The present investigation did not give favourable results with CME, although good results have been reported by Clamohoy et al. (1962).

In conclusion it may be stated that boar semen can be stored at 15°C with good results upto 60 hours in Kiew-I, Kiew-II and GGEBC extenders and 12 to 24 hours in CME, EYGC and IVT extenders. In all the extenders preservation at 5°C have been found to be less favourable beyond 12 hours storage.

S U M M A R Y

S U M M A R Y

In the present investigation an attempt has been made to study the utility of six different extenders in preserving boar semen with the ultimate object of evolving a suitable extender for boar semen.

Six healthy boars (4 Large White Yorkshire and 2 Landrace cross-bred boars) were selected at random from the Pig Breeding Farm attached to the Kerala Agricultural University, Mannuthy and 72 semen samples were collected from them using an artificial vagina of Norwegian type. Semen was extended in 13 different extenders using split sample technique at a proportion of 1:2. Each of the extended semen sample was then divided into two parts and stored at 15° and 5°C. Motility was assessed at every 12 hours upto 96 hours of storage or until total cessation of motility.

Thirteen extenders originally used for the study were Whole milk extender (WME), Citric acid whey diluent (CAW), Egg yolk-citrate diluent (EYC), Egg yolk-glycine diluent (EYG), Egg yolk-glucose-citrate diluent (EYGC), Egg yolk-glucose-glycine-citrate diluent (EYGGC), Egg yolk-glucose-sodium bicarbonate diluent (EYGB), Egg yolk-glucose-sodium bicarbonate-milk diluent (EYGBM), Illini variable temperature diluent (IVT), Kiew or Varohm diluent-1 (Kiew-I), Kiew or Varohm diluent-II (Kiew-II) and Glucose-glycine-EDTA-bicarbonate-citrate diluent (GGEBC).

During the course of the experiment seven extenders viz., WMC, CAM, EYG, EYGC, EYGGC, EYGB and EYGBM were eliminated from further studies due to poor preservability. The data obtained from study of 72 ejaculates were analysed.

Preservation at 15°C

It was observed that the initial motility of semen diluted with EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC was 59.17, 63.68, 55.76, 76.53, 76.81 and 76.88 per cent respectively. On storage upto 12 hours the motility decreased to 53.89, 58.47, 50.28, 72.64, 71.94 and 71.94 per cent respectively with EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders. At 24 hours storage the percentage of motile sperms was 49.03, 52.50, 44.38, 68.06, 66.60 and 67.15 in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders respectively. At 36 hours storage motility was 43.75, 46.60, 38.61, 62.08, 60.90 and 61.25 per cent respectively in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders. On storage upto 48 hours the percentage of motility in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders was 37.43, 40.97, 32.43, 55.69, 55.63 and 55.42 respectively. Percentage of motility declined to 30.63, 33.33, 25.63, 49.58, 50.28 and 50.07 respectively in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders after 60 hours of storage. Semen preserved for 72 hours in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders respectively retained 22.50, 25.49, 18.26, 44.03, 43.75 and 44.65 per cent

motility. On 84 hours storage the percentage of sperm motility in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders was 15.49, 18.54, 12.50, 34.86, 35.90 and 36.18 respectively. At 96 hours storage in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders the percentage of motility was 7.99, 11.46, 6.25, 26.74, 28.47 and 28.82 respectively.

Preservation at 5°C

The motility of semen at the onset of storage was 59.10, 63.60, 55.70, 76.50, 76.80 and 76.80 per cent respectively in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders. The motility was reduced to 45.20, 45.90, 40.60, 59.30, 62.30 and 58.50 per cent respectively in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders on preservation upto 12 hours. Semen preserved for 24 hours in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders maintained 20.20, 23.40, 24.40, 32.00, 36.70 and 30.00 per cent motility respectively. After 36 hours storage the percentage of motile sperm observed in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders was 10.00, 11.70, 11.10, 16.00, 15.20 and 14.50 respectively. At 48 hours storage, the percentage of motile sperms was 3.80, 4.50, 4.60, 7.90, 7.70 and 6.60 respectively in EYGC, IVT, CME, Kiew-I, Kiew-II and GGEBC extenders the percentage of spermatozoa having progressive motility decreased to 0.40, 0.40, 0.90, 2.20, 2.40 and 2.00 respectively.

In general, the preservation of semen at 15°C was found

to be better than at 5°C. Motility was found to be declining sharply at 5°C compared to preservation at 15°C. Among the six extenders studied, GGEBC, Kiew-II and Kiew-I diluents were found to be superior both at 15° and 5°C than the other extenders. The difference between the three extenders was not statistically significant. It was concluded that boar semen can be successfully preserved upto 60 hours in the three diluents viz., GGEBC, Kiew-II and Kiew-I at 15°C storage.

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STUDIES ON

THE PRESERVATION OF BOAR SEMEN IN VARIOUS EXTENDERS

By

V. VIJAYA KUMARAN

ABSTRACT OF A THESIS

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ABSTRACT

With the ultimate object of evolving a suitable extender for boar semen 72 ejaculates from six boars selected at random from University Pig Breeding Farm, Mannuthy were used for the preservation studies. Six diluents viz., Egg yolk-glucose-citrate, Illini variable temperature diluent, Coconut milk extender, Kiew-I, Kiew-II and Glucose-glycine-EDTA-bicarbonate-citrate diluent were used to study the keeping quality of boar semen at two temperatures of storage (15° and 5°C). The highest percentage of progressively motile sperms were observed in glucose-glycine-EDTA-bicarbonate-citrate, Kiew-II and Kiew-I extenders. At 15°C semen could be stored in these extenders upto 60 hours with good motility. Coconut milk extender proved to be least suitable for the preservation of boar semen both at 15° and 5°C of storage. Progressive motility in all the diluents was higher at 15°C than at 5°C.