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FERTILITY OF OESTRUS SYNCHRONISED CROSSBRED MALABARI DOES INSEMINATED WITH BOER BUCK SEMEN

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Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

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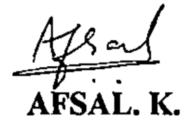
DECLARATION

I hereby declare that the thesis entitled **"FERTILITY OF OESTRUS SYNCHRONISED CROSSBRED MALABARI DOES INSEMINATED WITH BOER BUCK SEMEN"** is a bonafide record of research work done by me during the course of research and that this 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.



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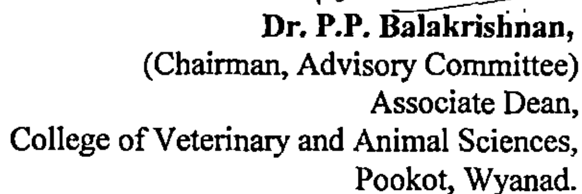

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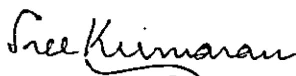

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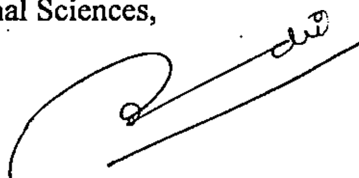

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Introduction

1. INTRODUCTION

Goats constitute a very important species of livestock in India, mainly on account of their short generation interval, higher rates of prolificacy and the ease with which the goats and their products can be marketed. Goat is one of the main source of meat in India and its meat has highest market value. Almost 95 per cent of the goat meat produced in the country is consumed locally. But the per capita availability of meat is still far below the requirement recommended. Therefore considerable attention is required for developing goat meat production not only for internal consumption but also for export. India has around 20 distinct breeds of goats and Malabari breed is the only breed native to Kerala, which is a prolific dual-purpose breed.

The meat industry in countries like Australia and New Zealand have been improved considerably by the introduction of Boer goats where the grading up of local feral goats with Boer sires improved the growth rate and meat production. India lacks a specific high producing meat type breed of goat. This has increased the interest in Boer goats for development of goat sector to appropriate climatic conditions. The Boer goats developed solely for meat production has been looked upon as a potential breed to enhance the meat production ability of the Indian goat breeds by means of cross breeding programme. It has the added advantage of hailing from a tropical country and hence there will not be many problems in acclimatisation in the harsh climatic conditions of India especially that of Kerala.

Oestrus synchronisation using hormonal methods are of practical interest as the means of facilitating the application of Artificial Insemination in goats. Oestrus cycle of does can be successfully controlled with the use of Prostaglandin so as to implement fixed time artificial insemination (AI) programmes to enhance the reproductive efficiency in goat herds (Gordon, 1997).

In Kerala, Artificial Insemination using frozen semen is well established in cows. But in goats this programme is not implemented widely as the technique of freezing buck semen is yet to be fully functional. Once the technique becomes operational, the facilities available for bull semen preservation and insemination can be well utilised for buck semen.

Availability of Boer bucks in our country at present is limited. This necessitates development of appropriate semen extender for AI purpose and dissemination of Boer germplasm and this makes it mandatory to study the semen characteristics especially in a humid tropical environment like that of Kerala.

The major constrained in deep freezing of buck semen is the presence of egg yolk coagulating enzyme in the seminal plasma. Removal of seminal plasma from the goat semen improved the motility at preservation (Iritani and Nishikawa, 1961). But removal of seminal plasma by washing was detrimental for the survival of sperms (Tuli and Holtz, 1994). So a ready to use, manufacturer certified extender available in the market will be a boon to the goat industry. Hence Biociphos Plus (IMV, France), an extender containing soybean extract as a substitute for egg yolk was used for extending goat semen.

Realising the high potential for the cross breeding programme between Malabari and Boer breed of goats in meat goat production programme of the state, present investigation was undertaken with the following objectives.

1. To study the semen characteristics of Boer buck semen.
2. To evaluate the freezability of Boer buck semen preserved in Tris extender and Biociphos extender.
3. To find out the conception rate in Malabari crossbred goats using frozen Boer buck semen under Kerala condition.

Review of Literature

2. REVIEW OF LITERATURE

2.1 SEMEN EVALUATION

Semen samples collected from Boer bucks were evaluated for volume, colour, mass activity, sperm concentration, live sperm percentage, pH and sperm abnormalities.

2.1.1 Volume

Volume of goat semen ranged from 0.25 to 5 ml and the quality declined when the volume of ejaculate exceeded 2 ml (Blokhuis, 1962). Igboeli (1974) compared the seminal characteristics of Zambian and Boer bucks and recorded an average ejaculate volume of 0.67 $\pm$ 0.03 and 1.34 $\pm$ 0.05 ml respectively. Patil and Raja (1978) studied the semen characteristics of Malabari bucks and found the volume to range from 0.2 ml to 1.2 ml with an average of 0.50 $\pm$ 0.13 ml. Volume of ejaculate of Alpine x Saanen x Malabari crossbred goat was reported to be 0.52 $\pm$ 0.19 and 0.48 + 0.19 ml for the first and second ejaculates when semen collection was performed twice daily (Joseph and Nair, 1991). The average volume of buck semen was 1.33 $\pm$ 0.07 ml for Malabari crossbred bucks (Ranjini, 1998) and 0.75 ml in Malabari and Alpine x Malabari breeds of goats (Kutty and Mathew, 2000). The ejaculate volume was found to be 0.61 $\pm$ 0.22 ml in Barbari bucks (Yadav and Pandey, 2000).

Tuli and Holtz (1992) studied the semen characteristics of Boer bucks in different seasons of the year and found that ejaculate volume did not differ much between summer (1.79 $\pm$ 0.15 ml) and winter (1.77 $\pm$ 0.14 ml) months. Singh and Purbey (1994) conducted a study in indigenous bucks and opined that the ejaculate volume was highest in wet summer (0.83 $\pm$ 0.04 ml) and lowest in winter (0.58 $\pm$ 0.02 ml). Srinivas *et al.* (2002) studied the effect of season on semen volume of

native bucks of Andhra Pradesh and found to be 0.70 ± 0.10 in winter and 0.60 ± 0.02 in summer seasons.

2.1.2 Colour

Normally the buck semen is opaque and creamy white in colour due to high concentration of spermatozoa in the semen of this species (Roberts, 1971). Ejaculate Colour of Boer buck semen ranged from creamy white to yellow and varied considerably between goats of the same breed (Igboeli, 1974). Colour of semen of Jamnapari and Barbari bucks varied between breeds, bucks and within the ejaculates of the same buck (Singh *et al.*, 2000).

Colour of ejaculate was creamy for Saanen bucks, yellowish to creamy for Barbari bucks (Pandey *et al.*, 1985) and creamy for Malabari crossbred bucks (Ranjini, 1998). Kutty and Mathew (2000) reported that the colour of ejaculate of Malabari and its alpine cross varied from yellowish white to deep yellow. The intensity of yellow colour was more during December, January and February and lesser during March, April and May.

2.1.3 Mass Activity

Goat semen samples with mass activity of + + + + or + + + only could be diluted with good prosperity of fertilisation (Blokhuis, 1962). The initial motility of semen was in the range of 40 to 85 per cent with a mean of 66.14 ± 1.34 per cent in Malabari (Patil and Raja, 1978), 89.66 per cent in Saanen and 78.85 per cent in Barbari bucks (Pandey *et al.*, 1985). The average mass activity of semen was 4.16 ± 0.08 in Saanen, 4.35 ± 0.06 in Barbari and 4.18 ± 0.07 in Saanen x Barbari crossbred bucks (Prasad *et al.*, 1986). Mass activity of Black Bengal goat semen was best during spring season followed by summer, winter and autumn (Baruah *et al.*, 1992). Tuli and Holtz (1992) reported the average mass activity of Boer buck semen in

summer and winter to be 3.75 ± 0.10 and 3.64 ± 0.14 respectively on a 0-5-point scale. The mass activity of semen of Malabari crossbred goat was found to be + + + + (Ranjini, 1998). Kutty and Mathew (2000) opined that the average mass activity of semen in Malabari x alpine crossbred buck was 3.31. The mass activity of semen was 4.46 ± 0.06 in winter and 4.36 ± 0.08 in summer in native bucks of Andhra Pradesh (Srinivas *et al.*, 2002).

2.1.4 Sperm Concentration

Sperm concentration along with volume of semen determines how many females could be inseminated using diluted semen. Sperm concentration could be measured using haemocytometer, calorimeter or spectrophotometer. The average sperm concentration of semen was 2700 ± 0.03 millions per ml in Boer goats (Igboeli, 1974). Patil and Raja (1978) estimated the sperm concentration of Malabari bucks in the range of 1100 to 7490 millions per ml with an average of 3534 ± 176.10 millions per ml. Prasad *et al.* (1986) recorded the spermatozoal concentration of Saanen, Barbari and their crossbreds to be 2820.96 ± 7.43, 2117.65 ± 32.45 and 2375.47 ± 7.10 millions per ml respectively. The normal sperm concentration for Boer goat semen was 3750 ± 0.10 millions per ml (Tuli and Holtz, 1992). The average sperm concentration was found to be 2972 ± 293 millions per ml in Malabari crossbred goats (Ranjini, 1998). In a study on the effect of season on sperm quality, Kutty and Mathew (2000) found that the sperm concentration did not vary significantly between different seasons of the year. They recorded maximum sperm concentration in summer (3133 millions per ml) and minimum in post monsoon season (2105 millions per ml). Semen concentration of native goats of Andhra Pradesh was 2450 ± 0.31 millions per ml in winter and 2350 ± 0.02 millions per ml in summer (Srinivas *et al.*, 2002).

2.1.5 Live Sperms

Assessment of viability along with acrosomal status of spermatozoa is essential for the assessment of semen quality for predicting the fertility of a particular male animal. Nigrosin-Eosin staining is the most commonly used technique for distinguishing the live and dead spermatozoa in a dried smear (Roberts, 1971). The normal live sperm percentage was found to be 87.70 $\pm$ 1.00 in Boer bucks (Igboeli, 1974) and 63.38 $\pm$ 2.58 in Malabari goats with a range of 22 to 88 per cent (Patil and Raja, 1978). Tuli and Holtz (1992) studied the seminal characteristics of Boer buck semen in different seasons of the year and found that there was no significant difference in the percentage of live spermatozoa among different seasons of the year and opined the values to be 75.08 $\pm$ 2.53 per cent in summer and 73.05 $\pm$ 3.10 per cent in winter. Tuli and Holtz (1995) studied the effect of season on the percentage of live spermatozoa of Boer semen and found to be 61, 71, 75 and 76 per cent respectively in spring, summer, autumn and winter months of the year. The average percentage of live spermatozoa in fresh semen of Malabari crossbred buck was 90.03 $\pm$ 0.80 (Ranjini, 1998). Srinivas *et al.* (2002) could obtain 87.05 $\pm$ 0.62 and 81.11 $\pm$ 0.51 per cent of live spermatozoa in summer and winter seasons in native goats of Andhra Pradesh.

2.1.6 Hydrogen ion Concentration

The average pH value of semen was reported to be 6.47 $\pm$ 0.16 with a range of 6.4 to 7 in Malabari (Patil and Raja, 1978), 6.60 $\pm$ 0.02 in Saanen and 6.78 $\pm$ 0.02 in Barbari bucks (Prasad *et al.*, 1986). In a study Prasanth (1995) found the mean pH value of semen of Alpine x Malabari buck to be 6.85 + 0.01. Ranjini (1998) reported the pH value of Malabari crossbred goat semen to be 7.28 $\pm$ 0.04 while Kutty and Mathew (2000) could obtain 6.16 during spring and 6.39 during monsoon in Alpine x Malabari crossbred goats.

2.1.7 Sperm Abnormalities

The average percentage of abnormal count of spermatozoa was 4.34 ± 0.48 for Malabari buck semen (Patil and Raja, 1978). The percentage of abnormal spermatozoa in fresh semen was 7.54 ± 0.45 in Saanen, 5.94 ± 0.25 in Barbari, 7.63 ± 0.44 in Saanen x Barbari crossbred goats (Prasad *et al.*, 1986). The mean percentage of abnormal spermatozoa was 3.05 ± 0.25 in Malabari crossbred goats (Ranjini, 1998). Srinivas *et al.* (2002) found that the native goat semen of Andhra Pradesh had 8.98 ± 0.23 per cent abnormal spermatozoa in winter and 9.28 ± 0.20 per cent in summer seasons.

2.1.8 Acrosome Morphology

2.1.8.1 *Acrosome Staining*

Acrosomal structure of ram spermatozoa was prominently stained when air dried smears of diluted semen was fixed for 15 minutes in buffered formol saline and stained for 90 minutes in six per cent buffered solution of Giemsa stain (Watson, 1975). He classified the stained acrosomes as intact, damaged, and entirely lost ones.

Sokolovskaya *et al.* (1981) classified the abnormal acrosomes as swollen with indistinct contour, exfoliation commencing, lost acrosomes and lost head. He suggested that thawed semen is suitable for insemination only if minimum 30 per cent of spermatozoa have normal acrosomes.

Benjamin (1985) compared various staining methods for acrosome and classified acrosomal abnormalities in to four categories viz., knobbed, incomplete, ruffled and abnormal nuclear cap. Acrosomal changes in chilled and frozen spermatozoa observed after staining the semen smear using Giemsa stain were swelling, degeneration, tearing and crinkling of acrosomal membrane (Saulankhi *et al.*, 1992).

Madan and Watson (1994) combined Nigrosin-Eosin stain and Giemsa stain in order to determine the acrosomal status of live spermatozoa. They classified spermatozoa into four categories – live acrosome intact, live acrosome reacted or damaged, dead acrosome intact and dead acrosome reacted or damaged.

Sarma (1995) fixed the semen smear in methanol for 15 minutes and stained using Giemsa for 3-4 hrs at 37°C, followed by washing in tap water. Kutty *et al.* (1996) described a rapid and simple staining procedure to distinguish the acrosomal damage of spermatozoa using Giemsa stain in Eosin -Nigrosin stained smears.

2.1.8.2 ***Acrosomal Abnormalities***

Deka and Rao (1984) found that 1.57 $\pm$ 0.28 per cent spermatozoa in fresh buck semen had damaged acrosomes. Deka and Rao (1985) opined that fresh bucks semen contained 1.18 $\pm$ 0.20 per cent damaged acrosomes. In a study Deka and Rao (1986a) stained acrosomes of buck semen using Giemsa stain and found that 0.43 $\pm$ 0.12 per cent showed swollen acrosome, 0.04 $\pm$ 0.06 per cent separating acrosome, 0.43 $\pm$ 0.13 per cent entirely lost acrosome and 0.93 $\pm$ 0.8 per cent entirely damaged acrosome. Average percentage of abnormal acrosomes in the fresh buck semen stained with Geimsa was 8.82 $\pm$ 0.64 (Ranjini, 1998) and 0.70 $\pm$ 0.15 (Simon, 1999). Srinivas *et al.* (2002) recorded that 72.68 $\pm$ 0.97 per cent of spermatozoa in summer and 77.26 $\pm$ 0.76 per cent in winter had intact acrosome in semen of native goats of Andhra Pradesh.

2.2 OESTRUS SYNCHRONISATION IN GOATS USING PROSTAGLANDIN $F_{2\alpha}$

Oestrus synchronisation developed as a method for reducing the problems associated with Oestrus detection and thereby increasing the utilisation of Artificial Insemination. In addition the oestrus synchronisation technology can be used to

improve the management of reproduction and thus to improve the reproductive performance of the herd.

2.2.1 Response to Prostaglandin Treatment

Ahmad and Bhattacharya (1983) used double injection of PGF2α 11 days apart and could synchronise oestrus in all the treated goats. The mean interval from the second prostaglandin injection to onset of oestrus was 66.40 $\pm$ 4.22 hours. Pandey *et al.* (1985) conducted a study on oestrus synchronisation in goats using two injections of PGF2α at 11 days interval and brought 80 non cycling, 86 cycling and 100 per cent non-cycling and nymphomaniac goats in to oestrus.

In a study by Mahmood *et al.* (1990), 56.14 per cent of Pashmina goats and 38.46 per cent of Jamnapari goats could be brought in to oestrus within an average time of 68.40 $\pm$ 1.98 and 67.94 $\pm$ 3.50 hours respectively after injection with a single dose of PGF2α.

There was no significant difference in the oestrus response after administration of two doses of PGF2α at a low dose rate (40 μg Cloprostenol) by intravulvo submucosal route and at the normal dose rate (100 μg Cloprostenol) by intra muscular route. Eighty eight percent of animals showed oestrus signs after intravulvo submucosal injection against 84 per cent after intra muscular injection (Gonzalez and Ramon, 1992)

Synchronisation rate of 83.30 per cent could be obtained in indigenous goats after double injection of PGF2α at 11 days interval along with Pregnant Mare Serum Gonadotropin one day prior to the second injection (Thilagar *et al.*, 1992). Animals came in to oestrus within 32 $\pm$ 1.22 hours after the second dose of PGF2α.

The effectiveness of a single injection of PGF2α by intravulvo submucosal route for oestrus synchronisation in 20 does in luteal phase was studied. All the animals came in to oestrus after the administration of prostaglandin (Kutty and Mathew, 1996)

An oestrus synchronisation rate of 94.60 per cent could be obtained in goats after the administration of double dose of PGF2α at 11 days interval (Shenglin *et al.*, 1999). Kusina *et al.* (2000) reported that 91.70 per cent of goats came into oestrus within four days of administration of second dose of PGF2α in a double injection regime of PGF2α at 11 days interval. Goel and Agarwal (2000) administered a naturally occurring prostaglandin to does in mid-luteal phase and found that 80 per cent of the animals came into oestrus within 48-96 hours.

Bharali and Dutta (2001) conducted a study on oestrus synchronisation in goats in mid-luteal phase using single dose of PGF2α and it's three different combinations with Human Chorionic Gonadotropin (at six hours post onset of oestrus) and Pregnant Mare Serum Gonadotropin (before 24 hours of PGF2α injection). All the treated goats exhibited oestrus when different hormonal combinations were used and the mean time taken for onset of oestrus after the treatment did not differ significantly with different hormonal combinations.

Chede *et al.* (2002) in a study propounded that 63.40 per cent of animals responded to oestrus synchronisation with double dose of PGF2α at 11 days interval. The animals exhibited oestrus signs 55.38 $\pm$ 2.22 hours after the second dose of prostaglandin. He opined that the low response to hormone for oestrus synchronisation might be due to variation in breed, plane of nutrition, environmental factors, managemental practices and hormonal profile in individual animals.

Senthilkumar (2002) compared the efficacy of prostaglandin-PMSG combination and prostaglandin alone on oestrus synchronisation in 48 goats. He

observed that 100 per cent of the animals came into oestrus when prostaglandin-PMSG was used against 91.70 per cent animals when Prostaglandin alone was used.

2.2.2 Duration of Oestrus

Geyling and Van Niekerk (1986) synchronised oestrus in Boer goats using double dose of prostaglandin and reported that the oestrus duration was 41.90 hours after the first dose of prostaglandin and 30.90 hours after the second. Duration of oestrus non-descript synchronised goats was 35.52 ± 2.39 hours (Goel *et al.*, 1992). Romano (1993) propounded that duration of oestrus in goat was shortened by serving and there was no difference in duration between multiparous and nulliparous goats. Selvaraju *et al.* (1997) conducted an experiment to study the effect of method of breeding on the duration of oestrus and found that shorter duration of 24.33 ± 1.58 hours was observed in goats that were subjected to natural service compared to31.33 ± 2.51 hours by artificial insemination.

Goel and Agarawal (2000) opined that duration of oestrus in goats was 34.50 ± 3.31 hours in natural oestrum as compared to 28.0 ± 3.26 hours in prostaglandin induced oestrus. Bharali and Dutta (2001) recorded that mean duration of oestrus in natural oestrus was 31 ± 1.75 hours against 23.50 ± 0.99 hours in PGF2α induced oestrus. Average duration of oestrus in prostaglandin synchronised does was found to be 34.91 ± 4.97 hours (Senthil Kumar, 2002).

In a study by Chede *et al.* (2002) the mean duration of prostaglandin induced oestrus was 35.38 ± 1.55 hours with a range of 24 to 48 hrs against 24 hours with a range of 20-24 hours in natural oestrum. Mean oestrus duration in Jakhrana goats averaged 27.97 ± 1.43 hours at puberty and 29.28 ± 0.98 hours at post puberty (Goel and Agarwal, 2002)

2.2.3 Oestrus Behaviour in Goats

Goats remain in oestrus for about 36 hours and exhibit various oestrus signs, which are more pronounced in meat type breeds compared to dairy type goats (Kumar and Yadav, 2000). They recorded signs of oestrus as frequent bleating, switching of tail, restlessness in seeking the buck, arching and stretching of the body, mounting and allowing mounting by other does. Rajkonwar and Borgohain (1978) described the oestrus signs in local goats as frequent bleating, restlessness, searching for the buck, swelling and congestion of vulva with flow of colourless transparent mucus from genital tract.

Smith (1980) described the signs of oestrus in goats as swollen, reddened and moist external genitalia and rapid side to side or up and down flagging of tail. Restlessness, tendency to be more vocal and frequent urination also could be observed. In a study by Mehta *et al.* (1991) smelling, mounting, aggressiveness by buck, bleating and switching of tail were the prominent signs of oestrus in goats. Vulval swelling and vaginal discharge were not the consistent signs of oestrus in goats. Goel and Agarwal (1994) found that vulval discharge was thin and watery during early oestrus, mucinous during mid oestrus and thick and cheesy in late oestrus.

Kutty and Mathew (1996) opined that only 25 per cent of goats administered with prostaglandin through intravulvo submucosal route exhibited oestrus signs, which included wagging of tail and standing to be mounted while rest of the treated goats failed to show any behavioural sign and failed to be detected by the buck. Intensity of oestrus in goats was classified by Senthilkumar *et al.* (1998) as very good, good, fair and poor based on the oestrus signs.

Senthilkumar (2002) graded the intensity of oestrus in goats by giving score to behavioural sign and physiological changes associated with oestrus in goats. He

reported the mean score obtained as 8.82 $\pm$ 1.59 in the animals which were administered with double dose of PGF2α. Wagging of tail, vulval redness and oedema were the predominant signs observed in those animals.

2.3 FREEZING OF SEMEN USING TRIS DILUENT

Preservation of semen has interested livestock breeders since artificial insemination was first considered. The large scale artificial insemination programmes in the twentieth century required preservation of spermatozoa under artificial conditions for extended period of time. This could be achieved by freezing of semen under ideal conditions thereby prolonging the fertile life of spermatozoa. (Salamon and Maxwell, 1995a)

2.3.1 Washing of Spermatozoa

A thermolabile egg yolk coagulating factor was found in goat semen but not in bull, ram, boar and rabbit semen; the factor was present in the seminal plasma and was derived from the secretion of cowpers gland; spermatozoa washed once did not coagulate egg yolk and remained motile for 10-15 days in egg yolk citrate at 4°c; twice washed spermatozoa were motile for one month (Iritani *et al.*, 1961). The optimum conditions of temperature, egg yolk concentration and pH for coagulation were investigated; the factor had properties similar to those of enzymes (Iritani and Nishikawa, 1961).

Westhuysen (1978) reported that washing goat semen before freezing significantly increased post-thaw motility and removal of seminal plasma by centrifugation of goat semen was beneficial for the survival of spermatozoa after thawing. When the semen was diluted six to eleven fold, double washing was more effective than single washing. (Ritar and Salamon, 1982).

The percentage of motile sperms did not vary significantly between semen frozen in Tris egg yolk citric acid fructose glycerol diluent with and without seminal plasma (Deka and Rao, 1984). Percentage of damaged acrosome was significantly higher in spermatozoa frozen with out seminal plasma.

Memon *et al.* (1985) recorded significantly higher percentage of post-thaw motility and normal acrosomes in goat semen washed twice with Ringer's solution than in unwashed semen. Perez (1985) noted that ejaculates from goats washed twice had higher sperm motility and a higher percentage of acrosome defects than non-washed semen after freezing. The percentage of live and progressively motile spermatozoa up to two months of storage at -196°C did not differ between buck semen frozen with and without seminal plasma (Deka and Rao, 1987a).

Machado and Simplicio (1992) opined that Kreb's Ringer-phosphate solution along with 4.92 per cent solution of Sodium citrate was more efficient as washing medium when compared to Kreb's Ringer's phosphate solution. In a study Misra *et al.* (1993) reported that motility of spermatozoa and intact acrosomes were higher in washed compared to unwashed frozen semen. No significant difference was noticed in post thaw motility of buck spermatozoa frozen with or without washing the spermatozoa (Purohit *et al.*, 1992).

Removal of seminal plasma prior to semen freezing in an extender containing egg yolk had an unfavourable effect on post thaw motility and integrity of spermatozoa when Boer buck semen was used (Tuli and Holtz, 1994).

2.3.2 Glycerolisation

Glycerol is used almost universally as the cryoprotective agent for freezing semen. It can be added to semen at 5°C slowly by dripping or by adding small amounts over a period of one hour or as one step addition (Blokhuis, 1962).

For buck semen frozen with Tris-egg yolk citric acid fructose-glycerol extender, the post thaw motility was higher with 6.4 per cent glycerol than with four or nine percent glycerol (Deka and Rao, 1986b). Four per cent glycerol gave best post thaw motility percentage when buck semen was frozen in Tris buffer (Deshpande and Mehta, 1991). Purohit *et al.* (1992) propounded that five percent level of glycerol was superior to three or four percent levels for freezing buck semen.

In a study by Sinha *et al.* (1992) six percent glycerol in Tris extender resulted in frozen semen with good motility and more intact acrosomes. Stepwise glycerolisation at 37°C gave higher progressive motility and percentage of live spermatozoa both before freezing and after thawing than one step glycerolisation at 37°C or stepwise extension with glycerol at 5°C for Boer buck semen (Tuli and Holtz, 1994).

Prasanth (1995) reported that better post thaw motility was obtained when six percent glycerol was used as cryoprotectant than five or seven percent levels in freezing Malabari buck semen. Sivaselvam *et al.* (2000) studied the freezing protocol for Tellicherry buck semen and found that seven percent glycerol protected the goat spermatozoa better than five or ten percent levels.

2.3.3 Equilibration

Equilibration time is the period needed before freezing for the sperm cells to become adjusted to the extender so that on freezing excessive loss of sperm cells does not occur. Equilibration is usually conducted at 5°C for four to six hours.

Westhuysen (1978) reported that increasing the equilibration time caused a significant increase in post-thaw motility though it did not have any adverse effect on pre-freezing sperm motility when Tris egg yolk glycerol was used as extender. Post thaw motility of buck semen frozen with Tris-egg yolk-citric acid-fructose-glycerol

extender was higher for six hours equilibrated semen than for one or three hours equilibration (Deka and Rao, 1986b). Sinha *et al.* (1987) opined that four or six hours of equilibration provided high percentage of post thaw motility for buck semen.

Purohit *et al.* (1992) concluded that five hours equilibration period showed high post-thaw semen quality for frozen buck semen. In a study on preservation of buck semen in skim milk with combination of six percent glycerol, Sinha *et al.* (1992) opined that four hours equilibration resulted in better quality chilled semen. He also found that four hours of equilibration period resulted in good quality frozen semen when Tris diluent was used. Sivaselvam *et al.* (2000) reported that four hours equilibration period resulted in good quality frozen buck semen in Tris extender.

2.3.4 Thawing

Deka and Rao (1987b) compared the post thaw motility of goat semen after rapid thawing (37°C for 12-15 seconds) and slow thawing (5°C for 2 minutes). Rapid thawing resulted in significantly higher sperm motility during post thawing preservation than slow thawing. Frozen buck semen was thawed at 37°C for 10 seconds (Choudhary *et al.*, 1987), 37 °C for 30 seconds (Deshpande and Mehta, 1991 and Sivaselvam *et al.*, 2000) and 37°C for 2minutes (Tuli and Holtz, 1994)

2.3.5 Motility of Tris Diluted Semen

Westhuysen (1978) reported 18-40 per cent post thaw motility for Angora goat semen frozen with Tris based diluent containing egg yolk and glycerol. Buck semen could be stored at 5°C with good motility up to 60 hours in Tris (Balakrishnan, 1979). Deka and Rao (1984) recorded 63.25 $\pm$ 1.83 and 64.15 $\pm$ 2.85 post thaw motility per cent for the buck semen frozen with and without seminal plasma. The efficacy of Egg yolk citrate fructose glycerol (EYCFG) and Tris egg

yolk citric acid fructose glycerol (TEYCFG) diluents for buck semen freezing was compared by Deka and Rao (1985) and found that the percentage of live spermatozoa and those with forward motility were significantly higher in semen frozen in Tris egg yolk citric acid fructose glycerol diluent.

Post thaw motility of 55 to 65 per cent was recorded when German improved White buck semen was frozen in a modified Tris diluent (Kolk, 1985). Sinha *et al.* (1987) compared the post thaw motility of spermatozoa of different goat breeds frozen in Tris egg yolk citric acid fructose glycerol extender and found to be 56.15, 54.58 and 47.85 per cent respectively for Jamnapari, Barbari and Black Bengal bucks. Percentage of post thaw motility after freezing was 64 $\pm$ 1.05 and 61.10 $\pm$ 1.10 respectively for Beetal got semen diluted in Tris egg yolk citric acid fructose glycerol and Egg yolk citric acid fructose glycerol extender (Choudhury *et al.*, 1987).

Percentage of post thaw motility of buck semen when frozen in Tris egg yolk citric acid fructose glycerol extender was 65.20 $\pm$ 0.80 (Sinha *et al.*, 1991) and 51.83 $\pm$ 2.65 (Deshpande and Mehta, 1991). Singh *et al.* (1992) could obtain significant difference, 52.96 $\pm$ 0.836 Vs 38.70 $\pm$ 0.897 per cent in the post thaw motility when buck semen was frozen in Tris egg yolk citric acid fructose glycerol and egg yolk citric acid fructose glycerol. Sivaselvam *et al.* (2000) obtained post thaw motility of 54.50 $\pm$ 0.01 per cent for Tellicherry buck semen frozen in Tris based diluent containing 20 per cent egg yolk and seven per cent glycerol. They also found that there was a gradual decline in motility, which became significant after nine months of storage.

2.4 FREEZING OF BUCK SEMEN USING BIOCIPHOS

Biociphos is an extender of non-animal origin containing soybean extract as a substitute for egg yolk. It is a ready to use, manufacturer certified extender that would minimise handling and accelerate preparation of the extender in the

laboratory. Further it can obviate the risk of microbial contamination of semen from the extender (Van Wagtendonk *et al.*,2000).

2.4.1 Motility of Semen Diluted in Biociphos

Bohm *et al.* (1995) compared the motility of frozen thawed bovine spermatozoa diluted in Biociphos and Tris based diluents. Biociphos diluted semen showed 60 and Tris diluted showed 62 per cent motility after freeze thawing.

Van Wagtendonk *et al.* (2000) stated that a well-standardised non-animal origin tissue would present a valuable contribution to the AI industry by preventing the risk of microbial contamination. He evaluated the motility of bovine semen cryopreserved in Tris standard, Tris concentrate and Biociphos extenders and obtained post thaw motility percentage of 39.20 $\pm$ 0.50 with Biociphos, which was significantly lower than that of Tris standard and Tris concentrate. He ascribed this to the higher viscosity and to the presence of particulate debris in the Biociphos extender, which resulted in a relatively lower, subjective evaluation of motility.

Gil *et al.* (2000) performed the computerised motility evaluation of bull semen diluted in Biociphos and Triladyl. Biociphos extended semen showed higher motility value for the velocity patterns and lateral sperm head deviations. In a study Singh *et al.* (2000) compared the freezability of buffalo bull semen in three different extenders viz., Tris buffer, Laiciphos and Biociphos. Tris buffer exhibited significantly better freezability compared to the other two extenders. They reported post thaw motility of 43.92 $\pm$ 1.75 with Tris buffer, 37.85 $\pm$ 3.73 with Laiciphos and 37.50 $\pm$ 2.90 per cent with Biociphos.

Post thaw semen characteristics of Murrah and Jersey bull semen diluted in Biociphos and Tris extender containing egg yolk was performed by Rao *et al.* (2002) and found that post thaw motility was significantly higher when the semen was

diluted in Biociphos compared to Tris extender for both the breeds. Thun *et al.* (2002) compared the post thaw motility of bull semen extended in Tris egg yolk extender packaged at 4°C, at room temperature and Biociphos packaged at room temperature. Post thaw motility was better when Tris egg yolk was used compared to Biociphos.

2.5 ARTIFICIAL INSEMINATION IN GOATS

Artificial insemination is the most important single technique devised for genetic improvement of animals because a few select males produce enough sperms to inseminate thousands of females per year. There are four methods for artificial insemination in the goat: vaginal, cervical, transcervical and laparoscopic or intra uterine methods (Ax *et al.*, 2000).

Patel (1967) conducted an experiment in Jamnapari bucks to study the results of artificial insemination when chilled semen extended in egg yolk citrate was used. He could obtain a conception rate of 68.98 per cent after first insemination and 7.04 per cent after the second in those that failed to conceive with the first, with an overall conception rate of 76.02 per cent. Kolk (1985) carried out artificial insemination in goats using frozen semen of German improved White buck. Of the 40 does inseminated, six required repeat insemination. The non-return rate over the two breeding seasons was eighty five per cent.

A study on the status of artificial insemination in goats in New Zealand revealed that an average conception rate of 71.60 per cent was obtained by artificial insemination (Summermatter, 1986). Conception rates after cervical and laparoscopic inseminations were compared in oestrus synchronised goats (Moore *et al,* 1989) and observed that laparoscopic AI resulted in a higher kidding rate than cervical AI.

Mowlem *et al.* (1992) studied the role of AI in improving the goat production in various tropical countries and concluded that AI using frozen and fresh semen would be an useful technique for spreading superior genetic material over a wider area in those countries.

Guelph system for transcervical AI was found to be successful for cervical penetration in ewes. The animal was restrained in dorsal recumbency and AI was performed using Bozeman forceps. Cervical penetration could be achieved in 87.80 per cent of the ewes with a conception rate of 50.70 in season and 24.40 out of season (Buckrell *et al.*, 1994).

In a study Eppleston *et al.* (1994) examined the relationship between fertility of frozen thawed ram semen and the depth of cervical insemination, the fertility increased by 7-12 per cent for each cm increase in depth of insemination.

Salamon and Maxwell (1995b) studied various methods to improve fertility in ewes and suggested that the most effective method is to increase the depth of deposition of frozen thawed semen in to the cervical canal. However the best method was found to be intra uterine insemination by laparoscopy.

2.6 PREGNANCY RATE AFTER INSEMINATION

2.6.1 Tris Diluted Semen

Balakrishnan (1979) obtained 44 per cent pregnancy rate in Malabari x saanen crossbred goats after insemination with chilled semen diluted in Tris extender. Cetinkaya (1980) conducted a study on the fertility of Angora goat semen frozen in Tris egg yolk sodium citrate glycerol diluent and reported 37.5 per cent conception rate after insemination with a kidding rate of 22.2 per cent. Eighty five percent non-return rate was observed when artificial insemination was performed using chilled semen of German improved White buck (Kolk, 1985).

Choudhury *et al.* (1987) compared the fertility of Beetal goat semen frozen in egg yolk citrate fructose glycerol diluent and Tris egg yolk citrate fructose glycerol diluent and reported non-return rate of 71.95 per cent and 88.46 per cent respectively. The fertility of semen of Jamnapari, Barbari and Black Bengal breeds of goats diluted in Tris egg yolk fructose citric acid glycerol extender was 45.16, 44.00 and 42.10 per cent respectively (Sinha *et al.*, 1987). Prasanth (1995) recorded a conception percentage of 47.36 in Malabari x Alpine crossbred goats after insemination using frozen semen. Senthilkumar (2002) observed conception percentage of 45.45 after insemination with chilled semen in Malabari crossbred goats.

2.6.2 Biociphos Diluted Semen

Bhom *et al.* (1995) reported a 60/90 days non return rate of 67.8 and 68.3 per cent when bull semen was cryopreserved in Biociphos and Tris based diluents. He concluded that Biociphos extender could be used as an alternative for animal protein constituent extenders for cryopeservation of semen.

Biociphos extender included soybean lecithin as a substitute for egg yolk to prevent or repair damage to the spermatozoal plasma membrane during cryopreservation (Van Wagtendonk *et al.*, 2000). A 56 days non-return rate of 64.3 ± 0.6 per cent was obtained with Biociphos diluent compared to the non-return rate of 68.7 ± 0.6 and 68.2 ± 0.6 per cent respectively with Tris standard and with Tris concentrate. The fertility rate obtained with bull semen cryopreserved in Triladyl and Biociphos was 69.1± 0.8 and 69.2 ± 0.8 per cent respectively (Gil *et al.*, 2000).

Rao *et al.* (2002) compared the fertility of frozen semen extended in Biociphos and Tris for Murrah and Jersey bull semen. Fertility rates of 45.33 ± 2.43 and 40.33 ± 2.70 per cent respectively were obtained when Biociphos extender was

used in Murrah and Jersey bull semen against 40.33 $\pm$ 2.70 and 44.67 $\pm$ 2.67 per cent when Tris extender was used in Murrah and Jersey bull semen.

Thun *et al.* (2002) studied the fertility of semen diluted in Tris egg yolk extender packaged at 4° C, room temperature and Biociphos extender packaged at room temperature for different breeds of bulls based on 75 days non return rate. Best results were obtained in Simmental breed using the Tris egg yolk extender packaged at 4° C and in Holstein-Friesians using Biociphos extender packaged at room temperature.

2.7 PREGNANCY DIAGNOSIS IN GOATS USING ABDOMINAL PALPATION

Abdominal palpation is a cheap and simple method for pregnancy diagnosis in goats. The accuracy of the diagnosis increases as the pregnancy gets advanced.

Smith (1980) reported that the fetus could not be detected by abdominal palpation in goats before 110 days of pregnancy. He also described various methods of palpation such as attempting to touch both hands together through the animal's abdomen, encircling the abdomen with both arms and lifting upwards and abdominal ballotment.

Abdominal palpation in late pregnancy was possible in slab sided, thin relaxed goats, but was very difficult in big bodied, strong willed goats because they resisted the palpation by tightening the abdominal muscles (Williams, 1986).

Goel and Agrawal (1990) conducted abdominal palpation in goats that were in natural standing position by pressing the abdominal wall on both sides to feel the fetal mass. He concluded that diagnosis by abdominal palpation was not possible at 51 to 60 days of pregnancy but the percentage of accuracy increased to 70 at 61-70 days, 90.3 at 71-80 days and 95.4 at 80 days and later.

Rajasekaran *et al.* (1992) used abdominal palpation as a method for pregnancy diagnosis in sixty-five goats at various reproductive stages. Percentage of animals diagnosed as pregnant was 27.5, as non-pregnant was 45 and as doubtful was 27.5. Out of the doubtful cases 71.4 per cent were diagnosed as pregnant and 28.6 per cent as non-pregnant by the use of X-ray.

Kutty(1999) opined that bimanual method is a simple, effective and inexpensive clinical method for diagnosing early pregnancy in small ruminants and for assessing approximate stage of gestation.

2.8 GESTATION LENGTH IN GOATS

Mean gestation period in goats was reported to be 148 to 156 days in goats (Roberts, 1971). Average gestation length in Malabari, Alpine x Malabari and Saanen x Malabari does was found to be 146.66 $\pm$ 0.53 days (Kuriakose, 1981). Prasanth (1995) opined that the average gestation period of 149.85 $\pm$ 4.45 days for Alpine x Malabari crossbred does. Mean gestation period in Boer goats was recorded as 150 $\pm$ 0.19 days (Seabo *et al*, 1999). Jainudheen and Hafez (2000) concluded that the average gestation length in goat was 150 days.

2.9 LITTER SIZE AT BIRTH

Van Niekerk and Casey (1988) stated that the average litter size at birth in Boer goat was 1.93 and the percentage of single, twin, triplet and quadruplets produced were 24, 58, 15 and one respectively. The incidence of single, twin and triplet birth was 40, 48 and 12 per cent respectively in Malabari x Alpine crossbred goats with an average number of kids per kidding of 1.7 (Prasanth, 1995). Frequency of birth of singles, twins, triplets and quadruplets in Boer goat was 27.97, 54.57, 16.04 and 1.43 respectively (Seabo *et al.*, 1999).

Materials and Methods

3. MATERIALS AND METHODS

3.1 SEMEN COLLECTION

Normal semen ejaculates from six Boer bucks aged two to three years maintained at the Goat Breeding centre, Kerala Livestock Development Board (KLDB), Dhoni, Palakkad were used for the study. The bucks selected were apparently normal, healthy and maintained at standard and uniform feeding and management practices. These animals were imported from Australia and were trained for semen collection using an artificial vagina. The study was conducted over a period of 11 months from August 2002 to June 2003.

Semen collection was performed by artificial vagina method using a Danish type of artificial vagina (Perry, 1969).

3.2 SEMEN EVALUATION

For the study of normal characteristics of Boer buck semen, six ejaculates collected from six Boer bucks at weekly intervals were subjected to preliminary evaluation by observing volume, colour, density, pH, mass activity and motility following the standard procedures (Roberts, 1971).

Sperm vitality was estimated using Nigrosin- Eosin stain (Ranjini, 1998) and Acrosome abnormality of spermatozoa was assessed by Giemsa staining technique (Sarma, 1995).

3.3 PROCESSING AND FREEZING OF THE SEMEN

Processing and freezing of the semen was carried out at the Central Frozen Semen laboratory, KLDB, Dhoni. Six semen samples from each buck were frozen in Tris diluent and six samples in Biociphos extender.

3.3.1 In Tris Extender

3.3.1.1 *Preparation of Tris extender*

Tris egg yolk extender was prepared as per the method described by Simon (1999). Tris hydroxy methyl amino methane (2.42 g), citric acid (1.34 g) and fructose (1.0g) were weighed accurately in to two sterile 100 ml volumetric flasks numbered I and II. Then 25 ml double glass distilled water was added to the flasks and stirred well. Fresh egg yolk was collected aseptically and 10 ml was added to the contents of the flasks. The contents were mixed well using a sterile glass rod. Benzyl penicillin and streptomycin were added at the rate of 750 IU/ml and 750 µg/ml of the diluent in the flasks. Twelve ml of glycerol was added to the flask numbered II and stirred well. Then the volumes of the extender in both the flasks were made up to 100 ml by adding double distilled water. Extenders were placed in a water bath maintained at 37°C. Solution in the flask I was used for initial dilution of semen and that in the flask II was used for glycerolisation of the extender.

3.3.1.2 *Selection of Samples*

Based on the preliminary evaluation, semen samples with more than 70 per cent initial motility were used for freezing trials.

3.3.1.3 *Washing and Freezing of Spermatozoa*

Semen samples were extended ten times with Tris buffer and centrifuged at 3000 rpm for five minutes. The supernatant fluid was pipetted out. The sediment was extended ten folds by mixing with Tris buffer and repeated the centrifugation at 3000 rpm for five minutes. The clear supernatant fluid was removed. Semen pellet after washing was extended five times with non-glycerolated Tris extender and the initial motility of the sample was assessed under microscope. The washed semen samples were diluted in Tris extender to a final dilution ratio of 1:10. Freezing and storing of

the semen samples were carried out as per the standard procedure followed by Ranjini (1998).

3.3.2 In Biociphos extender

3.3.2.1 *Preparation of Biociphos Extender*

Biociphos extender was prepared as follows. Hundred ml of concentrated extender was placed in water bath at 37°C for ten minutes. Four hundred ml of double distilled water also was placed in the same water bath at 37°C. The concentrated extender was then transferred to the double distilled water. The bottle, which contained the concentrated extender, was then rinsed twice with the final solution. The solution was then thoroughly mixed using a magnetic stirrer and used for extending semen samples.

3.3.2.2 *Dilution and Processing of Semen Samples in Biociphos Extender*

Semen sample for freezing was selected after microscopic examination and pre-dilution was performed with Biociphos extender at 1:1 ratio. The pre-diluted semen sample was kept in water bath at 37°C for ten minutes. Final dilution was performed by adding the pre-diluted semen sample into the remaining extender so that the final dilution ratio is 1:10. The flask containing the diluted semen was then transferred to a plastic container containing water at 37°C. The container was then transferred into a cold cabinet at 5°C so as to bring the temperature of the diluted semen to 5°C within one to one and half hours. The diluted semen was left in the cabinet for equilibration at 5°C for four hours. The semen sample was then packed into straws and frozen, stored and thawed following the same procedure as with the Tris diluent.

3.4 OESTRUS SYNCHRONISATION OF DOES

Hundred and fifty Malabari parous does with body weight of above 20 kg, belonging to Goat and Sheep farm, Kerala Agricultural University, Mannuthy were used for the study. The does for the study were maintained in the farm under identical conditions of feeding and management throughout the period of study. The does were maintained under semi-intensive system of management. Oestrus synchronisation was carried out in the does by intramuscular administration of two doses of 0.147 mg Tiaprost trometamol (0.75 ml of Iliren), a prostaglandin analogue at 11 days interval as suggested by Senthilkumar (2002).

3.4.1 Time Taken for Oestrus

Each doe after the administration of second dose of $PGF_{2\alpha}$ was closely observed for the onset of oestrus by noting the oestrus associated behavioural and physiological changes. An apronised buck was used to detect oestrus changes in the does. Onset of oestrus was confirmed by observing relaxation of the cervical canal by using a vaginal speculum. The doe was secured by lifting both hind limbs. A sterile vaginal speculum lubricated using sterile jelly was used to dilate the vaginal cavity and to observe dilated cervical opening and other physiological changes associated with oestrus. A headlamp was used to provide light in the dilated vaginal cavity. The time interval between the time of administration of second dose of prostaglandin and the time of onset of oestrus was recorded.

3.4.2 Duration of Oestrus

The period from the time of onset of oestrus signs to the end of behavioural and physiological signs of oestrus was considered as the duration of oestrus.

3.4.3 Intensity of Oestrus

Intensity of oestrus in the does was graded by assigning scores (Senthilkumar, 2002) to behavioural and physiological changes associated with oestrus.

3.4.3.1 *Oestrus Signs and Their Scores*

Parameters	Score
I Behavioural signs	
Wagging of tail	2
Mounting on other animals	1
Bleating	1
Circling with the buck	1
Standing to be mounted	5
II Physiological signs	
Vulval redness	2
Vulval oedema	3
Vulval discharge	5
Total	20

3.5 ARTIFICIAL INSEMINATION

The does were divided into two groups, Group I with 100 does and Group II with 50 does. Group I does were inseminated using frozen semen diluted in Tris and Group II does using frozen semen diluted in Biociphos extender. Frozen semen sample which had minimum of 40 percent post-thaw motility were used for artificial insemination. Only the does that exhibited oestrus signs were inseminated. The doe was secured by lifting both hind limbs of the animal. A sterile vaginal speculum lubricated with sterile jelly was used to dilate the vaginal cavity and to confirm whether the animal is in heat. Artificial insemination was carried out by speculum method (Balakrishnan, 1979).

3.6 PREGNANCY DIAGNOSIS

Pregnancy diagnosis was performed in all the inseminated animals at three months of pregnancy using abdominal palpation method (Sudarsanan, 1970).

3.7 FERTILITY STUDY

Fertility of the inseminated does was assessed after kidding. Duration of pregnancy was calculated as the time interval between the last insemination date and the kidding date. Litter size at birth and the birth weight of kids were recorded. The results were statistically analysed as per methods described by Snedecor and Cochran (1985).

Fig. 1. Boer Buck (No. 5)

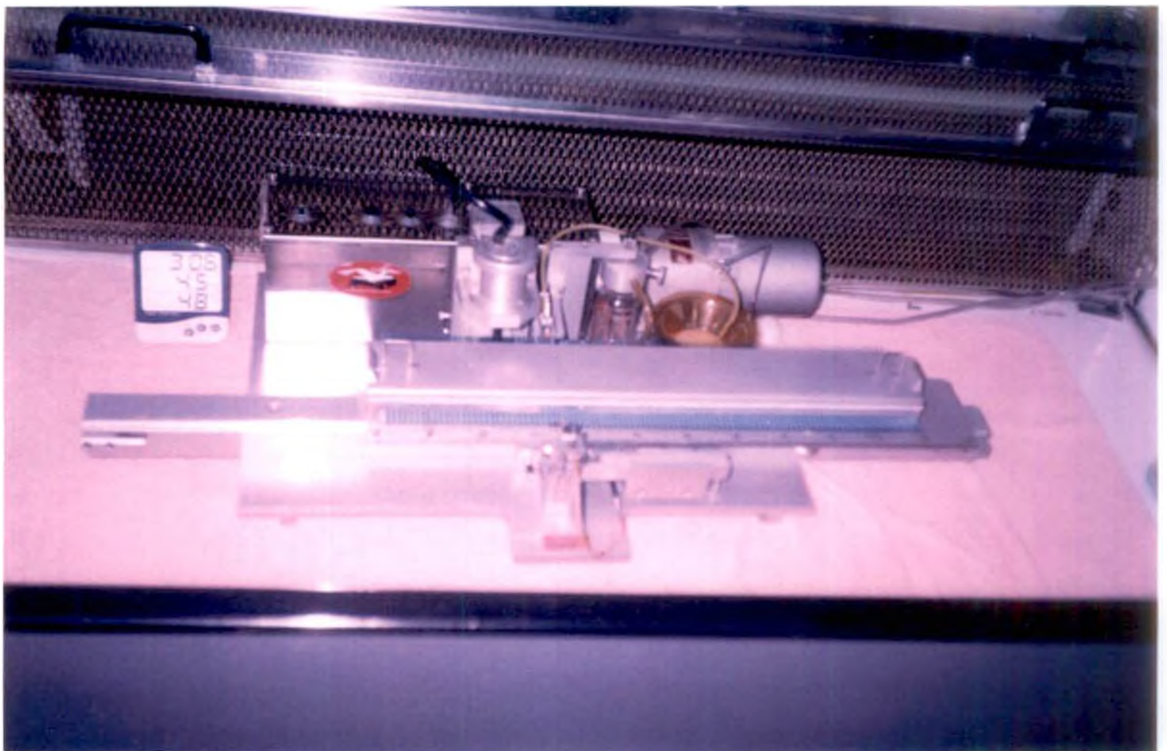

Fig. 2. Automatic filling and sealing machine

Results

4. RESULT

A study was carried out to find out the fertility of oestrus synchronised crossbred Malabari does maintained at Goat and sheep farm, Kerala Agricultural University, Mannuthy. Frozen semen samples of six Boer bucks maintained at Goat Breeding Centre, Kerala Livestock Development Board, Dhoni were used for artificial insemination. Semen characteristics and freezability of the samples were also studied. Data obtained on semen evaluation, oestrus synchronisation and fertility are presented in Table 1 to 9 and Figure 1 to 6.

4.1 SEMEN EVALUATION

The results of semen evaluation of six Boer bucks are presented in Table 1. Average volume of Boer buck semen was 1.27 ± 0.06 ml with a range of 0.5 ml to 2 ml. Average semen volume was found to be 1.48 ± 0.06 ml, 1.14 ± 0.18 ml, 0.94 ± 0.08 ml, 1.33 ± 0.18 ml, 1.33 ± 0.11 ml and 1.42 ± 0.16 ml for the bucks 1, 2, 3, 4, 5 and 6 respectively. There was no significant difference in the volume of semen among different bucks ($P>0.05$).

Colour of all ejaculates was found to be creamy and density was DDDD. Mass activity of various samples found to vary from + + to + + + +.

Average concentration of spermatozoa was 2956.67 ± 81.74 millions per ml and was found to range from 1640 to 3840 millions per ml. The average sperm concentration of semen of bucks 1, 2, 3, 4, 5 and 6 was found to be 2902.50 ± 104.06, 2810.00 ± 191.48, 2504.44 ± 209.86, 3401.25 ± 118.43, 3155.00 ± 153.89 and 3032.22 ± 250.13 millions per ml respectively. There was no significant difference in sperm concentration among different bucks ($P>0.05$).

Mean live sperm per cent of buck semen was 88.16 ± 0.50 with a range of 80 to 95 per cent. The average percentage of live spermatozoa in fresh semen of bucks

1, 2, 3, 4, 5 and 6 were 88.75 ± 0.84, 90.11 ± 1.03, 88.56 ± 0.90, 87.38 ± 1.54, 86.38 ± 0.98 and 87.56 ± 1.72 respectively. Between bucks there was no significant difference in percentage of live sperms (P>0.05).

Mean pH of semen was 6.98 ± 0.03 with a range of 6.60 to 7.30. The average pH of semen of bucks 1, 2, 3, 4, 5 and 6 was found to be 6.90 ± 0.08, 7.05 ± 0.08, 6.96 ± 0.08, 6.96 ± 0.08, 6.99 ± 0.07 and 6.99 ± 0.06. No significant difference was noticed in the pH of semen among different bucks (P>0.05).

Average percentage of abnormal spermatozoa in the fresh semen as observed in the Nigrosin-Eosin stained smear was 3.20 ± 0.27. The percentage of abnormal spermatozoa was 2.75 ± 0.45, 2.11 ± 0.46, 2.67 ± 0.47, 3.50 ± 0.87, 5.25 ± 0.80 and 3.11 ± 0.56 for bucks 1, 2, 3, 4, 5 and 6 respectively.

Mean percentage of spermatozoa in the fresh semen with abnormal acrosome was 1.00 ± 0.13. The average percentage of spermatozoa with abnormal acrosome was found to be 1.38 ± 0.32, 0.89 ± 0.31, 1.00 ± 0.37, 1.13 ± 0.35, 0.88 ± 0.35 and 0.78 ± 0.22 in bucks 1, 2, 3, 4, 5 and 6 respectively.

4.2 OESTRUS SYNCHRONISATION OF DOES

Oestrus synchronisation of the does was performed by intramuscular administration of two doses of prostaglandin analogue, 0.147 mg Tiaprost trometamol (0.75 ml of Iliren) at 11 days interval.

4.2.1 Response to Oestrus Synchronisation

Perusal of Table 2. indicate that 96 does (96 per cent) from Group I and 47 does (94 per cent) from Group II exhibited oestrus. Out of the total 150 does, 143 (95.33 per cent) exhibited oestrus. Average time taken for the onset of oestrus after administration of second dose of prostaglandin was 51.02 ± 0.99 hours with a range

of 36 to 96 hours (Table 2.). Mean interval in group I and II does was 51.92 ± 1.29 and 49.25 ± 1.49.

4.2.2 Duration of Oestrus

Mean duration of oestrus in the does was 35.81 ± 0.86 hours (Table 2.). The duration of oestrus varied from 18 to 54 hours. Average duration in Group I and II does was 35.21 ± 1.01 and 37.00 ± 1.60 hours.

4.2.3 Intensity of Oestrus

Percentage of animals that exhibited various oestrus signs is given in the Table 3. and figure 1. Oestrus intensity scores of the does which exhibited oestrus signs ranged from One to 18 .The average oestrus score obtained in the goats which came in to oestrus was 5.97 ± 0.41. Behavioural signs and physiological changes noticed in the oestrus does were wagging of tail (65.33 per cent), frequent bleating (56.67 per cent), vulval oedema (43.33 per cent), standing to be mounted (34 per cent), circling with the buck (26 per cent), vulval redness (21.33 per cent), mounting on other animals (14 per cent) and discharge from genitalia (four per cent). One hundred and forty three does (95.33 per cent) were found to be in oestrus by speculum examination. Thirty one does (21.68 per cent) did not show any sign of oestrus even in the presence of buck. Among them 24 does were found to be in oestrus by speculum examination. This indicated that 16.78 per cent of oestrus does did not reveal any external oestrus sign.

4.3 Freezing of Semen

Semen samples from the six selected Boer bucks were frozen separately in Tris and Biociphos extenders and the observations are presented in Table 4 to 6 and Figure 2 and 3.

4.3.1 Tris Extender

Results of freezing of semen in Tris extender is presented in Table 4 and Figure 2. Average percentage of sperm motility of buck semen after washing twice in Tris buffer was 67.00 ± 1.47. Mean motility percentage of spermatozoa after freezing in Tris extender was 43.91 ± 1.86. There was significant reduction in motility of spermatozoa after freezing as compared to that of washed spermatozoa ($P<0.05$). But no significant difference was noticed in the motility of spermatozoa among different bucks ($P>0.05$).

Mean percentage of live spermatozoa in the fresh and freeze thawed semen samples were 87.83 ± 0.75 and 52.61 ± 1.56 respectively. While no significant variation was observed in the percentage of live spermatozoa of fresh semen between bucks ($P>0.05$) highly significant variation was observed after freezing ($P<0.01$).

4.3.2 Biociphos Extender

Results of freezing of semen in Biociphos extender is presented in Table 4 and Figure 3. Average percentage of sperm motility after initial dilution and after freezing in Biociphos extender was 80.56 ± 1.11 and 40.19 ± 1.87. Between bucks there was no significant difference in the post thaw motility of spermatozoa ($P>0.05$). Statistical analysis (Table 5.) revealed that there was no significant difference in sperm motility between the semen frozen in Tris and Biociphos extenders ($P>0.05$).

Mean percentage of live spermatozoa in fresh semen used for dilution in Biociphos extender was 87.64 ± 0.76. Variation between bucks in the percentage was not statistically significant ($P>0.05$). After freezing in Biociphos extender mean percentage of live spermatozoa was 43.64 ± 1.49. Significant difference was noticed among bucks in the percentage of live spermatozoa after freezing in Biociphos

extender ($P<0.05$). Statistical analysis (Table 6.) revealed that there was highly significant difference in the percentage of live spermatozoa between semen diluted in Tris and Biociphos extenders ($P<0.01$).

4.4 PREGNANCY DIAGNOSIS BY ABDOMINAL PALPATION

Pregnancy diagnosis was performed by abdominal palpation at three months of gestation in all the inseminated does. Twenty one does (21.88 per cent) in Group I and eight does (17.02 per cent) in Group II were diagnosed as pregnant. The result obtained was compared with number of does kidded and the accuracy of the method was found to be 93.55 per cent.

4.5 PREGNANCY RATE AFTER INSEMINATION

Details regarding the conception rate are presented in Table 7. and Figure 4. The does in oestrus in Group I and Group II were inseminated intra cervically using frozen Boer buck semen. In Group I, 96 does (96 per cent) were found to be in oestrus and inseminated. Twenty two does conceived. Thirty six kids were produced out of which three kids were born dead. The conception rate obtained was 22.92 per cent.

Out of 50 does in Group II, 47 (94 per cent) were in oestrus and were inseminated. Nine animals conceived and kidded. Nineteen kids were produced, of which two were stillborn. Conception rate in Group II was 19.15 per cent.

Of the total 150 animals selected for the study 143 were inseminated. Thirty one animals (21.68 per cent) conceived. Fifty five kids were produced. Five were born dead. There was no statistically significant difference between the two groups in the conception rate ($P>0.05$).

4.6 GESTATION LENGTH IN GOATS

Average gestation length in the does was 147.23 ± 0.76 days (Table 7). Mean gestation length in Group I and Group II was 146.00 ± 0.800 and 150.22 ± 1.34 days.

4.7 LITTER SIZE AT BIRTH

Incidence of multiple births in the study is presented in the Table 8. and Figure 5. In Group I, one doe (4.55 per cent) gave birth to triplets, 12 does to twins (54.55 per cent) and nine does to single kid (40.91 per cent). Out of 36 kids born to Group I does, 19 were male (52.78 per cent) and 17 female (47.22 per cent). Mean litter size at birth was 1.64.

In the group II animals, two does gave birth to triplet (22.22 per cent), six does to twins (66.67 per cent) and one doe to single kid (11.11 per cent). Number of kids per kidding averaged 2.11. Total kids born were 19, of which nine were males (47.37 per cent) and 10 females (52.63 per cent).

Out of the total 31 pregnant animals; three does (9.67 per cent) gave birth to triplets, 18 does (58.07 per cent) to twins and 10 does (32.26 per cent) to single kid. Average number of kids per kidding averaged 1.77. In total 55 kids were born of which 28 were males and 27 females. Percentage of male and female kids in the total number of kids born was 50.91 and 49.09 respectively.

4.8 BIRTH WEIGHT OF KIDS

Average birth weight of kids was 2.38 ± 0.08 kg (Table 9. and Figure 6). Mean birth weight of male kids was 2.36 ± 0.12 kg and female kids was 2.40 ± 0.11 kg. Average birth weight of single born kids was 2.97 ± 0.13 kg while twins and triplets had 2.39 ± 0.08 and 1.57 ± 0.20 kg.

Table. 1 Evaluation of buck semen

Buck no.	Volume	Colour	Density	Mass activity	pH	Sperm concentration	Live sperms (%)	Abnormal sperms (%)	Abnormal acrosomes (%)
1	1.48 ± 0.06	Creamy	DDDD	++++	6.90 ± 0.08	2902.50 ± 104.06	88.75 ± 0.84	2.75 ± 0.45	1.38 ± 0.32
2	1.14 ± 0.18	Creamy	DDDD	++++	7.05 ± 0.08	2810.00 ± 191.48	90.11 ± 1.03	2.11 ± 0.46	0.89 ± 0.31
3	0.94 ± 0.08	Creamy	DDDD	+++	6.96 ± 0.08	2504.44 ± 209.86	88.56 ± 0.90	2.67 ± 0.47	1.00 ± 0.37
4	1.33 ± 0.18	Creamy	DDDD	++++	6.96 ± 0.08	3401.25 ± 18.43	87.38 ± 1.54	3.50 ± 0.87	1.13 ± 0.35
5	1.33 ± 0.11	Creamy	DDDD	++++	6.99 ± 0.07	3155.00 ± 153.89	86.38 ± 0.98	5.25 ± 0.80	0.88 ± 0.35
6	1.42 ± 0.16	Creamy	DDDD	++++	6.99 ± 0.06	3032.22 ± 250.13	87.56 ± 1.72	3.11 ± 0.56	0.78 ± 0.22
Mean	1.27 ± 0.06	Creamy	DDDD	++++	6.98 ± 003	2956.67 ± 81.74	88.16 ± 0.50	3.20 ± 0.27	1.00 ± 0.13

Table 2. Oestrus characteristics in does

Group	Number of animals administered Prostaglandin	Number of animals exhibited oestrus	Oestrus response (%)	Time for onset of oestrus after last Prostaglandin dose (hours)	Duration of oestrus (hours)
Group I	100	96	96	51.92 ± 1.29	35.21 ± 1.01
Group II	50	47	94	49.25 ± 1.49	37.00 ± 1.60
Total	150	143	95.33	51.02 ± 0.99	35.81 ± 0.86

Table 3. Incidence of various oestrus signs

Oestrus sign	Percentage of animals exhibited the sign
Wagging of tail	65.33 (98)
Mounting	14 (21)
Bleating	56.67 (85)
Circling with the buck	26 (39)
Standing to be mounted	34 (51)
Vulval oedema	43.33 (65)
Vulval redness	21.33 (32)
Discharge from genitalia	4 (6)

(Values in the parenthesis indicate the actual number of animals)

Table 4. Average percentage of spermatozoa motility and live count before and after freezing.

	Tris extended semen		Biociphos extended semen	
	After washing of semen	After freezing of semen	After initial dilution	After freezing
Motility %	67.00 ± 1.47	43.91 ± 1.86	80.56 ± 1.11	40.19 ± 1.87
Live spermatozoa %	87.83 ± 0.75	52.61 ± 1.56	87.64 ± 0.76	43.64 ± 1.49

Table 5. Analysis of Variance- Motility percentage of spermatozoa frozen in Tris and Biociphos extenders

Source	Degrees of Freedom	Sum of squares	Mean square	F value	Probability
Buck (A)	5	889.24	177.85	1.69	0.1513*
Treatment (B)	1	183.68	183.68	1.74	0.1917*
AB	5	439.24	87.85	0.83	
Error	60	6320.83	105.35		

* P >0.05

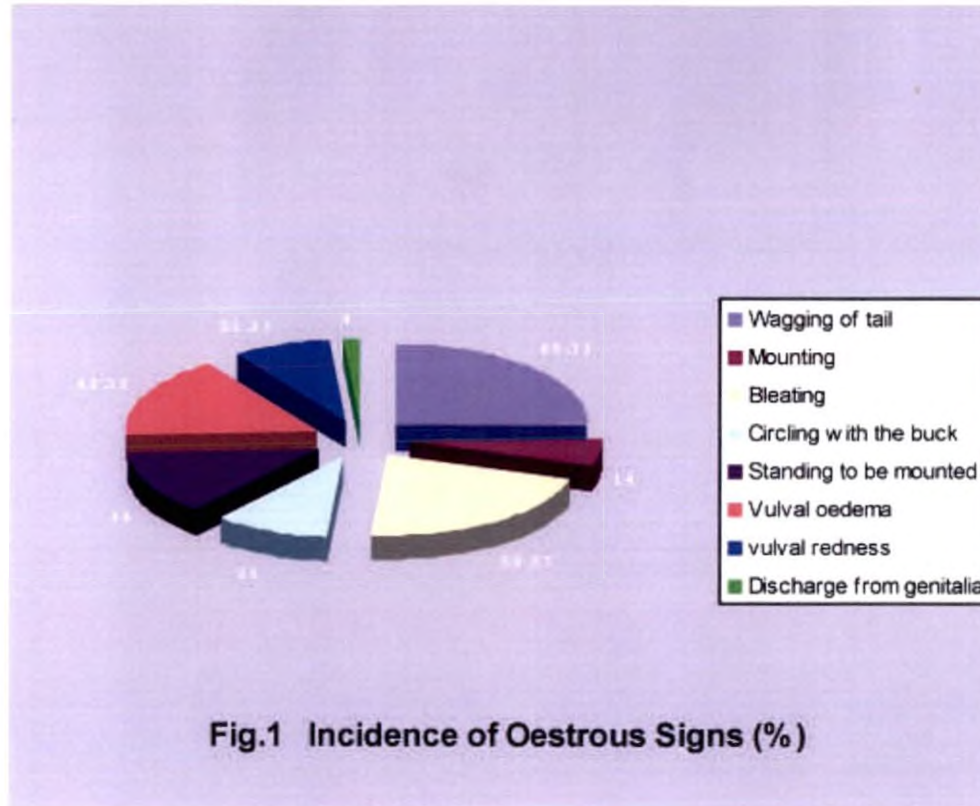

Fig.1 Incidence of Oestrous Signs (%)

Table 6. Analysis of Variance- Live percentage of spermatozoa frozen in Tris and Biociphos extenders

Source	Degrees of Freedom	Sum of squares	Mean square	F value	Probability
Buck (A)	5	1090.13	218.03	3.35	0.0098**
Treatment (B)	1	1449.01	1449.01	22.28	0.0000**
AB	5	876.90	175.39	2.70	0.0290
Error	60	3901.83	765.03		

** P <0.01

Table 7. Conception rate and gestation length in the does

Group	Number of animals in oestrus	Number of animals inseminated	Number of animals conceived	Conception percentage	Gestation Length (days)
Group I	96	96	22	22.92	146.00 ± 0.800
Group II	47	47	9	19.15	150.22 ± 1.34
Total	143	143	31	21.68	147.23 ± 0.76

Table 8. Incidence of multiple births

Group	Percentage		
	Singleton	Twins	Triplets
Group I	40.91(9)	54.55(12)	4.55(1)
Group II	11.11(1)	66.67(6)	22.22(2)
Total	32.26(10)	58.07 (18)	9.68 (3)

Table9. Birth weight of kids, kg

Type of birth	Weight (kg)		
	Male	Female	Total
Average	2.36 ± 0.12	2.40 ± 0.11	2.38 ± 0.08
Singles	2.95 ± 0.27	2.99 ± 0.08	2.97 ± 0.13
Twins	2.36 ± 0.12	2.39 ± 0.11	2.39 ± 0.08
Triplets	1.76 ± 0.20	1.59 ± 0.19	1.57 ± 0.20

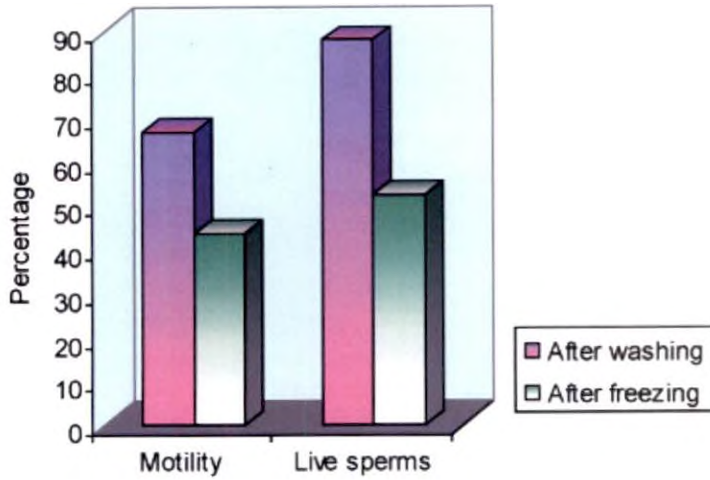

Fig.2. Average Motility and Live Sperm Count of Tris Extended Semen

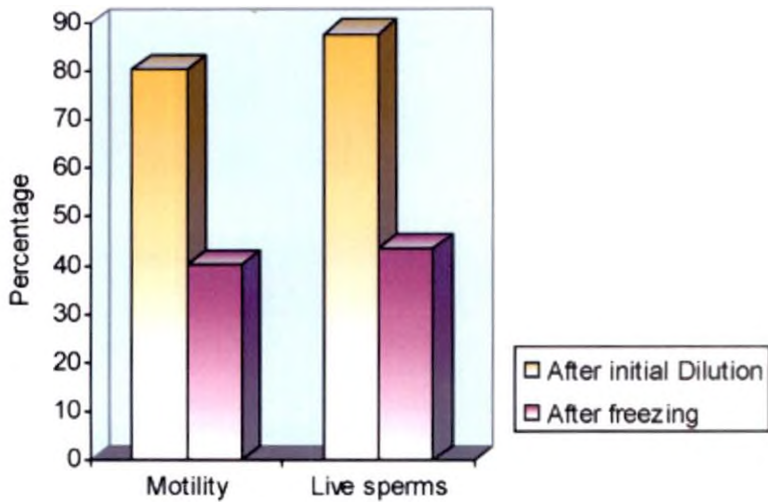

Fig.3. Average Motility and Live Sperm Count of Biociphos Extended Semen

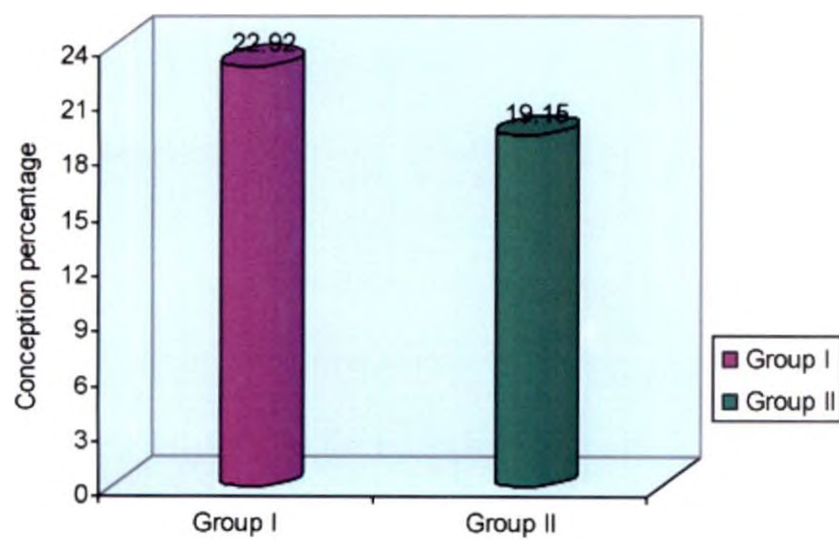

Fig.4. Conception Rate, (%)

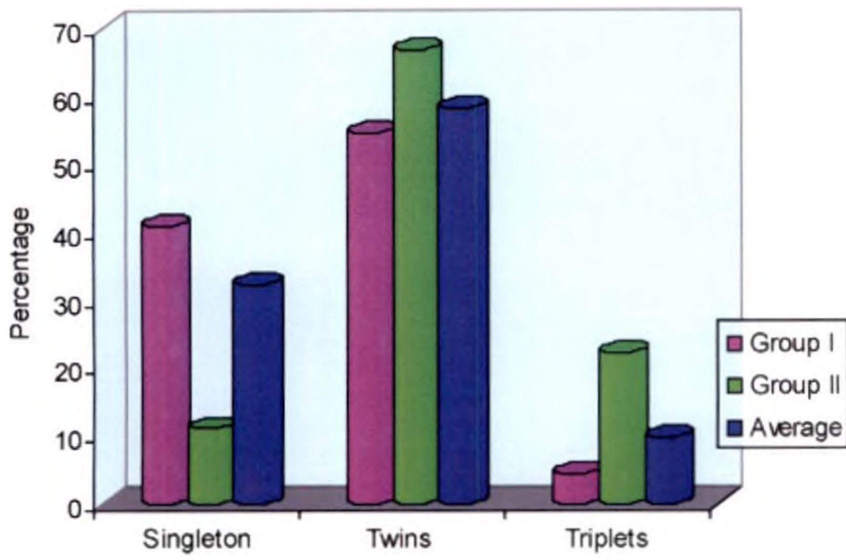

Fig.5 Incidence of Multiple Births

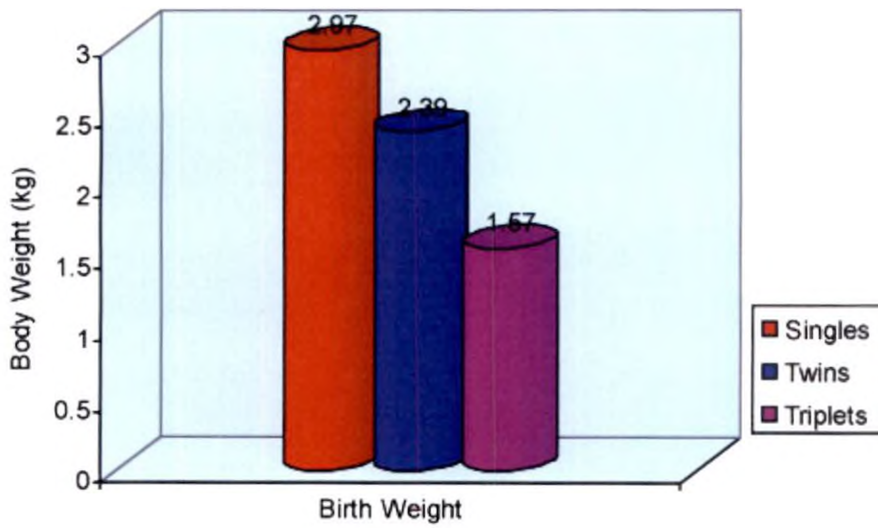

Fig.6. Birth Weight of Kids

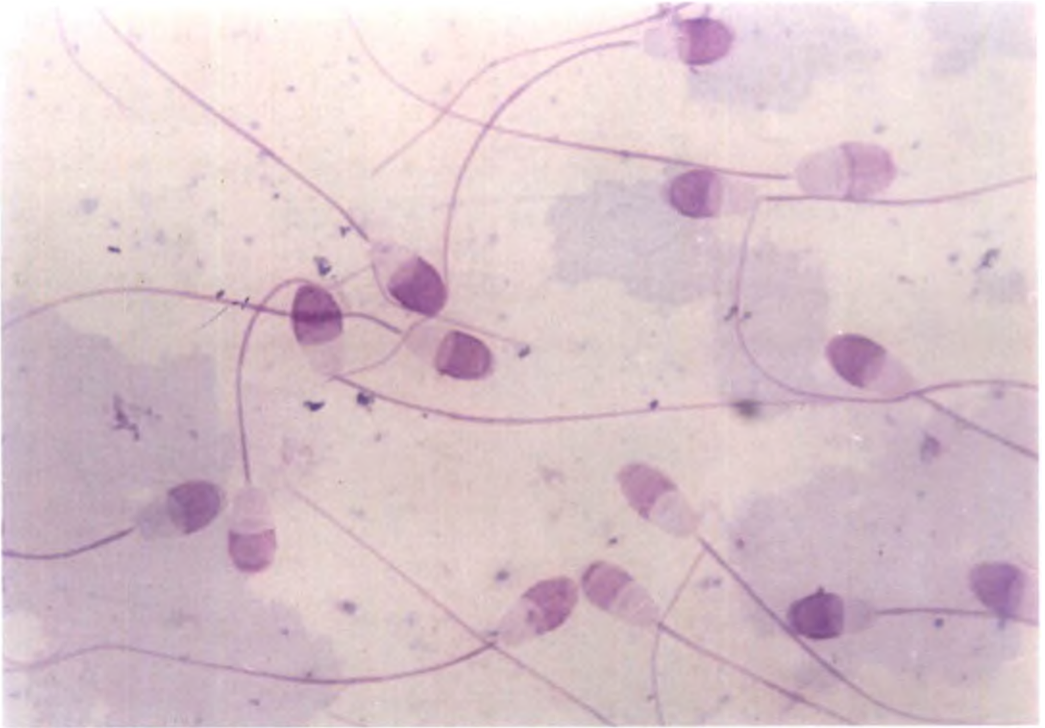

Fig. 1. Buck spermatozoa showing normal acrosome -Giemsa staining

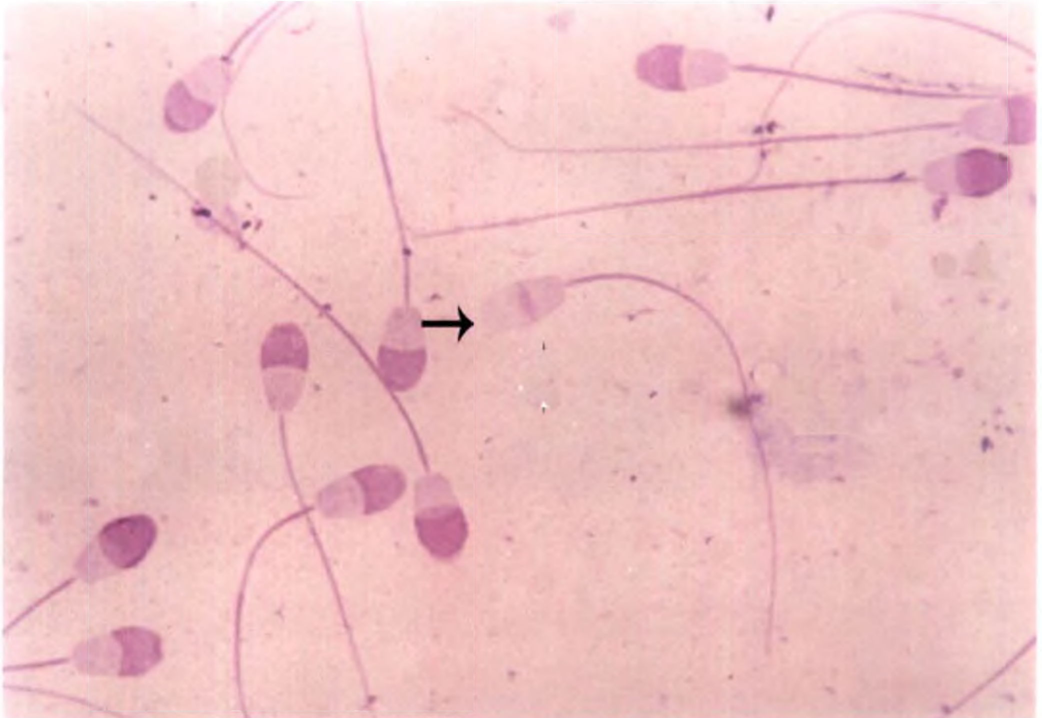

Fig. 2. Buck spermatozoa showing lost acrosome- Giemsa staining

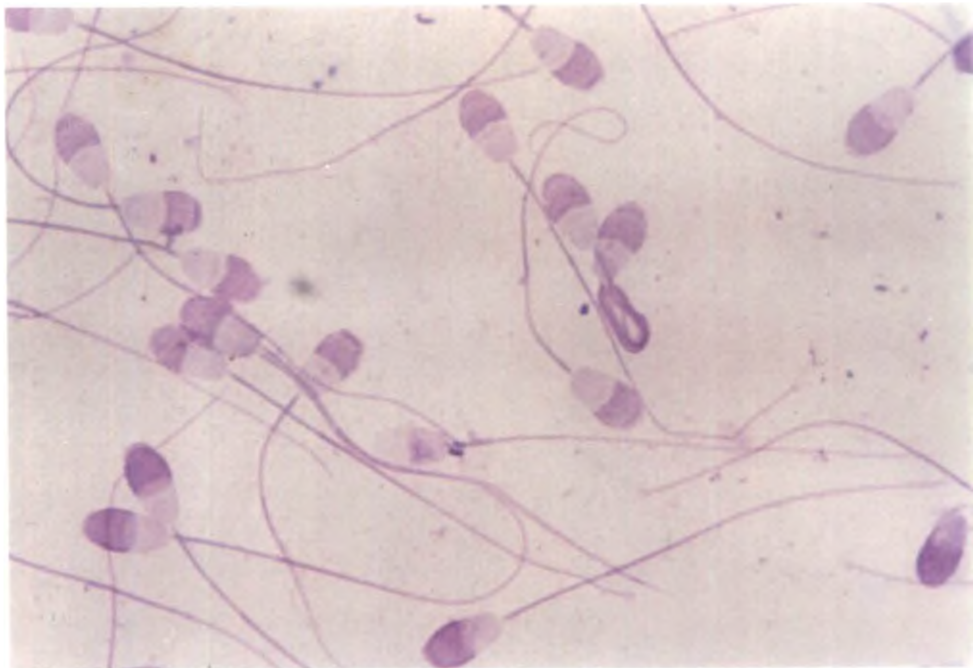

Fig. 1. Buck Spermatozoa with coiled tail- Giemsa staining

Fig.2. Boer x Malabari Crossbred kids

Discussion

5. DISCUSSION

The developments of methods that permit inseminations to be carried out at a predetermined time may be of value in goat breeding programmes (Gordon, 1997). Oestrus control measure is a valuable mean to facilitate the application of artificial insemination in goats.

A study was conducted to learn about the characteristics and cryopreservation of Boer buck semen under Kerala condition. Fertility of frozen Boer buck semen used for insemination in oestrus synchronised Malabari crossbred does as a part of Boer x Malabari cross breeding programme was studied.

5.1 SEMEN EVALUATION

In the present study mean volume of semen was found to be 1.27 $\pm$ 0.06 ml (Table 1.). The value is in agreement with the results recorded by Blokhuis (1962), Igboeli (1974) in Boer bucks and Ranjini (1998) in Malabari crossbred bucks. Lower values have been observed by Patil and Raja (1978), Joseph and Nair (1991) in Malabari, Singh and Purbey (1994) in indigenous bucks, Kutty and Mathew (2000) in Malabari crossbred bucks, Yadav and Pandey (2000) in Barbari bucks and Srinivas *et al.* (2002) in indigenous bucks of Andhra Pradesh. However higher values have been reported by Tuli and Holtz (1992) in Boer bucks. The volume of the ejaculate and the number of spermatozoa per ejaculate are inversely proportional to the numbers of ejaculates per day and the number days the males have been used for semen collection (Lindsay, 1991). It can also be affected by breed, age of bucks and the method of semen collection. Teasing of bucks before service can improve the semen volume (Roberts, 1971). The conflicting results obtained for semen volume by different workers might be attributed to variation in these factors.

From the results recorded in Table 1., colour the semen samples was creamy. This observation in bucks compares favourably with the findings of Roberts (1971), Igboeli (1974) in Boer, Pandey *et al.* (1985) in Saanen, Ranjini (1998) and Kutty and Mathew (2000) in Malabari crossbred bucks.

Overall mass activity of buck semen was + + + + which is in agreement with the observation of Ranjini (1998).

Average sperm concentration of Boer buck semen was 2956.67 $\pm$ 81.74 millions per ml which is comparable to the values recorded by Igboeli (1974) in Boer and Ranjini (1998) in Malabari crossbred bucks. Higher values have been observed by Patil and Raja (1978) in Malabari bucks and Tuli and Holtz (1992) in Boer bucks. However Prasad *et al.* (1986) in Saanen and Srinivas *et al.* (2002) in indigenous bucks of Andhra Pradesh obtained lower sperm concentration. In genotypes that are only mildly influenced by photoperiod, variation in nutrition is rapidly reflected in variation in testicular volume. The testicular volume and the daily production of spermatozoa are highly correlated (Lindsay, 1991). Variation observed in the results in different studies might be due to the variation in the seasonal availability of nutrients for bucks. Sperm concentration might also be affected by frequency of ejaculation, age and breed of the animal.

Mean percentage of live sperms of fresh Boer buck semen was 88.16 $\pm$ 0.50 which is within the range of values reported by Igboeli (1974) in Boer bucks, Ranjini (1998) in Malabari crossbred bucks and Srinivas *et al.* (2002) in indigenous bucks of Andhra Pradesh . Lower values have been recorded by Patil and Raja (1978) in malabari and Tuli and Holtz (1992 and 1995) in Boer breeds of goats.

Mean pH of the semen was 6.98 $\pm$ 0.03, which is comparable to the values observed by Prasad *et al.* (1986) in Saanen and Prasanth (1995) in Malabari crossbred bucks. Lower values have been reported by Patil and Raja (1978) in

Malabari and Kutty and Mathew (2000), while higher semen pH was recorded by Ranjini (1998) in Malabari crossbred bucks. Variations in pH value of semen are due to excessive usage of animals, incomplete ejaculation and pathological or inflammatory conditions affecting testis, epididymis, ampulla or seminal vesicle. These factors might have lead to varying results noticed by different workers.

Average percentage of abnormal spermatozoa in the fresh buck semen was 3.20 $\pm$ 0.27, which is comparable to the value observed by Ranjini (1998) in Malabari crossbred goats. Many workers reported higher percentage of abnormal spermatozoa (Patil and Raja, 1978, Prasad *et al.*, 1986 and Srinivas *et al.*, 2002). Major difference arises in the abnormality count of spermatozoa due to variations in the technique of handling and preparing slides (Roberts, 1971). Increased frequency of abnormal spermatozoa was noticed with increased frequency of ejaculation and with advancing age of the male (Sane *et al*, 1991).

Mean percentage of spermatozoa with abnormal acrosome in the fresh semen was 1.00 $\pm$ 0.13. Comparable result was reported by Deka and Rao (1984), Deka and Rao (1985) in indigenous bucks and Simon (1999) in Malabari crossbred bucks. However higher values have been reported by Ranjini (1998) in Malabari crossbred and Srinivas *et al.* (2002) in indigenous goats of Andhra pradesh. Number of spermatozoa with acrosomal abnormality increases in semen due to epididymal ageing of spermatozoa or improper handling of semen (Sane *et al.*, 1991).

5.2 OESTRUS SYNCHRONISATION IN DOES

5.2.1 Response to Oestrus Synchronisation

Perusal of Table 2. indicates that 143 (95.33 per cent) out of the total 150 does administered with prostaglandin came to oestrus. Similar results were recorded by Shenglin *et al.* (1999), Kusina *et al.* (2000) and Senthilkumar (2002). Cent per

cent synchronisation rate was observed in seven goats by Ahmad and Bhattacharya (1983) after administration of double dose of prostaglandin at 11 days interval. Lower oestrus response in goats was recorded by Gonzalez and Ramon (1992) and Chede *et al.* (2002) using the same treatment in goats.

The response to synchronisation protocol might be affected by the presence of bucks with does. All senses especially olfactory stimulus play a role in the response of intact does to male effect. Keeping does with male in the same pen stimulate LH concentration in blood, leads to oestrus and ovulation (Keskin, 2003). In the present study, does were kept away from the bucks throughout the experiment period except for short periods when apronised buck was brought to the does for oestrus detection. This might have reduced the number of animals synchronised by prostaglandin treatment.

In the present study, average time required for the onset of oestrus in does after the administration of second dose of prostaglandin was 51.02 $\pm$ 0.99 hours (Table 2.). The result was in consonance with the earlier findings of Goel and agarwal (2000) and Chede *et al.* (2002). The interval for onset of oestrus was shorter in the findings of Thilagar *et al.* (1992) and Senthilkumar (2002). Wildeus (1999) opined that the time required for onset of oestrus was reduced by the continuous exposure of does to bucks following synchronisation regimen using cloprostenol in Nubian goats. Bucks kept away from the prostaglandin treated does throughout the study might have prolonged the time for onset of oestrus in the present study.

5.2.2 Duration of Oestrus

Data presented in Table 2. revealed that the mean duration of oestrus in does in prostaglandin induced oestrus was 35.81 $\pm$ 0.86 hours. The finding is in agreement with the results obtained by Goel *et al.* (1992), Chede *et al.* (2002) and Senthilkumar (2002). However shorter duration of oestrus in goats at prostaglandin synchronised

oestrus was recorded by Goel and Agarwal (2000) and Bharali and Dutta (2001). Duration of oestrus in goats in natural oestrus observed by various workers was 34.50 ± 3.31 hours (Goel and Agarwal, 2000), 31 ± 1.75 hours (Bharali and Dutta, 2001) and 29.28 ± 0.98 hours (Goel and Agarwal, 2002). Selvaraju *et al.* (1997) reported 24.33 ± 1.3 hours in goats subjected to natural breeding compared to 31.33 ± 2.51 hours in those subjected to artificial insemination.

Conflicting results observed in the duration by various workers might be attributed to variation in breed, age, season and presence of buck near the does in oestrus. Shorter duration of oestrus was observed at the beginning and end of breeding season and in the first breeding season of young females (Jainudeen *et al.*, 2000). In hot humid climate, goats exhibited oestrus for a significantly shorter duration as compared to cool dry and moderate-medium seasons (Kumar and Yadav, 2000). The duration of oestrus was reduced when bucks were allowed to serve the does, as opposed to mere mounting and the response was mediated through mechanical action of penis against vagina (Wildeus, 1999).

5.2.3 Intensity of Oestrus in Goats

Perusal of data presented in Table 3. indicates that the oestrus signs observed in the does in the induced oestrus were wagging of tail (65.33 per cent), bleating (56.67 per cent), vulval oedema (43.33 per cent), standing to be mounted (34 per cent), circling with the buck (26 per cent), vulval redness (21.33 per cent), mounting (14 per cent) and discharge from genitalia (four per cent). The result is comparable to the observations of Rajkonwar and Borgohain (1978), Smith (1980) and Kumar and Yadav (2000) at natural oestrus in goats. On the contrary, wagging of tail was noticed in all the goats and bleating in 40 per cent of goats at prostaglandin induced oestrus (Shiv Kumar, 1993). Senthilkumar (2002) reported wagging of tail in all 12 experimental does in prostaglandin induced oestrus.

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In the present study average intensity score in does calculated as per the method given by Senthilkumar (2002) was 5.97 $\pm$ 0.41. Higher oestrus score (8.82 $\pm$ 1.59) was reported by Senthilkumar (2002) in induced oestrus. Oestrus signs were accentuated by the presence of pheromones from bucks (Noakes, 2001). In the present study the does were kept away from bucks during the period of study which might have led to lower oestrus intensity score.

5.3 PROCESSING AND FREEZING OF SEMEN

5.3.1 Tris Extender

Progressive motility of the samples was evaluated after washing in Tris buffer and after freeze thawing. Sperm motility of 67.00 $\pm$ 1.47 per cent observed at the end of washing was found to drop to 43.91 $\pm$ 1.86 per cent at the end of freezing and thawing (Table 4. and Figure 2.). There was significant reduction in motility of spermatozoa between washing and freeze thawing. Mean percentage of live spermatozoa in the fresh and freeze-thawed semen samples was 87.83 $\pm$ 0.75 and 52.61 $\pm$ 1.56.

The post thaw motility observed in the study is comparable to the observations of Westhuysen (1978), Deshpande and Mehta (1991) and Singh *et al.* (1992). Many workers have recorded higher percentage of post thaw motility of spermatozoa (Deka and Rao, 1984; Deka and Rao, 1985; Kolk, 1985; Deka and Rao, 1986; Chaudhury *et al.*, 1987, Sinha *et al.*, 1991 and Sivaselvam *et al.*, 2000).

Removal of seminal plasma prior to semen freezing in an extender containing egg yolk had an unfavourable effect on post thaw motility and integrity of spermatozoa when Boer buck semen was used (Tuli and Holtz, 1994). In the present study Boer semen samples were subjected to freezing after washing twice in Tris buffer. Lower progressive motility and percentage of live spermatozoa obtained after

washing might be associated with the elimination of fructose and other substrates present in seminal plasma as described by Tuli and Holtz (1994).

5.3.2 Biociphos Extender

Average percentage of sperm motility after initial dilution in Biociphos extender and after freeze thawing was 80.56 $\pm$ 1.11 and 40.19 $\pm$ 1.87(Table 4. and Figure 3.). Comparable post thaw motility was observed for bull semen extended in Biociphos extender by Van Wagtendonk *et al.* (2000) and Singh *et al.* (2000). However Bhom *et al.* (1995) obtained higher post thaw motility of 60 per cent in frozen thawed bull semen diluted in Biociphos extender.

In the present study there was no significant difference in average sperm motility between the semen frozen in Tris and Biociphos extenders ($P>0.05$). Lower post thaw motility was observed for bull semen frozen in Biociphos extender compared to Tris extended semen (Bhom *et al.*, 1995; Van Wagendonk *et al.*, 2000 and Singh *et al.*, 2000). In contrast higher post thaw motility was obtained for Murrah and Jersey semen samples frozen in Biociphos as compared to Tris (Rao *et al.*, 2002).

Mean percentage of live spermatozoa in fresh semen used for dilution in Biociphos extender was 87.64 $\pm$ 0.76. After freezing in Biociphos extender mean percentage of live spermatozoa was 43.64 $\pm$ 1.49. Highly significant difference was noticed in the average percentage of live spermatozoa between semen diluted in Tris and Biociphos extenders ($P<0.01$). Studies on the use of Biociphos for goat semen extension are scanty.

5.4 PREGNANCY DIAGNOSIS BY ABDOMINAL PALPATION

Pregnancy diagnosis was performed by abdominal palpation at three months of gestation. Twenty nine does were diagnosed as pregnant. The result obtained was

compared with the number of does kidded and the accuracy of the method was found to be 93.55 per cent. According to Goel and Agarwal (1990), accuracy of abdominal palpation in goats for pregnancy diagnosis was 90.30 per cent at 71-80 days and 95.38 at 80 days and later.

5.5 PREGNANCY RATE AFTER INSEMINATION

5.5.1 Tris Extended Semen

Out of 96 does inseminated with frozen semen diluted in Tris extender 22 conceived with a conception rate of 22.92 per cent. Higher conception rate was recorded in goats by Balakrishnan (1979), Cetinkaya (1980), Kolk (1985), Choudhury (1987) and Sinha *et al.* (1987).

Lower pregnancy rate obtained served in the present study might be associated with ultra structural, biochemical and functional damage occurring to a significant proportion of spermatozoa during freeze thawing. The cryopreservation process can also alter the ability of spermatozoa to ascend the female reproductive tract. The problem can be bypassed by placing the thawed cryopreserved spermatozoa directly into uterine lumen by aid of a laparoscopy (Garner, 1991).

The low fertility might also have been resulted from cervical insemination resulting in impaired transport of frozen thawed spermatozoa through cervix and their reduced viability in the female genital tract (Salamon and Maxwell, 1995b). Conception rate after artificial insemination was lower when prostaglandin therapy was used for oestrus synchronisation than with progesterone pessary treatment (Ax *et al.*, 2000).

5.5.2 Biociphos Extended Semen

In Group II, 47 does were inseminated using frozen semen extended in Biociphos. Nine animals (19.15 per cent) conceived. Higher conception rate was recorded by Bhom *et al.* (1995), Wagtendonk *et al.* (2000), Gil *et al.* (2000) and Rao *et al.* (2002) in bovines. Studies on the use of Biociphos extender for preservation of goat semen are scanty. Further investigations are required to study more about the efficacy of Biociphos as goat semen extender.

5.6 GESTATION LENGTH IN GOATS

Mean gestation length in does under study was 147.23 $\pm$ 0.76 days. Gestation length in days in Malabari crossbred does reported by earlier workers were 146.66 $\pm$ 0.53 (Kuriakose, 1981) and 149.85 $\pm$ 4.45 days (Prasanth, 1995). Seabo *et al.* (1999) observed 150.00 $\pm$ 0.19 days gestation length in Boer goats. Variation observed in the results of various workers might be attributed to difference of breed, litter size, parity of does and month of mating. Shortest gestation period was observed when animals were bred from December to January (Amoah, 1996). Gestation period decreases as the number of offspring increases and increased slightly with increasing parity.

5.7 LITTER SIZE AT BIRTH

Perusal of Table 8. and Figure 5. revealed that the incidence of single, twin and triplet obtained in the study was 32.26, 58.07 and 9.68 respectively. The percentage of incidence of single, twin, triplet and quadruplets in Boer goats recorded by earlier workers were 24, 58, 15 and one (Van Niekerk and Casey, 1988) and 27.97, 54.57, 16.04 and 1.43 respectively (Seabo *et al.*, 1999). Incidence of single, twins and triplets in Malabari x Alpine crossbred goat was 40, 48 and 12 per cent respectively (Prasanth, 1995). Higher twinning percentage was reported in Boer

goats maintained at Kerala (James *et al.*, 2002). The present finding also reveals a high percentage of twins as compared to singles and triplets.

Average number of kids produced per kidding was 1.77. Comparable results were recorded in Malabari crossbred goats by Prasanth (1995) and Senthil Kumar (2002).

5.8 BIRTH WEIGHT OF KIDS

In the present study, mean birth weight of kids was 2.38 $\pm$ 0.08 kg. Birth weight of male and female kids was 2.36 $\pm$ 0.12 and 2.40 $\pm$ 0.11 kg respectively. Birth weight of kids born as single, twin and triplet was 2.97 $\pm$ 0.13, 2.39 $\pm$ 0.08 and 1.57 $\pm$ 0.20 kg respectively. Higher birth weight was recorded in Boer kids by Van Niekerk and Casey (1988), Seabo *et al.* (1999), James *et al.* (2002) and Sundaram (2002). Comparable result was recorded by Gajendran (2000) in Boer x Tamil Nadu local crossbred goats and by Kuriakose (1981) in Malabari goats.

Summary

6. SUMMARY

A study was carried out to ascertain the fertility of oestrus synchronised crossbred Malabari does inseminated using frozen Boer buck semen. A total of 150 crossbred Malabari does maintained at Goat and Sheep Farm, Kerala Agricultural University, Mannuthy, Thrissur, were oestrus synchronised by administration of double dose of 0.147 mg Tiaprost Trometamol, a prostaglandin analogue intramuscularly at 11 days interval. The does were grouped into two; first comprising of 100 does that were inseminated using semen frozen in Tris diluent and second comprising of 50 does inseminated using semen frozen in Biociphos extender. Semen samples were collected from six Boer bucks maintained at Goat Breeding Centre, Dhoni Farm, Kerala Livestock Development Board.

Average volume of the semen was 1.27 ± 0.06 ml. No significant difference was observed in semen volume between bucks. The Colour of semen was creamy and density DDDD. Mass activity was found to range from + + to + + + +. Mean pH of buck semen was 6.98 ± 0.03. Between bucks there was no significant difference in pH of semen. Average sperm concentration was 2956.67 ± 81.74 millions per ml. There was no significant difference in sperm concentration between bucks. Mean live sperm percentage was 88.16 ± 0.50. No significant difference was noticed in the per cent of live sperm between bucks. Average per cent of abnormal spermatozoa in the fresh semen was 3.20 ± 0.27. Mean per cent of acrosomal abnormality of sperms was 1.00 ± 0.13.

After preliminary evaluation, semen samples were processed and frozen. Six semen samples from each buck were frozen in Tris diluent and six samples in Biociphos extender.

The semen samples were washed twice in Tris buffer and diluted at 1:10 ratio before being frozen and stored in liquid nitrogen. For freezing in Biociphos extender, pre dilution of the semen samples was performed with the extender at 1:1 ratio and kept at 37°C for 10 minutes. Final dilution was performed by mixing with remaining extender. The diluted semen was chilled at 5°C and equilibrated at the same temperature for four hours. Freezing was performed in liquid nitrogen vapour for 10 minutes.

Average percentage of sperm motility after washing twice in Tris buffer was 67.00 ± 1.47 and after thawing was 43.91 ± 1.86. There was significant reduction in motility of spermatozoa after freezing as compared to that of washing. Average percentage of sperm motility after initial dilution in Biociphos extender was 80.56 ± 1.11 and after thawing was 40.19 ± 1.87. There was no significant difference in post thaw motility of spermatozoa frozen in Tris and Biociphos extenders.

Mean percentage of live sperms in fresh semen used for dilution in Tris extender was 87.83 ± 0.75 and after thawing was 52.61 ± 1.56 per cent. Average percentage of live sperms in semen used for dilution in Biociphos extender was 87.64 ± 0.76 and after freezing was 43.64 ± 1.49. Highly significant difference was noticed in the percentage of live spermatozoa between semen frozen in Tris and Biociphos extenders.

All the selected crossbred Malabari does which were administered the prostaglandin analogue were observed for any oestrus signs. Among the treated animals 95.33 per cent exhibited oestrus. Average time taken for the onset of oestrus was 51.02 ± 0.99 hours and duration of oestrus was 35.81 ± 0.41 hours. Mean intensity of oestrus was 5.97 ± 0.41. Major behavioural signs and physiological changes noticed in oestrus does were wagging of tail (65.33 per cent), frequent

bleating (56.67 per cent), vulval oedema (43.33 per cent) and standing to be mounted (34 per cent).

Artificial insemination was carried out in the does in oestrus by speculum method. Pregnancy diagnosis was performed at three months of gestation by abdominal palpation and the accuracy was found to be 93.55 per cent. Conception rate in Groups I and II was 22.92 and 19.15 per cent. There was no significant difference in the conception rate between the two groups. Average gestation length in does was 147.23 ± 0.76 days. In group I, the percentage of single, twins and triplets were 40.91, 54.55 and 4.55 per cent and in group II the corresponding values were 11.11, 66.67 and 22.22 per cent respectively. Average birth weight of kids was 2.38 ± 0.08 kg.

Controlled breading in goats was standardised oestrus synchronisation and application of frozen semen technology. This has wider ramification in intensive goat production programmes in Kerala State. Oestrus synchronisation protocols were capable of induction of oestrus in 95.33 per cent does. Tris and Biociphos extenders were found to have no significant influence on semen viability and fertility. Considering the cost effectiveness, Tris diluent appears to have a better application as buck semen extender in frozen semen technology. Conception rate among the oestrus synchronised does inseminated with frozen semen was 21.68 per cent which necessitates improvement for widespread application of these technologies for goat breading programmes.

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FERTILITY OF OESTRUS SYNCHRONISED CROSSBRED MALABARI DOES INSEMINATED WITH BOER BUCK SEMEN

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ABSTRACT

With the objective of studying the fertility of frozen Boer buck semen, six Boer bucks maintained at Goat Breeding Station, Dhoni farm, Kerala Livestock Development Board and 150 Malabari crossbred does maintained at Sheep and Goat farm, Mannuthy were selected. Semen collection was carried out at weekly intervals from the bucks and preserved under freezing.

Average volume of buck semen was 1.27 ± 0.06 ml. Colour of the semen was creamy, density DDDD and mass activity + + + +. Average sperm concentration of the semen samples was 2956.67 ± 81.74 millions per ml and mean live sperm percentage was 88.16 ± 0.50. Average semen pH was 6.98 ± 0.03. No significant difference was found in these semen characters between bucks (P>0.05). Average percentage of abnormal spermatozoa in the fresh semen was 3.20 ± 0.27. Mean percentage of sperms with abnormal acrosome was 1.00 ± 0.13.

The does selected were administered 0.147 mg Tiaprost Trometamol, a prostaglandin analogue (0.75 ml Iliren) intramuscularly at 11 days interval for oestrus synchronisation. The does were divided into two groups, Group I with 100 does and Group II with 50 does. Ninety six does in Group I and 47 does in group II responded to the treatment. Incidence of oestrus in the does was 95.33 per cent. Average time taken for the does to come to oestrus was 51.02 ± 0.99 hours. Mean duration of oestrus in the does was 35.81 ± 0.86 hours. Average oestrus intensity score in the does was 5.97 ± 0.41. Wagging of tail (65.33 per cent), frequent bleating (56.67 per cent), vulal oedema (43.33 per cent) and standing to be mounted (34 per cent) were the prominent signs of oestrus exhibited by the does.

Six semen samples from each buck were frozen in Tris and Biociphos extenders separately. Progressive motility of semen samples after washing twice in Tris buffer and after thawing was 67.00 ± 1.47 and 43.91 ± 1.86 per cent. Average

percentage of live sperms in the fresh semen and after thawing was 87.83 ± 0.75 and 52.61 ± 1.56.

Mean percentage of sperm motility after initial dilution and freezing and thawing in Biociphos extender was 80.56 ± 1.11 and 40.19 ± 1.87. Percentage of live spermatozoa in fresh semen and thawed semen was 87.64 ± 0.76 and 43.64 ± 1.49. Though no significant difference was noticed in post thaw motility of spermatozoa, highly significant variation was observed in the percentage of live spermatozoa between the semen frozen in Tris and Biociphos extenders ($P>0.05$).

Artificial insemination was carried out in all the oestrus does by speculum method. Group I does were inseminated with semen extended in Tris and Group II does with semen extended in Biociphos. Pregnancy diagnosis was done at three months of gestation by abdominal palpation and the accuracy of the method was found to be 93.55 per cent. Conception percentage in Group I and Group II was 22.92 and 19.15. There was no significant difference in the conception rate between the two groups. Mean gestation length was 147.23 ± 0.76 days. Number of kids per kidding averaged 1.77. Percentage of male and female kids was 50.91 and 49.09. Average birth weight of kids was 2.38 ± 0.08 kg.