The study was conducted on six apparently healthy pure bred Jersey breeding bulls, maintained at Intensive Livestock Improvement Programme, Semen Processing Laboratory, Palampur. A total of 36 ejaculates were investigated in this study. Semen was collected twice a week from each bull. Collected semen was subjected to the various evaluation tests before processing, that included volume, colour, mass movement, concentration of sperm cells, progressive motility, and percentage of live spermatozoa, acrosomal integrity and hypo-osmotic swelling test.

After evaluation, neat semen was extended in Tris based extender and extended semen was divided into three aliquots. First aliquot (10 ml diluted semen) served as control (G1), while other two were modified either with Chloroquine diphosphate (G2) or with Ascorbic acid (G3) for having desired concentration of additives. The final concentrations of Chloroquine diphosphate and Ascorbic acid were $10^{-5}$M and 0.02 percent in the G2 and G3 groups, respectively. All the dilution, extension and modified extension procedures of the semen were carried out at 37°C. Each semen sample was diluted in such a way that it contained around 60 millions spermatozoa in one ml of diluted semen. Temperature of the diluted semen was brought down from 37°C to 4°C by gradual cooling (slow cooling) in the cooling cabinet in about 4 hours. All three groups were again subjected to the evaluation of progressive motility, live dead count; acrosomal integrity evaluation and hypo-osmotic swelling test at 37°C and 4°C. semen samples were filled in the 0.5 ml French medium plastic straws. Filled straws were kept again in the cooling cabinet at 4°C for a few minutes in the trays. Trays from the cooling cabinet were then shifted in to the liquid nitrogen storage tank and frozen at -196°C.

Semen straws from all three groups were thawed at 37°C for 30 seconds and semen samples were evaluated at 0, 1 and 2 hours post-thaw for progressive motility, differential staining of live dead spermatozoa, acrosomal characteristics and for reaction to hypo-osmotic solution.
Fertility trials were conducted in the University Dairy Farm, Govt. Jersey Farm at Palampur, College Clinical Complex and field Veterinary institutions located in the periphery of this University. Total of 90 animals were inseminated in the farms ($G_1=30$, $G_2=30$ and $G_3=30$) and 75 animals ($G_1=25$, $G_2=25$ and $G_3=25$) were inseminated under field conditions. Pregnancy diagnosis was done 60 days post artificial insemination by per rectal method.

In neat semen, the mean live sperm percentage was 81.39 ± 0.88 percent. At 37°C post dilution, the mean live sperm percentage was 80.53 ± 0.76, 81.17 ± 0.83 and 80.49 ± 0.82 percent for control ($G_1$), Chloroquine diphosphate ($G_2$) and Ascorbic acid ($G_3$) added semen, respectively. At post-equilibration (4°C) evaluation, corresponding values were 76.77 ± 0.68, 78.64 ± 0.78 and 78.86 ± 0.76 percent for the three groups, respectively. The evaluation of frozen semen post thawing at 0 hr revealed that the mean live sperm percentage was 53.16 ± 1.19, 56 ± 1.14 and 57.39 ± 1.02 percent for $G_1$, $G_2$ and $G_3$, respectively. $G_3$ differed significantly (P<0.05) from $G_1$. However, there was no significant difference either between $G_1$ and $G_2$ or $G_2$ and $G_3$. On incubation of frozen thawed semen at 37°C for 1 hr, live sperms further decreased to 44.50 ± 1.16, 47.39 ± 1.19 and 47.55 ± 1.07 percent for $G_1$, $G_2$ and $G_3$ groups, respectively. Semen added with Ascorbic acid ($G_3$) was found significantly (P<0.05) better as compared to control group ($G_1$). There was no statistical difference between any other groups. Following 2 hours incubation, mean live sperm percentage in groups $G_1$, $G_2$ and $G_3$ was 36.22 ± 1.08, 38.80 ± 1.13 and 40.50 ± 0.96 percent, respectively. The difference between $G_1$ and $G_3$ was significant (P<0.05).

In neat semen the mean progressive motility percentage was 75.42 ± 0.55. At 37°C post dilution, the mean progressively motile sperms percentage was 74.31 ± 0.41, 74.97 ± 0.38 and 74.14 ± 0.39 percent for control ($G_1$), Chloroquine diphosphate ($G_2$) and Ascorbic acid ($G_3$) added semen, respectively. At post-equilibration (4°C) evaluation, corresponding values were 71.06 ± 0.65, 72.36 ± 0.57 and 71.61 ± 0.52 percent, respectively, for the three groups. At post-thaw 0 hour evaluation mean values for progressive motility percentage were 47.58 ± 1.08, 50.14 ± 1.01 and 51.28 ± 0.90 percent for $G_1$, $G_2$ and $G_3$, respectively. At 1 hour incubation corresponding values were 38.69 ± 0.92 percent for $G_1$, 40.97 ± 1.02 percent for $G_2$ and 41.86 ± 0.82 percent for $G_3$. At both stages, only $G_3$ differed significantly (P<0.05) from $G_1$. At 2 hours incubation, the mean progressively motile sperms were 28.28 ± 0.98, 32.03 ± 1.04 and 32.67 ± 0.90 percent for $G_1$, $G_2$ and $G_3$, respectively. The difference between $G_1$ and $G_3$ (P<0.01) and between $G_1$ and $G_2$ (P<0.05) was significant. However, difference between $G_2$ and $G_3$ was not significant.
In neat semen the percentage of HOS reactive spermatozoa was 66.42 ± 1.12. At 37°C mean HOST positive spermatozoa were 67.47 ± 0.05, 68.97 ± 1.14 and 69.17 ± 0.92 percent and at post-equilibration stage the corresponding values were 63.31 ± 1.11, 64.97 ± 0.97 and 64.58 ± 0.98 percent for G₁, G₂ and G₃, respectively. Difference among groups was non significant at both these stages. At 0 hr post thaw G₁, G₂ and G₃ had 40.78 ± 0.93, 45.97 ± 0.76 and 45.56 ± 0.99 percent HOS reactive spermatozoa, respectively. Following incubation these values were 33.06 ± 0.83, 39.22 ± 0.76 and 37.94 ± 0.93 percent at 1 hour and 25.36 ± 0.77, 31.22 ± 0.73 and 30.56 ± 0.86 percent at 2 hour for G₁, G₂ and G₃, respectively. At all post-thaw stages, G₂ and G₃ had significantly (P<0.01) higher HOS reactive spermatozoa in comparison to G₁. However, the difference between G₂ and G₃ was not significant.

In neat semen the mean acrosomal integrity was 87.28 ± 0.94 percent. At 37°C post dilution, the mean acrosomal integrity percentage was 86.17 ± 0.94, 86.94 ± 0.80 and 86.89 ± 0.92 percent for G₁, G₂ and G₃, respectively. At post-equilibration evaluation, corresponding values were 82.47 ± 1.05, 84.06 ± 0.82 and 84.47 ± 0.86 percent for the three groups, respectively. At 0 hr post-thaw, the percentages of normal acrosomes were 67.92 ± 0.93 for G₁, 68.36 ± 0.79 for G₂ and 69.97 ± 0.56 for G₃, respectively. There was no difference between groups at all these three stages. The mean acrosomal integrity was 59.69 ± 0.84, 62.06 ± 0.73 and 63.47 ± 0.76 percent following 1 hour incubation in G₁, G₂ and G₃, respectively. G₂ (P<0.05) and G₃ (P<0.01) were having significantly higher normal acrosome percentage in comparison to G₁. After 2 hour’s incubation, acrosomal integrity was 52.28 ± 0.83, 56.44 ± 0.98 and 56.72 ± 0.76 percent for the three groups, respectively. G₂ and G₃ were having significantly (P<0.01) higher acrosomal integrity than G₁. No significant difference was observed between G₂ and G₃ at any stage of evaluation.

All the semen quality assessment traits were negatively and highly significantly (P<0.01) correlated with the post-thaw incubation time. Irrespective of groups the fall in live sperm percentage was significantly correlated (P<0.01) with incubation time in G₁ (r = -0.72), G₂ (r = -0.73) and G₃ (r = -0.75). The overall regression coefficient of live sperm percentage on incubation time was -8.47 ± 0.81 for G₁, -8.89 ± 0.81 for G₂ and -8.44± 0.17 for G₃, respectively. Thus, per hour fall in percent live sperms in G₁, G₂, and G₃ were 8.47, 8.89 and 8.44, respectively. The progressive fall in the live sperm percentage was more in Choloroquine diphosphate added group (G₂) in comparison to G₁ and G₃.

Irrespective of groups the fall in progressive motility was significantly correlated (P<0.01) with incubation time in G₁ (r = -0.80), G₂ (r = -0.76) and G₃ (r = -0.83). The overall regression coefficient of progressively motile spermatozoa on incubation time was -9.65 ± 0.81.
0.71, -9.06 ± 0.72 and -9.31 ± 0.61 for G₁, G₂, and G₃, respectively. Thus, per hour decrease in progressive motility in G₁, G₂, and G₃ was 9.65, 9.06 and 9.31 percent, respectively. Semen added with Chloroquine diphosphate (G₂) provided comparatively better protection to motile spermatozoa than with Ascorbic acid (G₃).

Similarly, irrespective of groups, the fall in number of spermatozoa, which were reactive to hypo-osmotic solution, was significantly correlated (P<0.01) with incubation time in G₁ (r =- 0.78), G₂ (r =- 0.81) and G₃ (r =- 0.75). The overall regression coefficient of HOST reactive spermatozoa on incubation time was -7.71 ± 0.59 for G₁, -7.38 ± 0.53 for G₂ and -7.53 ± 0.65 for G₃, respectively. Thus, per hour decrease in the percent HOST reactive spermatozoa was 7.71, 7.38 and 7.53 in G₁, G₂, and G₃, respectively. Membrane stabilizer (Chloroquine diphosphate) appeared to be better for longer incubation than either antioxidant (Ascorbic acid) or semen without any additive (control).

Irrespective of groups the fall in percent intact acrosome was significantly correlated (P<0.01) with incubation time in G₁ (r =- 0.78), G₂ (r =- 0.69) and G₃ (r =- 0.79). The overall regression coefficient of spermatozoa with intact acrosome on incubation time was -7.82 ± 0.61, -5.96 ± 0.59 and -6.63 ± 0.49 for G₁, G₂, and G₃, respectively. Thus, per hour decrease in the percent spermatozoa with intact acrosome in G₁, G₂, and G₃ were 7.82, 5.96 and 6.63, respectively. Semen added with chloroquine diphosphate (G₂) provided comparatively better protection to acrosomes than with Ascorbic acid (G₃).

Live sperm percentage was significantly correlated (P<0.01) with progressively motile spermatozoa (r=0.91, n=36). The overall regression coefficient of progressive motility on live sperm percentage was 0.92 ± 0.02. HOST reactive spermatozoa were significantly correlated (P<0.01) with Live sperm percentage (r=0.83, n=36). The overall regression coefficient of live sperm percentage on HOST reactive spermatozoa was 0.71 ± 0.03 (n=36). Similarly, HOST reactive spermatozoa were significantly correlated with progressively motile spermatozoa (r=0.86, n=36). The overall regression coefficient of progressively motile spermatozoa on HOST reactive spermatozoa was 0.74 ± 0.03 (n=36).

In all 165 cows were inseminated during this study to evaluate the effect of semen additives on conception that included 30, 15 and 10 (total 55) cows inseminated in each of the three groups at university dairy farm, clinical complex and rural institutions, respectively. Following artificial insemination in control group (G₁), 12 (40.0%), 8 (53.34%) and 5 (50.0%) cows conceived out of 30, 15 and 10 animals inseminated at dairy farm, clinical complex and rural institutions, respectively. Overall conception percentage was 45.56 percent. In group second (G₂), 16 (53.34%), 9 (60.0%) and 6 (60.0%) cows conceived with Chloroquine diphosphate added semen, out of 30, 15 and 10 animals
inseminated at dairy farm, clinical complex and rural institutions, respectively. Overall conception percentage was 56.37 percent. In group G3 19 (63.34%), 11 (73.34%) and 6 (60.0%) cows conceived with Ascorbic acid added semen, out of 30, 15 and 10 animals inseminated at dairy farm, clinical complex and rural institutions, respectively. Overall conception percentage was 65.46 percent.

Conception with Ascorbic acid added semen (G3) was significantly (P<0.05) higher than that of control group (G1). However, there was no significant difference either between G1 and G2 or G2 and G3.

**Conclusions drawn from this study are;**

1. Ascorbic acid (anti-oxidant) as well as Chloroquine diphosphate (Membrane stabilizer) improved the post-thaw quality of frozen semen of Jersey bulls.
2. Ascorbic acid gave numerically better (however, non significant) protection to frozen spermatozoa of Jersey bulls as compared to Chloroquine diphosphate.
3. Live sperms, progressive motility, HOST reactive spermatozoa and acrosomal integrity had significant negative correlation with post thaw incubation time.
4. There was a highly significant correlation between percent live sperm, progressive motility and HOST reactive spermatozoa in semen of Jersey bulls.
5. There was significant improvement in the number of cows conceiving with semen added with Ascorbic acid (anti-oxidant) as compared to control group. Chloroquine diphosphate (Membrane stabilizer) also improved conception by protecting spermatozoa; however, it was not better than that with Ascorbic acid.