Tohoku Journal of Agricultural Research
Vol. 54 No. 1-2, November 2003
*Printed in Japan*

# Sheep Semen Characteristics and Artificial Insemination by Laparoscopy

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(Received, September, 2003)

## Summary

The semen characteristics and technology using the method of direct injection of the semen to the uterus by laparoscopy were examined to improve the conception rate in sheep. Fifty-two sheep in total were examined which were raised at the Faculty of Agriculture and Kawatabi Experimental Station of Tohoku University.

The semen characteristic was better in the breeding season than in the other seasons based on the semen volume, number of spermatozoa and the spermatozoa movement index. The method of direct injection of the semen to the uterus by laparoscopy produced a much better conception rate in sheep than the generally done vaginal insemination, and the cervical insemination. In addition, the new uterine insemination via the vagina was evaluated.

**Key words**: sheep, semen, artificial insemination, laparoscopy

## Introduction

It is reported that the conception rate of artificial insemination of sheep is low in comparison with that of the cow. It is reported that the conception rates are 45% on average. It is an important subject for sheep production to improve this low conception rate. Because sheep production on farms in Japan is small-scale, inbreeding occurs in their herds. Therefore, semen distribution based by artificial insemination is desired.

So, the technical development of artificial insemination by the laparoscopy was examined in this study with the purpose of increasing the conception rate in sheep. It is also important to improve the conventional technique of artificial insemination via the vagina.

## Materials and Methods

*The semen characteristics of the ram*

*Sheep breeds*

The breeds of Suffolk (SF) : 3 head, Corriedale (CO) : 1 head, Cheviot (CH) : 1 head, Perendale (PE) : 2 head and Cross bred ($F_1$) : 1 head raised at the Faculty of Agriculture in Sendai and Experimental Station of Tohoku University in Kawatabi were examined for the semen characteristics.

*Semen collection*

The semen collected one or twice every month from May to January in 1998 and 1999 using electroejaculation or the artificial vagina. They were examined for semen volume, number of spermatozoa and the spermatozoa movement index in our laboratory.

In case of the electroejaculation, semen collection was done using an electric ejaculator device manufactured by Western Instruments Co. in US. Rams were laid decumbent then the prepuce and glans were placed in a small test tube. The ejaculator rod was inserted into the rectum at a depth of 10-20 cm, and electrical stimulation was done at 11.2 volt. The electric stimulation was continued for three seconds and stopped for four seconds. This was repeated approximately ten times. The total semen collected was termed as the semen volume.

For making an artificial vagina, a thin rubber tube in the plastic pipe was used. When the teaser female fixed in a stand up position and the ram showed a sexual behavior of mounting, then the penis was lead into the artificial vagina. When the semen was successfully collected, the artificial vagina was introduced into the collection test tube toward the bottom, and entire semen was withdrawn.

Semen concentration was calculated by using the melangeurt pipette and hemacytometer under an optical microscope. The number of spermatozoa was found as the product of the semen volume and semen concentration. The motility of the spermatozoa was indicated by the rate of motile spermatozoa based on the total in the scope counted on the test plate by visual inspection. The motility index of spermatozoa was graded as four to one points about spermatozoa activity in which four is the highest activity and one is no activity, and individual motility index was calculated by multiplying the spermatozoa activity by their survival rate.

These data were analyzed using GLM (General Linear Model) procedure of SAS (Statistical Analysis System) by the UNIX computer system in the Education Center Information Processing at Tohoku University.

*Freezing preservation and fusion*

The collected semen was diluted five times by the Evans-Maxwell (4) primary diluents as shown in Table 1. The diluted semen was then gradually

*Table 1 Egg york-tris-glucose diluents*

| | |
|---|---|
| Tris (hydoroxymethyl) aminomethane | 3.634 g |
| Glucose | 0.500 g |
| Citric acid (monohydorate) | 1.990 g |
| Egg yolk | 15 ml |
| Glass distilled water | 100 ml (final volume) |

cooled to 4°C.

Thereafter, the diluted semen was dripped by secondary diluents which is 12-14% glycerin added to the primary diluents. Furthermore, the glycerin balance was done for 6-12 hours in a cold room at 5°C.

These semen diluents were put into 0.25 ml or 0.5 ml plastic straw and sealed with straw powder. To gradually freeze, these straws were enveloped with a liquid nitrogen steam for about one hour, and then placed in the liquid nitrogen.

Frozen semen straw was thawed in the lukewarm water of 38°C for about 15-30 seconds, and the sperm movement rate was scored in the same way before freezing. These data were analyzed using GLM (General Linear Model) of SAS (Statistical Analysis System) at the Tohoku University Education Center of Information Processing.

*Cervical insemination*

*Sheep*

A total 31 of ewes consisting of 14 Suffolk (SF), 5 Corriedale (CO), 1 Cheviot (CH), 1 Perendale (PE) and 10 crossbred ($F_1$) were inseminated by cervical insemination. Ram semen was used for the three breeds of SF, CO and PE.

*Synchronized estrous*

Every ewe was inserted with an intravaginal sponge (pessary) containing 60 mg of medroxyprogesteron acetate (MAP). The sponge insertion period was 9-12 days. The sponge was pull out from the vagina and 500 IU PMSG (Sankyo Pharmacy in Japan) injected into the muscle of the ewe's buttocks. The fresh semen was injected into the cervical canal with the pipette of our own design form about 0.1 to 0.7 ml. The ewes were inseminated 24-48 hours after the sponge removal.

*Artificial insemination by the laparoscopy*

*Sheep*

A total sixteen of ewes were examined for artificial insemination by the laparoscopy. They included five SF and eleven Crossbreds. The semen used from the rams was from the SF and PE. They were raised at the Experimental Station in Kawatabi and at Faculty of Agriculture in Sendai. The synchronized

estrous was the same method as the cervical insemination.

*Anesthesia*

The ewes were abstained from food for at least twelve hours and given general anesthesia. First, the ewes were injected with 0.2-0.3 ml of 2% Seractar solution (Bayer AG) into the muscle for hypomyotonia. Then, 10 ml of Labonar (Tanabe Pharmaceutical Co. in Japan) was slowly injected into the jugular vein of ewe while checking the status of anesthesia.

When the anesthesia was effective, the ewe was restrained on its back on the operating table.

*Operation treatment*

The apparatus tools which we used for the operation of the laparoscopy were the Trocar (puncture device) and forceps developed for human manufactured by Johnson & Johnson Medical Co. in Japan (Fig. 1). The wool in front of the udder and on the abdomen was shaved and then the area sterilized with Isodine. The ewe was dissected about one cm that is 5-7 cm above the udder and 3-4 cm left from the median line. Then, the Trocar was then inserted into the abdominal cavity. The inner needle was removed from the Trocar and the abdominal cavity pumped with $CO_2$ gas.

The hysteroscope was injected into the abdominal cavity through the Trocar and scope out the uterine horn. Another place 3-4 cm right of the median line was dissected about several mm, the inner needle removed from the Trocar, and forceps inserted (Fig. 2). With this forceps, we found the uterine horn and held it. Fresh semen was injected through the abdominal epithelium and uterine horn epithelium with an 18G long needle (20 cm) and one ml tuberculin syringe (Fig.

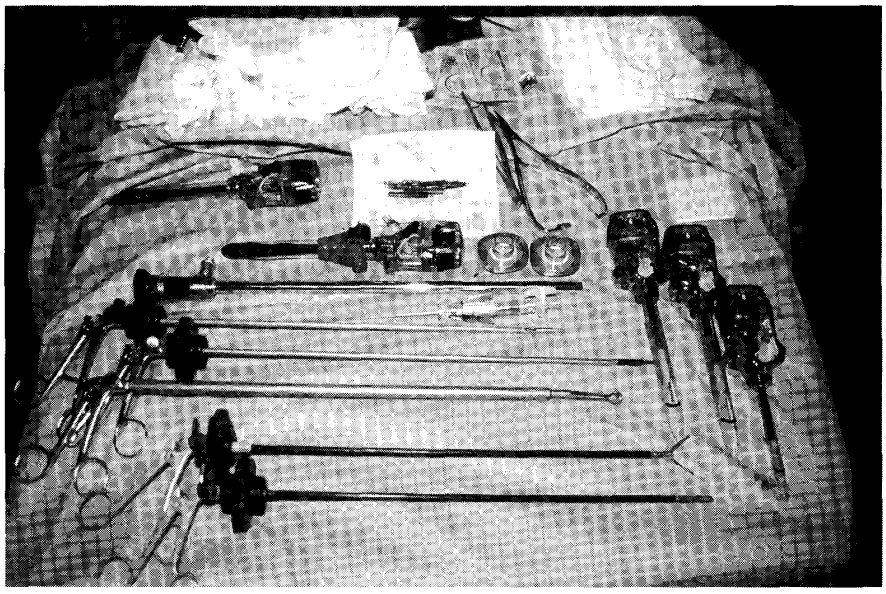


FIG. 1. Surgical tools for laparoscopy
Trocar (right and upper), Forceps (left)

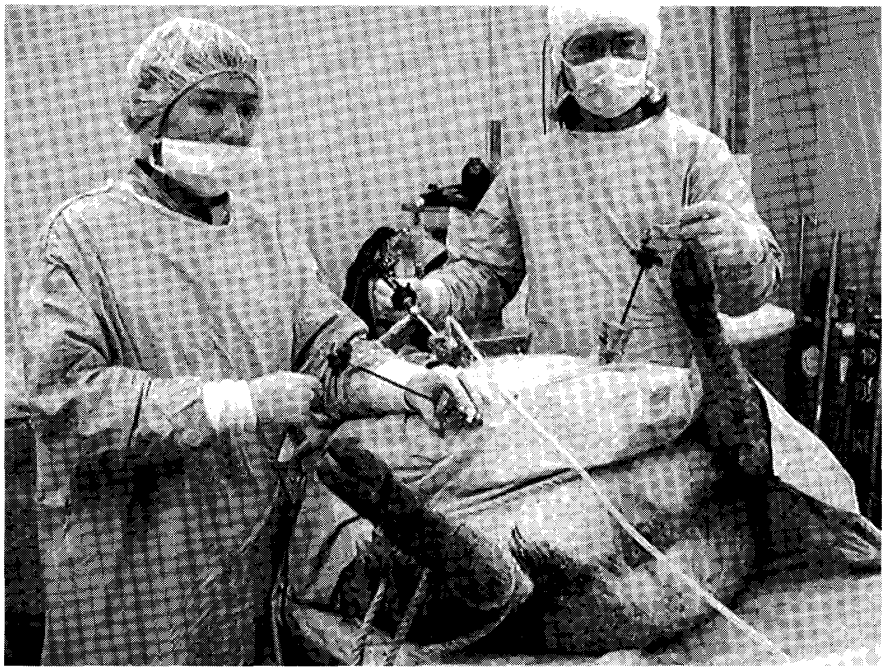


FIG. 2. Laparoscopy operation of sheep

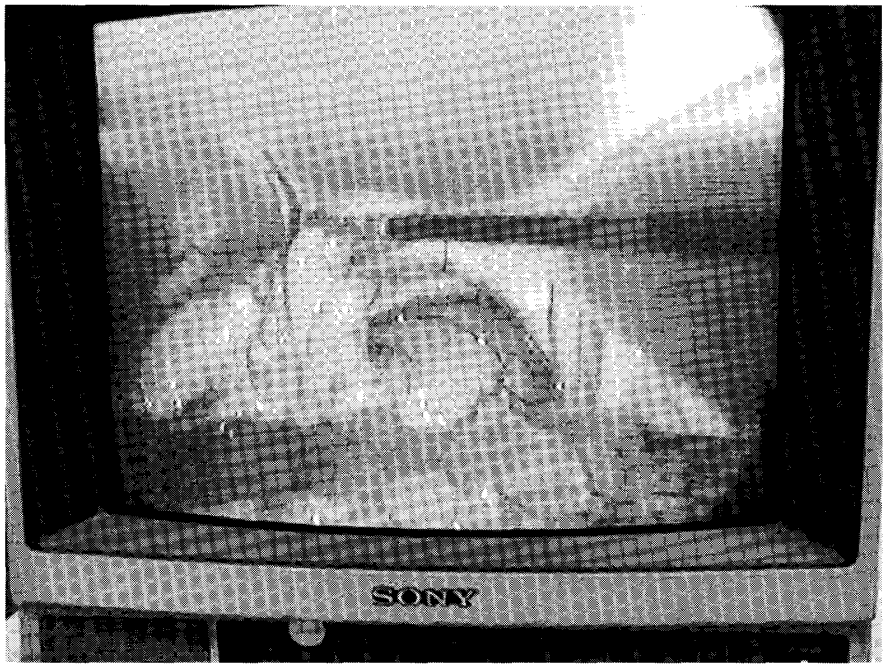



FIG. 3. Holding the uterine horn by forceps and injected semen into uterine in the abdominal cavity

3). This fresh semen was not diluted and 0.1-0.3 ml of it injected into the uterine. After the injection, dissections were stitched up and sterilized with Isodine. The ewes recovered from the anesthetic within 30 minuets, then taken to their pen and normally raised.

*Birth rate*

The birth rate achievement was calculated from the date of lambing backward to the birth date. However, we did not have such data for the reason of a shorter period, therefore we calculated the Birth rate achievement by the ultrasonic estimation of the sheep fetus growth. The lambing date was estimated by the growth curve of the sheep fetus developed by Yamaki.

## Results and Discussion

*The ram semen characteristics*

The monthly changes in the semen characteristics are shown in Fig. 4. The monthly semen characteristics were shown as their least square means values. The number of spermatozoa showed a significant difference, and October was the highest while May was the lowest. The semen concentration was the highest in October and low in August and May. The results of Duncan's multiple tests in GLM about the semen characteristics are shown in Table 2, and significant differences were recognized by month and season. These data were then summarized into the four seasons that is May is spring, July to August is summer, September to November is autumn and December to January is winter. The Semen volume was significantly higher in the autumn and winter, and the semen concentration was significantly higher in the autumn. The spermatozoa movement index also significantly higher in the autumn.

The spermatozoa movement index was high in September and October, and

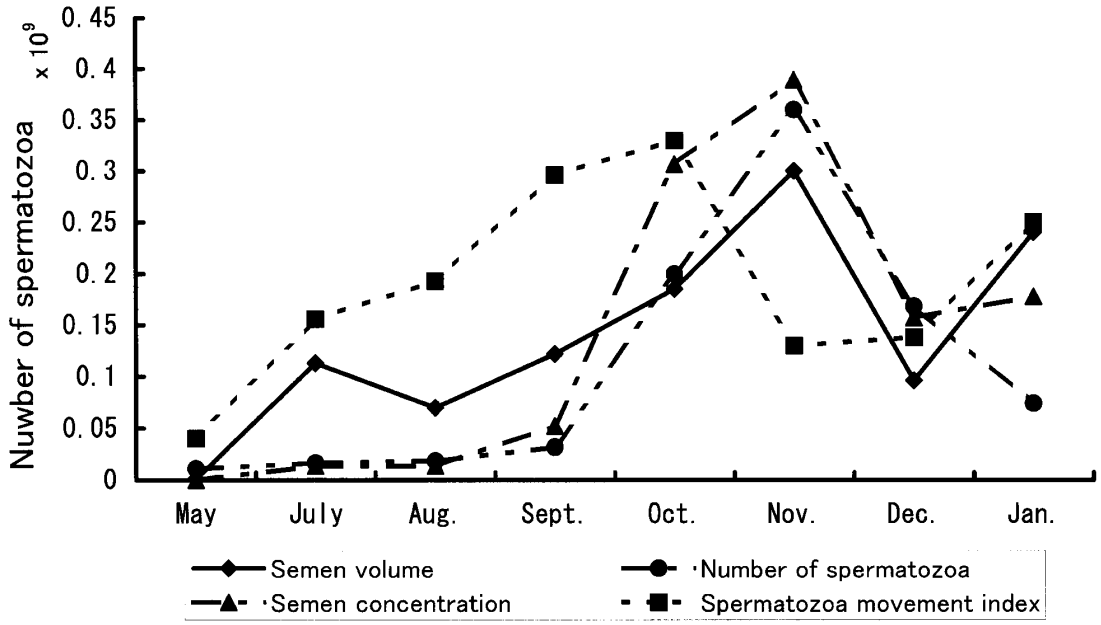



FIG. 4. Monthly semen characteristics

*Table 2 Least square means of semen characteristics*

| Month | Semen volume (ml) | Semen concentration ($10^9$/ml) | Number of spermatozoa ($10^9$) | Spermatozoa movement index |
|---|---|---|---|---|
| May | – | $0.57^{ab}$ | $0.04^{b}$ | $0.05^{b}$ |
| July | 0.77 | $0.37^{b}$ | – | $1.41^{b}$ |
| August | 0.45 | $0.20^{b}$ | – | $1.93^{ab}$ |
| September | 0.91 | $0.43^{b}$ | $0.01^{b}$ | $2.90^{ab}$ |
| October | 1.99 | $2.56^{a}$ | $4.16^{a}$ | $3.35^{a}$ |
| November | 3.47 | $0.82^{ab}$ | $2.54^{ab}$ | $1.47^{ab}$ |
| December | 0.93 | $1.68^{a}$ | $1.50^{b}$ | $1.35^{b}$ |
| January | 2.87 | $1.02^{ab}$ | $2.70^{ab}$ | $2.67^{ab}$ |

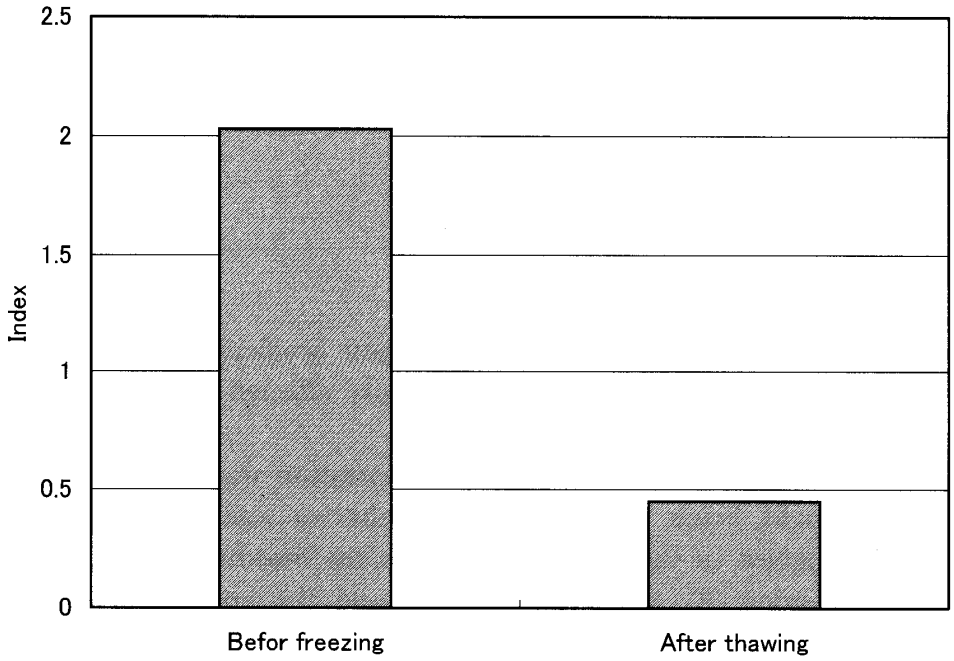
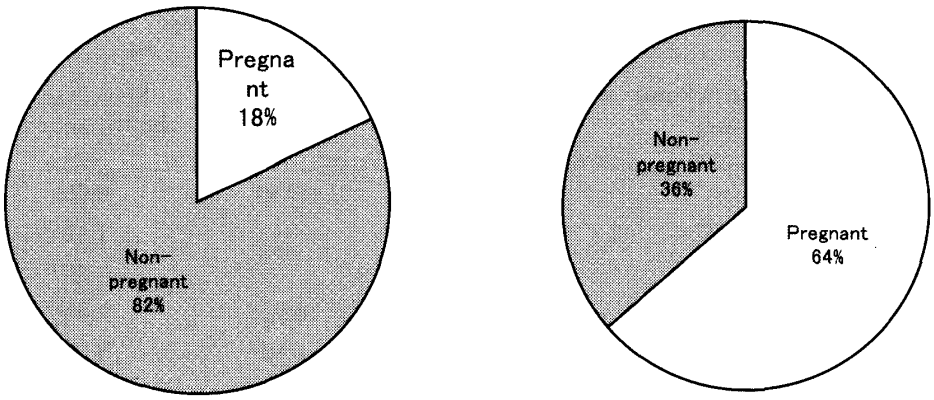



FIG. 5. Least square means of spermatozoa movement index ($P<0.05$)

the lowest in May. The spermatozoa movement rate before and after freezing was analyzed by a t-test, and a significant difference was recognized before or after (Fig. 5). This shows that freezing has a great influence on spermatozoa movement, and it was the cause of the low birth rate in sheep. Our results of birth rate both the cervical insemination and the insemination using laparoscopies were shown in Fig. 6.

A correlation between temperature and the semen characteristics is shown in Table 3. A significant correlation between the sperm concentration and average




The cervical insemination The insemination by laparoscopy

FIG. 6. Birth rate of the insemination method

*Table 3 Correlations of monthly average temperature and semen characteristics*

| | Semen volume (ml) | Semen concentration ($10^9$/ml) | Number of spermatozoa ($10^9$) | Spermatozoa movement index |
|---|---|---|---|---|
| Monthly Average Temperature | −0.064 | −0.358* | −0.270 | 0.256 |

*significant $P<0.05$

temperature was recognized, and the correlation coefficient was −0.385 ($P<0.05$). This showed that the temperature mainly affected the semen concentration.

Elwisby(1) reported that the semen concentration was high in the summer with long daylight hours, and the sexual behavior and semen volume were becoming the highest in the autumn, which is the breeding season. Moreover, according to Gordon (6), the semen volume and the number of spermatozoa decreased in August to September, and increased in October to December. Furthermore, the numbers of spermatozoa and sperm vitality were significantly lower in the non-breeding season compared to the breeding season.

In our experiments, the semen concentration, number of spermatozoa and spermatozoa movement index gradually rose from autumn, with the tendency to become high in the winter, and low in the summer. The semen volume showed same tendency as the other semen characteristics. For the semen characteristics, negative correlations were recognized between the semen concentration and monthly average temperature.

These results demonstrated that the condition of the semen was superior in the cooler climates than the warmer ones. This tendency was consistent with the results that the semen concentration in 7-9°C environments was higher than that

of non-treatment in the summer reported by Fukui(5).

Therefore, it is definite that the temperature is significantly involved as one of the factors involved in the decline of the semen quality and characteristics. A semen characteristic deteriorates due to high temperature or the influence of heat, and the function of antheridia is sometimes spoiled.

It is pointed out that frozen semen decreases the conception rate from the fresh semen, and it will be an infertility factor. Our experiments also showed that the cause of the low conception rate is due to the decline in the sperm activation when it was frozen.

To prevent the decline in the sperm activation, it is necessary to improve the dilution ratio, method of dilution and composition of the diluents. Furthermore, it is necessary to investigate how the decline in the sperm activation is affected by the season when the semen is frozen. When the various results are taken into consideration, and improved, it will raise the birth rate by artificial insemination in sheep.

*Artificial insemination by laparoscopy*

A comparison of the conception rate was examined for the artificial insemination by the cervical canal and the direct insemination into the uterus using laparoscopy. In the cervical canal insemination, 33 inside six ewes conceived, and this was 18% of the total. In addition, in the insemination by laparoscopy, 11 inside seven ewes conceived, and this was 64% of the total (Fig. 6). Based on analysis of the chi-square test applied to this result, the conception rate of insemination by artificial insemination using laparoscopy was significantly higher than that of the method of the cervical canal. Therefore, a normal pipette for semen insemination cannot easily be inserted into the uterine cavity.

Stoyanov(9) made a special pipette for the injection to the structure of the cervical canal of the sheep. It was reported that a high conception rate was achieved when this pipette is used. It is considered that the conception rate also rises, if artificial insemination is done twice every ten hours though it was only one time of the artificial insemination. In this case, the frozen effect of the conception rate appears more than the fresh semen as for the semen. It is reported that the conception rate improved by directly injected into the uterus by using the laparoscopy. Our result of artificial insemination by laparoscopy improved the conception rate and corresponded to their result.

Evans(2) reported that even if semen is injected only into one of the uterine horn, the conception rate was the same as that if it was injected into both uterine horns. Our experiment also injected semen into one of the uterine horn. Nevertheless, 85% of the ewes were successful in pregnancy by the artificial insemination using laparoscopy. Therefore, our result of conception rate is lower than the result they reported. One of reasons was the delay of days from the

natural mating so that the ewes were not easily conceived. Second, ewes were under two years old, so that they are difficult to conceive at that time.

Though such factors were taken into consideration, artificial insemination by laparoscopy was an excellent technology to get the birth rate close to 100%. The laparoscopy technology is scalable to embryo transfer. Moreover, according to the report of Maxwell (7), an artificial insemination by laparoscopy made the use of frozen semen effective. It was also found that insemination by laparoscopy was an excellent method for effective fertilization though it is a more advanced technology for artificial insemination.

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