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Clinical Application of Intrauterine Laparoscopic Insemination in Zaraiby Goats Using Different Concentrations of Zaraiby and Boer Bucks' Frozen Semen

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ABSTRACT

This study aimed to determine the proper sperm dose per laparoscopic intrauterine insemination (LAI) in Zaraiby goats. Thirty-six Zaraiby goats, two Zaraiby and two Boer bucks were used in the study. Does were divided into six groups; the first three groups were inseminated laparoscopically with frozen-thawed Zaraiby semen either at doses of 10 x 10^6 (GP1), 20 x 10^6 (GP 2), or 40 x 10^6 (GP 3). The other three groups were inseminated with Boer semen's same doses (GP 4, 5 &6). Results showed that goats laparoscopically inseminated with 20 x 10^6 Zaraiby spermatozoa or 10 x 10^6 Boer sperm cells both had the highest *(P ≤0.05)* pregnancy rate (about 70% & 60%, respectively). The multiple birth rate in goats inseminated by Boer buck's spermatozoa were generally higher than those inseminated with Zaraiby buck's spermatozoa (66.67 to 100.00% vs. 25.00 to 40.00%, respectively). In conclusion, the LAI is an efficient means of achieving high fertilization rates in goats. The recommended minimum necessary dose for laparoscopic artificial insemination in Zaraiby is 10 ×10^6 and 20×10^6 motile Boer and Zaraiby buck's spermatozoa, respectively.

Keywords: Goat, insemination, laparoscopic, semen dose, Zaraiby.

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INTRODUCTION

In Egypt, goat production is an important part of society and agriculture. Goats play a crucial role due to their high tolerance to heat stress and harsh conditions. In contrast to other ruminants, goats can easily convert limited forages and crops into the meat and milk **(Tekin, 2019)**. Boer goat (*Capra hircus)* is considered one of the most desirable goat breeds for meat production **(Lu, 2001)**. It has gained worldwide recognition for excellent body conformation, fast-growing rate and good carcass quality. Furthermore, Boer goats can improve many indigenous breeds' productive performance through cross-breeding **(Lu, 2001).**

Cross-breeding by the application of assisted reproduction technologies (ART) results in the utilization of hybrid vigor for commercial production **(Anakkul *et al.*, 2013).** Artificial insemination (AI) is a high-impact technique for genetic improvement worldwide, especially when frozen-thawed semen was applied **(Leboeuf *et al.*, 2000).** To get higher pregnancy rates with post-thaw semen, the spermatozoa should be deposited directly into the uterus using laparoscopic artificial insemination (LAI) **(Sohnrey and Holtz, 2005).** This cross-bred LAI improves genetics via male germplasm **(Anakkul *et al.*, 2013).**

There are several types of AI techniques in small ruminants, depending on the choice for semen deposition. Using fresh semen, by vaginal (pericervical deposition of semen), cervical (intracervical deposition of semen) or intrauterine (laparoscopic technique) deposition, satisfactory pregnancy rates are achieved (50-70%) **(Faigl *et al.*, 2012).** However, by using frozen semen, laparoscopic or transcervical intrauterine insemination techniques are the only means to achieve acceptable pregnancy rates **(Cseh *et al.*, 2012; Kumar**

and Naqvi, 2014) because cryopreservation render the spermatozoa are functionally compromised **(Salamon and Maxwell, 1995; Barbas and Mascarenhas, 2009)** and with disturbed semen transport through the reproductive tract after cervical AI. So, y depositing the frozen semen directly into the uterus, enhanced fertilization rates can be achieved **(Faigl *et al.*, 2012).**

The advantage of laparoscopic insemination is that the semen is deposited closer to the site of fertilization. Deep uterine insemination is advantageous in several domestic species, such as sheep **(Salamon and Maxwell, 1995; Wulster-Radcliffe *et al.*, 2004)**, goats **(Ritar and Salamon, 1983; Moore et al., 1988; Anakkul *et al.*, 2013)**, cattle **(Lopez-Gatius, 2000; Verberckmoes *et al.*, 2004),** horses **(Morris and Allen, 2002)**, and pigs **(Watson and Behan, 2003),** especially when sperm numbers are limited or sperm quality is suboptimal **(Salamon and Maxwell, 1995)**.

Generally, pregnancy rates have been reduced when insufficient sperm numbers are used for AI **(Eppleston *et al.*, 1994; Scenna *et al.*, 2005)**. Therefore, the determination of the optimum number of spermatozoa is required to achieve the highest pregnancy rates in goats. A wide range of insemination doses ($5x10^6$ to $200x10^6$ sperm) has been reported for frozen-thawed goat spermatozoa, and these doses depend largely on the AI technique that is employed **(Anakkul *et al.*, 2014)**. Therefore, this study's major objective was to compare the kidding rates of goats laparoscopically inseminated by different sperm doses and to determine the minimal sperm dose per intrauterine insemination to maximize the genetic diffusion of superior Boer and Zaraiby males, without decreasing AI success.

MATERIALS AND METHODS

Animals and treatment

The study was carried out during October and November 2019. Thirty-six mature, healthy Zaraiby goats (aged 1.5 - 3 years), 2 Zaraiby bucks (aged 2.5 – 3.5 years) and 2 Boer bucks (aged 1.5 – 2 years) were assigned to the study. Zaraiby breed was kept on the Animal Reproduction Research Institute (ARRI) experimental farm and private farm in Giza Governorate. Boer bucks were reared in the Jazerat Al-Shaer production farm of the Animal Production Research Institute (APRI). The estrous cycles of Zaraiby goats were synchronized using intravaginal sponges containing 40 mg of progesterone (Lutone, Nile Co., Egypt). The sponges were inserted for 6 days **(Fonseca *et al.*, 2017)**.

Each goat was injected intramuscularly with 250 IU ECG (Folligon; Germany) at the time of sponge removal. The animals were divided into six groups; the first three groups (7 goats in each) were inseminated laparoscopically 60 h after sponge removal with frozen-thawed Zaraiby semen either at doses of 10 x 10^6 (GP 1), 20 x 10^6 (GP 2) or 40 x 10^6 (GP 3). The other three groups (5 goats in each) were inseminated laparoscopically with the same doses of frozen-thawed Boer semen (GP 4, 5 &6).

Collection and processing of semen

Semen was collected from mature bucks with an artificial vagina that was adjusted to a proper condition. Semen collection was performed early in the morning by means of electro-ejaculator (Electrojac III®, Ideal Instruments Co., USA). Bucks were secured, then the rectum was cleaned of faces and the preputial area was shaved and washed with physiologic saline. For electroejaculation, a three-electrode probe was used. Probe diameter and length were 3.2 and 35.0 cm, respectively. The electroejaculation regime consisted of consecutive 4 series of 5-s pulses of 13 volts, each separated by a 5-s break. Immediately after collection samples were evaluated for volume, mass motility and individual motility using a prewarmed stage of the phase-contrast microscope (X400). The sperm concentration was determined by means of a hemocytometer.

Only ejaculates of more than 1 mL volume, spermatozoa with >80% progressive motility, and a higher concentration than 2.0 x 10^9 sperm cells/mL was used for the freezing protocol. Semen samples were diluted at 30°C with Tris-based diluent **(Evans and Maxwell, 1986)**. The extended semen was cooled to 5^oC for 60 minutes in a cold handling cabinet. The cooled semen was loaded into 0.25 ml French straws (IMV, L'Aigle, France), arranged horizontally on a cooling rack, then lowered into liquid nitrogen vapor inside a foam box **(Khalifa, 2001).** Then, the straws were immersed in liquid nitrogen and stored until use.

Laparoscopic artificial insemination

Laparoscopic procedures were done using Henke-Sass Wolf Laparoscope (Wolf Co., Germany) of 5 mm diameter, 33 cm length and 0^o scope viewing angle. An automatic insufflator was used to deliver the CO_2 intraperitoneally (pressure 8 mmHg). Laparoscopic insemination, in detail, was described by **Toni *et al.* (2012)**. Briefly, goats have fasted and restricted access to water at least 24 hours before laparoscopy and epidural anesthesia was induced by lumbosacral injection of 2 ml Lidocaine 2% ten minutes before the procedure was performed. The goat was then placed in a laparoscopy cradle. The abdominal region was surgically prepared by shaving the hair and disinfecting the skin. Using the cradle, the goat was positioned in a supine head-down (Trendelenburg) position to an approximate angle of

45°. A scalpel blade was used to make a small skin incision in order to create pneumoperitoneum with CO_2 using a verus needle 7-10 cm ventral to the udder and 5-10 cm on each side of the midline (*linea alba*). The 2-mm Verus needle connected with the CO_2 is first introduced and the abdomen was inflated to reduce the chance of injury to organs and facilitate trocar penetration (Fig. 1). The trocars and cannulae for introducing laparoscope and insemination pipette were inserted and the sharp trocar was withdrawn as soon as the abdominal wall has been penetrated. The blunt cannula was pushed well into the abdomen Endoscope.

Fig. 1: A small skin incision was done in order to facilitate trocar penetration.

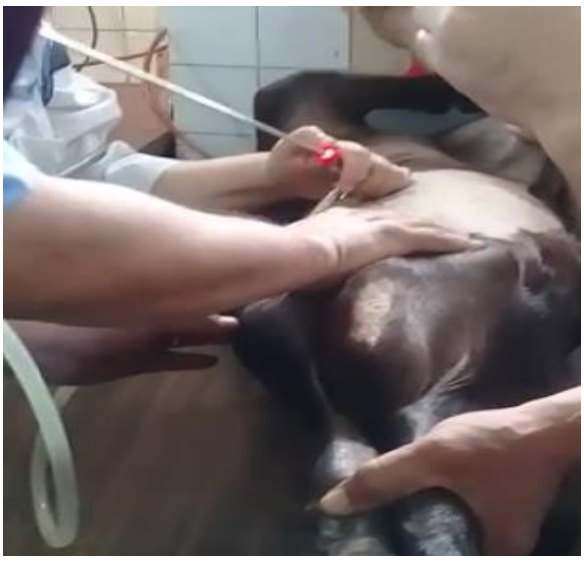

Fig. 2: Endoscope went through the cannula for visualization of uterus

An AI instrument passed through the cannulae (Fig. 2), and the uterus was located and fixed using the grasping forceps just ventral to the urinary bladder. Zaraiby and Boer frozen-thawed semen at different doses (According to the experimental design) was deposited in the lumen of each uterine horn approximately halfway between the uterine bifurcation and the utero-tubal junction (Fig. 3 & 4). Instruments were withdrawn and put into disinfectants between each animal. An antibiotic spray was applied to the wounds before it was sutured using absorbable sutures (Proline).

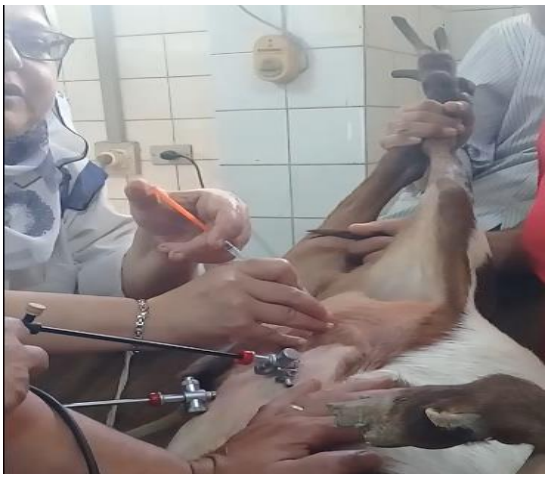

Fig. 3: Semen deposition inside the uterine horn

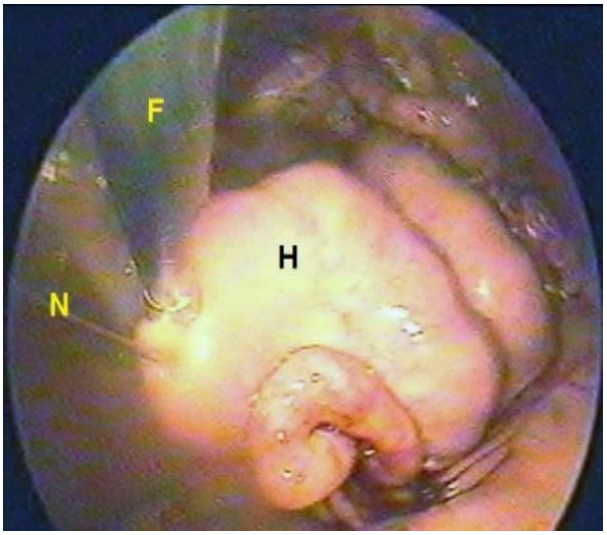

Fig. 4: The recommended site of puncture for semen deposition is the major curvature of the uterine horn (arrow)

Pregnancy diagnosis

Pregnancy was determined 35 days after AI using ultrasound equipment MyLabTM30 VET (Esaote S.P.A., Genova, Liguria Italy) with a micro convex multifrequency (3.5–8.5 MHz) probe.

Statistical analysis

The recorded data of pregnancy rates among all treatment groups were analyzed by Chi-square analysis, and $P < 0.05$ was considered statistically significant **(Snedecor and Cochran, 1989).**

RESULTS

As presented in table 1, intrauterine insemination with 20 x 10^6 Zaraiby spermatozoa resulted in the highest *(P ≤0.05)* pregnancy rate (71.43%). While using Boer spermatozoa, 10 x 10^6 sperm cells resulted in the highest *(P ≤0.05)* pregnancy rate (60.00%). Using a sperm dosage of 40 x 10^6 spermatozoa from either Zariaby or Boer bucks resulted in the lowest *(P ≤0.05)* pregnancy rate (0.00 to 14.29%).

Table 1: Kidding rate of Zaraiby goats after laparoscopic intrauterine insemination with frozen-thawed Zaraiby and Boer goat semen at different insemination doses.

Treatment groups	GP1		GP2		GP3	
Bucks	Zaraiby	Boer	Zaraiby	Boer	Zaraiby	Boer
No. of goats inseminated	7	5	7	5	7	5
No. of pregnant goats	4	3	5	2	1	0
(%)	(57.14) ^{b}	(60.0%) ^{A}	(71.4) ^{a}	(40%) ^{B}	(14.3) ^{c}	(0.0%) ^{C}
Multiple birth rate	(1/4)	(2/3)	(2/5)	(2/2)	(0/1)	0.0%
	25.0% ^{b}	66.6% ^{B}	40.00 % ^{a}	100% ^{A}	0.0% ^{c}	(0/0) ^{C}
No. of kids /goat)	5	8	7	6	1	0
	(1.25) ^{a}	(2.67) ^{A}	(1.4) ^{a}	(3.0) ^{A}	(100.0) ^{c}	(0.0) ^{B}
Single (%)	3	0	3	0	1	0
	(75.0%)	(0.0%)	(60.0%)	(0.0%)	(100.0%)	(0.0%)
Twin (%)	1	1	2	0	0	0
	(25.0%)	(33.3%)	(40.0%)	(0.0%)	(0.0%)	(0.0%)
Triplet (%)	0	2	0	2	0	0
	(0.0%)	(66.7%)	(0.0%)	(100.0%)	(0.0%)	(0.0%)
Male kid rate	2/5	4/8	3/7	2/6	1/1	0/1
(%)	(40%)	(50%)	(42.8)	(33.3%)	(100%)	(0.0%)
Female kid rate	3/5	4/8	4/7	4/6	0/1	0/1
(%)	(60%) ^{a}	(50%) ^{B}	(57.1%) ^{a}	(66.6%) ^{A}	(0.0%) ^{b}	(0.0%) ^{C}

Values with different superscripts a, b,c or A, B, C in the same raw differs significantly at least at P < 0.05

No significant differences were observed in the number of kids per goat when using either 10 x 10^6 or 20 x 10^6 spermatozoa laparoscopically inseminated by the two breeds semen. The multiple birth rate in goats inseminated by the fore-mentioned doses of the Boer buck's spermatozoa were generally higher than those inseminated with Zaraiby buck's spermatozoa (66.67 to 100.00% vs. 25.00 to 40.00%, respectively). The IUI of goats by 10 to 20 x 10^6 spermatozoa were belonging to the two breeds resulted in a higher ratio of female kids (50 to 66.67%).

DISCUSSION

The present study was carried out to validate the technique of intrauterine laparoscopic insemination in Zaraiby goats using different concentrations of Zaraiby and Boer bucks' frozen semen. Because of the anatomical structure of the cervix in small ruminants, transcervical/intrauterine AI application is extremely difficult. **Kulaksiz and Ari (2016)** stated that sperm cell concentration in cervical or vaginal AI should be at least 200 x10^6 to obtain satisfied pregnancy results in goats and sheep. This high dosage used in cervical or vaginal AI allows only 10-15 females to be inseminated with ejaculate from bucks or rams. In contrast, LAI warranties higher pregnancy rates using frozen-thawed/low doses (10-20x10^6) semen **(Anakkul *et al.*, 2014; Kulaksiz and Ari, 2016)**. In this way, LAI provides more efficient and widespread semen usage from an elite buck or ram for AI.

Using a proper insemination dose in the current study, LAI has a success rate of approximately 60–71.43%. Kidding rates similar to our results (59.5% to 71.4%) were recorded in different goat breeds by **Ritar *et al.* (1990), Dickson *et al.* (2001), Kulaksiz and Daskin (2012),** and **Bonato *et al.* (2019).** Lower values (about 40%) were obtained by **Goonewardene *et al.* (1997)** and **Lowinger *et al.* (2001).** On the other hand, other researchers obtained more modest results, which occasionally peaked at 85% **(Salamon and Maxwell, 2000; Toni *et al.*, 2012).** Although this rate can vary depending on the quality of semen, the female's body condition, lactation status, the skill of the inseminator**,** the number of previous parturitions, the interval from kidding to AI, reproductive seasonality, farm and buck fertility, as well as semen cryopreservation technique, are also factors affecting pregnancy rate **(Palacín *et al.*, 2012; Yotov *et al.*, 2016; Gibbons *et al.*, 2019**).

Generally, finding the optimum number of spermatozoa is of great interest in achieving the highest pregnancy rates in goats. Based on the present data, it can be recommended that the minimum necessary dose for laparoscopic artificial insemination in Zaraiby does is 10 ×10^6 and 20×10^6 motile Boer and Zaraiby buck's spermatozoa, respectively. Similarly, in ewes, **Evans and Maxwell (1987)** recommend a minimum dose of only 20 x 10^6 motile sperm while there are several reports of acceptable fertility (> 50%) using doses as low as 5 x 10^6 **(Eppleston *et al.*, 1986)** and 10 x 10^6 **(Salamon *et al.*, 1985)** motile spermatozoa. Furthermore, acceptable fertility levels were achieved after low dose insemination using flow cytometrically sorted ram sperm at a dose of 1 x 10^6 motile sperm per ewe **(de Graaf *et al.*, 2007).** No such data were available for goat semen doses. **Anakkul *et al.* (2014)** found no significant differences in kidding rates when 60 x 10^6 or 120 x 10^6 spermatozoa were used in LAI in Saanen goats (50.33% vs. 60%, respectively).

In the current study, the multiple birth rate in goats inseminated by the Boer buck's spermatozoa were generally higher than those inseminated with Zaraiby buck's spermatozoa (66.67 to 100.00% vs. 25.00 to 40.00%, respectively). These differences may be attributed to sperm quality factors such as viability, motility, and longevity **(Sohnrey and Holtz, 2005; Martínez-Rojero *et al.*, 2007).** In a recent study, **Al-Khawaga *et al.* (2020)** mentioned that spermatozoa collected from Boer bucks were better preserved than Zaraiby ones.

CONCLUSIONS

In conclusion, the LAI is a relatively simple and convenient means of achieving high fertilization rates and facilitating cross-breeding in goats. The recommended minimum necessary dose for laparoscopic artificial insemination in Zaraiby does is 10 ×10^6 and 20×10^6 motile Boer and Zaraiby buck's spermatozoa, respectively.

Declaration of Competing interest

On behalf of all authors, I hereby declare that no conflict of interest may interfere with the publication of the manuscript.

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