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Ovarian cauterization Versus $Cacl_2$ Injection For "In Situ Ovariectomy" In Female Cats

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ABSTRACT

Overpopulation of stray and unwanted cats has been a serious issue in every country and different individuals have different motivations for their spaying or neutering. The current experiment aimed to evaluate the feasibility and safety of ovarian cauterization compared to $CaCl_2$ injection for ovarian tissue damage in female cats. Each ovary of three cycling normal cats in reproductive age was punctured at four sites and 40 W was applied to 5 seconds at each point using monopolar electro-coagulation, resulting in 800 J (Joules) of thermal energy. Ovaries of another three cats were injected with 0.25 ml of 20% $CaCl_2$ in 95% ethanol solution. Macroscopic and microscopic lesions were assessed depending on a number of evaluating criteria. Operative time, intraoperative and postoperative complications, and estrogen level estimation showed significant difference between both techniques. The cauterization technique proved easy applicable and safe, and there were no intra-abdominal thermal injuries. Histologic evaluation proved essential to clarify the ovarian functional activity. Ovarian cauterization seems to be feasible and safe, and 40 W (800 J) thermal energy allowed incomplete ovarian thermal damage.

1. INTRODUCTION

Overpopulation of stray and unwanted cats has been a serious issue in every country in the world (Bloomberg, 1996). The control of free-roaming animals is a focus of great interest for public health and the welfare risk of these animals (Gerhold and Jessup, 2013). Numerous techniques are used for dogs and cats; however, the goals are the same-to remove the ovaries, with or without the uterine horns and body, with secure ligature placement (Fossum et al., 2013). All procedures may lead to postoperative problems, nevertheless, with bleeding being the most frequent and leading cause of death in postoperative ovariohysterectomy (Melo et al., 2018). Ovariectomy using snook hook, as a widely spread technique, entails complications regarding hemorrhage primarily from the ovarian pedicles when ligatures are improperly placed (Santos et al., 2009; Honsho et al, 2010). Developing of ovarian remnant syndrome (ORS) nevertheless has functional ovarian tissue (Muram and Drouin, 1982; Wallace, 1991).

Surgical procedures have a difficult access to the population with limited resources (Woodruf and Smith, 2020). Nevertheless, nonsurgical forms such as chemical agents may offer a less costly alternative for inducing sterilization (Jana and Samanta, 2006). Studies investigating chemosterilant methods have generally focused on males, mainly due to the ease of administration to the testicles (Cavalieri, 2017; Jana and samant., 2011; Leoci et al., 2014 and 2014). The difficulty in accessing ovaries without surgical exposure cited as the greatest difficulty in performing the technique in pets (Mogheiseh et al., 2017).

In laparoscopic ovarian diathermy for poly cystic ovary in women, the ovarian surface is punctured multiple times under direct visual inspection using a laser or diathermy as a single therapy to enhance ovulation (Duncan et al., 1994). Unfortunately, because it is an intrusive operation, there are more risks and more morbidity associated with the abdominal surgery and general anesthesia (Amer et al., 2002). Meirelles et al. (2007) employed a comparable method on cattle and noted that ovarian degeneration prevented estrus from occurring during the assessment period. The current investigation aimed to establish the feasibility and safety of ovarian electro-coagulation compared to $CaCl_2$ injection as an "in situ ovariectomy" techniques in female cats.

2. MATERIALS AND METHODS

Six cycling normal adult female cats aging 7-24 months and weighing 2.5-4 kg were used during their breeding season. The experiment was being approved by the Institutional Animal Use and Care Committee of Faculty of Veterinary Medicine, Alexandria University (AU-IAUCC). Cats were randomly classified into two groups (n=3 for each). All operations were performed by ventral laparotomy under the effect of general anesthesia with ketamine HCl (10 mg/kg) pre-medicated with xylazine HCl (1 mg/kg). Following laparotomy, an ovarian hook was inserted to locate the uterine horn that leads to the ovary**.** Fingers were then used to retract the uterine horn and the ovary outside the abdominal cavity (Fig., 1 a & b).

2.1. Cauterization technique

The ovary was grasped and punctured at four sites, using monopolar electro-coagulator (ALSATOM SU 100-M) device. 40 W was applied for 5 seconds at each point, resulting in a total of 800 J (Joules) of thermal energy (Fig., 2a). The amount of thermal energy was selected according to Pimentel et al. (2013). A similar procedure was repeated in the other ovary and the ovaries are repositioned to the abdomen.

2.2. Injection technique

Following manual grasping of ovary, a careful single intra ovarian injection of 0.25 ml of 20% $CaCl_2$ dehydrate diluted in 95% ethanol using sterile 29-gauge 1/2-inch syringe was done (Fig., 2b).

Figure (1): Showing ovarian identification and isolation (a&b)

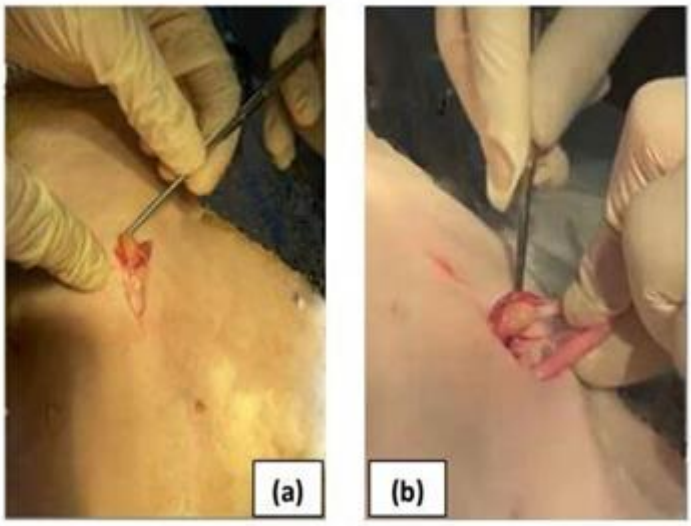
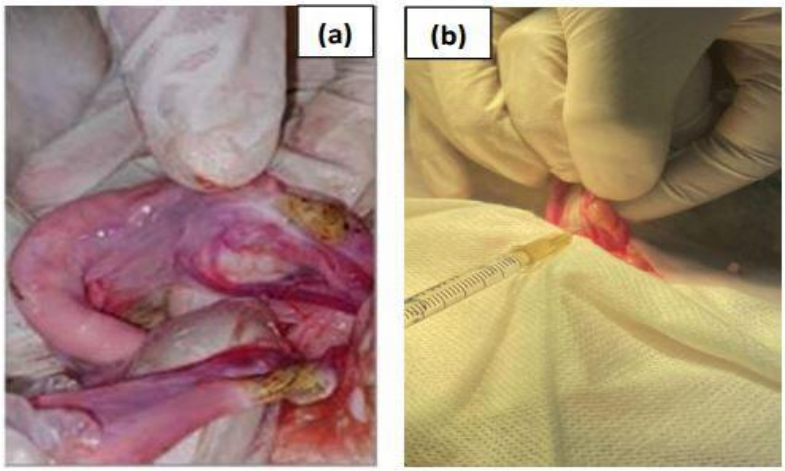


Figure (2): Showing Cauterized ovary (a) and intra ovarian injection (b)



Every precaution was taken to avoid the fluid from seeping out of the injection site by paying attention that the needle is being inside the ovary. The technique is adapted from Gomes et al. (2022). The same procedure is repeated in the ovary of the other side. Following surgery, the abdomen was checked for bleeding and routinely closed, received one shot of meloxicam (Meloxidyl®, as 0.3 ml/5kg) and antibiotic (Synolux®, as 0.25 ml/5kg) once daily for 5 consecutive days. The surgical wound managed with local antibiotic (Bivatracin® spray) till skin stitches removal.

A number of criteria were considered for techniques' assessment including; operative time (the time from isolation of ovary till complete cauterization or complete intra-ovarian injection), intra-operative bleeding depending on the scores described by Culp et al. (2009) and Dupré et al. (2009), animals' clinical evaluation considering estrous signs, estrogen level, adhesion formation, assessment of the ovarian diameter (cm), as well as the histological examination.

Blood samples were collected twice; at day 0 (day of the operation) and at day 30 later, for determination of serum estradiol level (pg/mL). Abdomen was thoroughly examined for ovarian and non-ovarian adhesions according to scores estimated by Shokeir et al. (2008) during re-laparotomy after 30 days. Ultrasound images of ovarian length and width were collected prior to injection of $CaCl_2$, immediately after injection and one month later. The ovarian diameter was estimated according to the formula described by Frattarelli et al. (2002), where: Ovarian diameter (D) = length (L) + width (W) /2, in cm.

Ovarian samples were collected at day 30, and rapidly preserved in 10% neutral buffered formalin for further processing (Bancroft et al., 2013) and descriptive tissue analysis. All data are subjected to statistical analysis using t-test and the values are expressed as M±SD.

3. RESULTS

The injectable $CaCl_2$ appeared as crystalized fluid within the ovary with transparent fibrous membrane indicating slow distribution of the fluid post injection (Fig., 3a). One cat showed post injection hematoma formation (Fig., 3b).

The Cauterization technique proved easy applicable, and there were no intra-abdominal thermal injuries secondary to ovarian electro-coagulation.

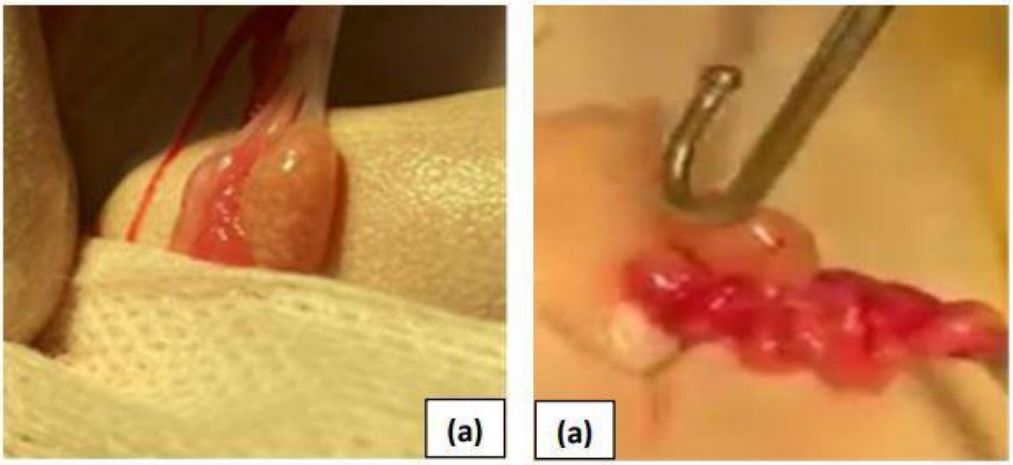
Operative time

The M±SD values of time of the chemical injection (0.86 ± 0.41 mins) was significantly shorter compared with the values of time required for cauterization (3.0 ± 1.0 mins) (Table, 1).

Intra operative complications

No major intra-operative complications were observed. One cat of that underwent cauterization showed the first bleeding score (minor bleeding) characterized by few residual drops of blood remaining in the left ovarian pedicle during firing and stopped immediately and did not require hemostatic intervention.

Figure (3): Showing crystalized fluid with transparent membrane snd post injection hematoma formation



All cats before operations exhibited normal estrous cycle signs; loud vocalizing, elevating the hindquarters (lordosis), when stroked along the back or spine, raise their rear quarters into the air, rolling on the floor and swelling of the vulva. After the operation, one cat subjected to $CaCl_2$ injection showed mild estrous signs, just vocalization.

Estrogen level

Values of serum estradiol following chemical and cauterization showed significant difference between day 0 and day 30 (Table, 2).

During re-laparotomy examination, two cases showed score (1) adhesion; one following cauterization and one following chemical injection, characterized by thin filmy adhesions involved peri-ovarian tissues. Adhesions were easily dissected and removed (Table, 3 and Fig., 4a&b).

The only noticeable changes following cauterization were in the shape and color of the ovarian surface that appeared reduced in size (Fig., 5a&b).

The extent of the ovarian damage was not detected macroscopically; therefore, the histological results were necessary. Following chemical injection, no gross macroscopic changes like encapsulation or calcified material were noticed in the ovarian or the related peri-ovarian structures in the injected cats.

Table (1): Showing M ± SD values of operative time (mins) in both techniques in female cats

Technique	Time (mins)	
	Min – Max	M ± SD
Cauterization	2.0 – 4.0	3.0 ± 1.0^{a}
Chemical injection	0.40 – 1.20	0.86 ± 0.41^{b}

M= Mean, SD= Standard deviation, Min= Minimum, Max= Maximum, mins= minutes
Means carrying different superscript small letter on the same column are significantly different (P<0.05).

Table (2): Showing M ± SD values of estradiol (pg/ml) in female cats before and after application of different ovariectomy techniques

Techniques	day 0	Day 30	t-value	significance
Cauterization	47.2 ± 13.81^{a}	7.4 ± 3.02^{c}	6.044	0.02
$CaCl_2$ injection	24.6 ± 13.41^{a}	9.63 ± 8.02^{b}	1.44	0.285

Means carrying different superscript small letter on the same row are significantly different (P<0.05).

Table (3): Showing post-operative adhesions in both techniques

Technique	adhesions			
	No.	Score	Features	Dissection
Cauterization	1	1	Thin film adhesions	Possible
Injection	1	1	Thin film adhesions	Possible

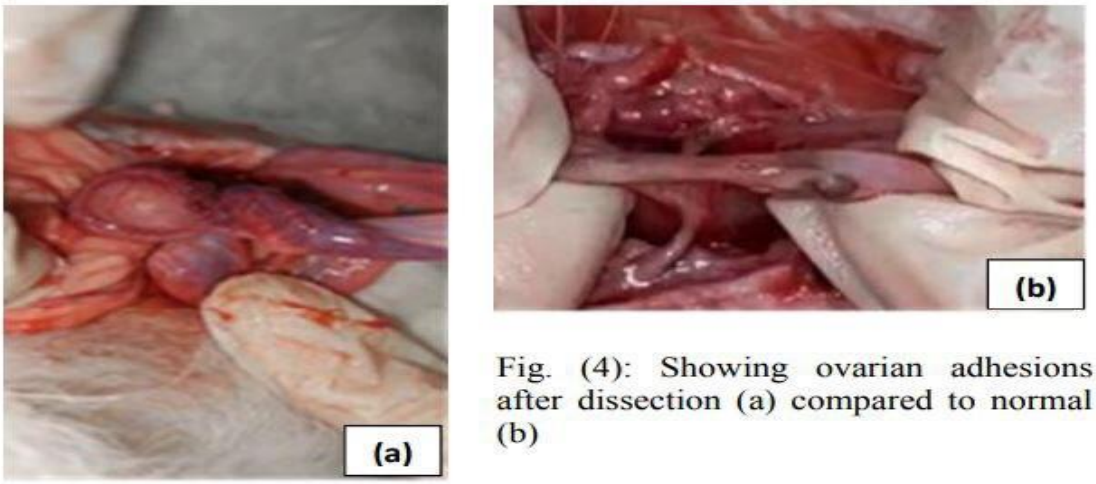


Fig. (4): Showing ovarian adhesions after dissection (a) compared to normal (b)

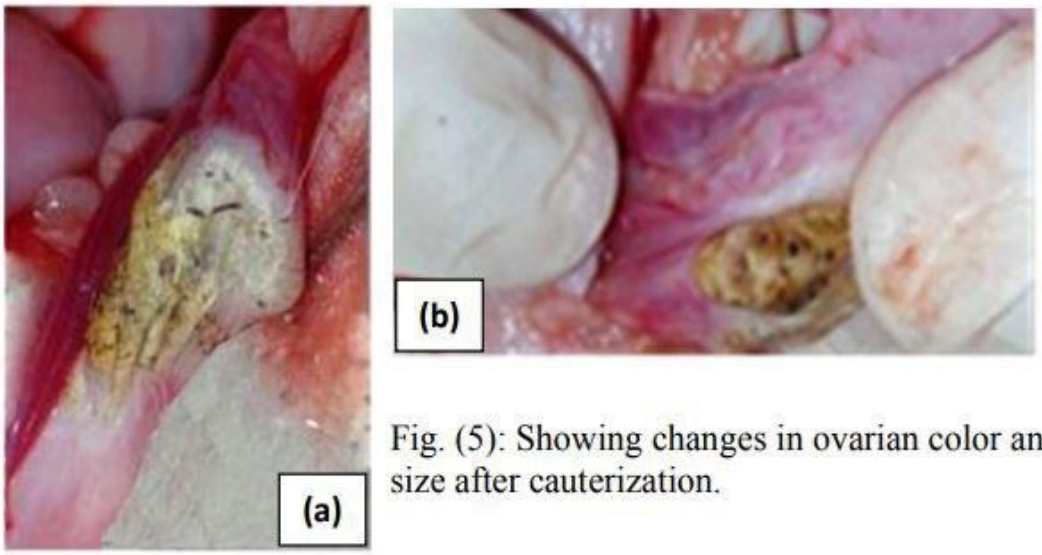


Fig. (5): Showing changes in ovarian color and size after cauterization.

Table (4): Showing M ± SD values of ovarian dimensions (cm) following $CaCl_2$ injection in female cats at different time points

Techniques	Ovarian dimensions (M ± SD)		
	Length	Width	Diameter
Before injection	0.371 ± 0.07^{a}	0.73 ± 0.22^{a}	0.55 ± 0.13^{a}
Immediately after injection	0.81 ± 0.43^{b}	1.27 ± 0.23^{b}	1.04 ± 0.19^{b}
30 days after injection	0.372 ± 0.21^{a}	0.73 ± 0.50^{a}	0.56 ± 0.34^{a}

Means carrying different superscript small letter on the same column are significantly different (P<0.05)

Ultra-sonographic measurements

Ovarian dimensions based on ultrasound images before and after ovarian injection showed significant changes. There was significant increase in the mean values of ovarian dimensions ($P<0.05$) immediately after injection compared to those before injection (Table, 4).

Histological findings

Large areas of connective tissue stroma with interstitial glandular cells, severe neutrophilic and eosinophilic infiltration were detected for electro-coagulated ovaries (Fig., 6).

While, chemically injected ovary, showed hemorrhage and vascular congestion, areas of necrotic and fibrotic tissue, degenerative oocytes with vacuolization in ooplasm and ganulosa cells (Fig., 7).

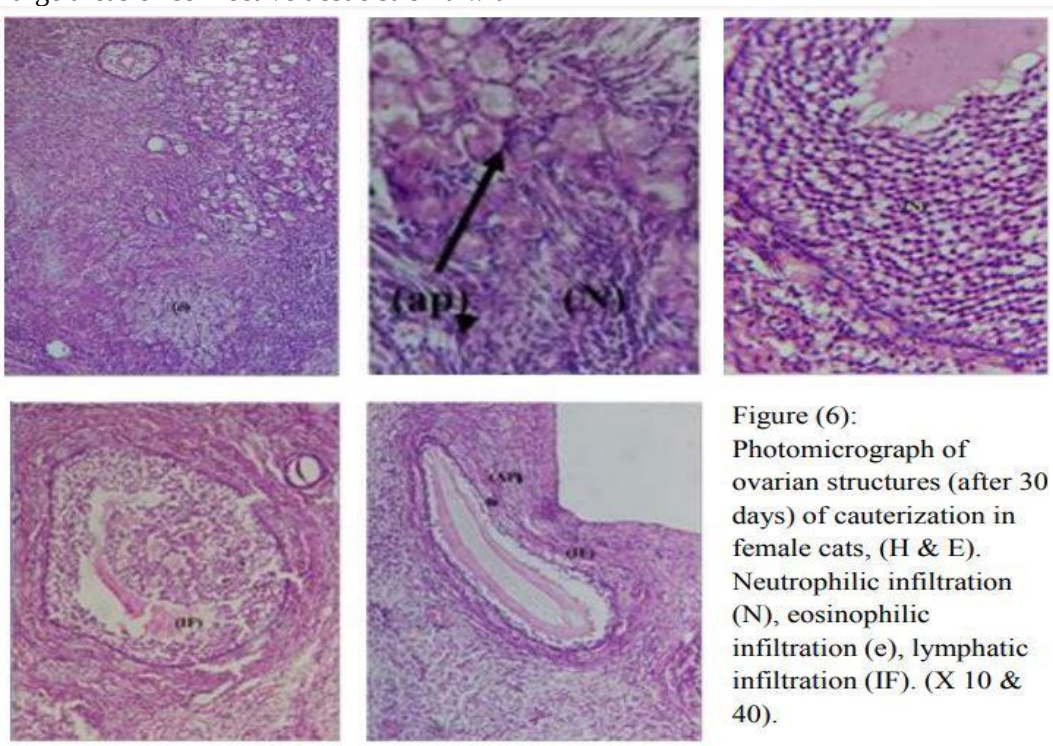
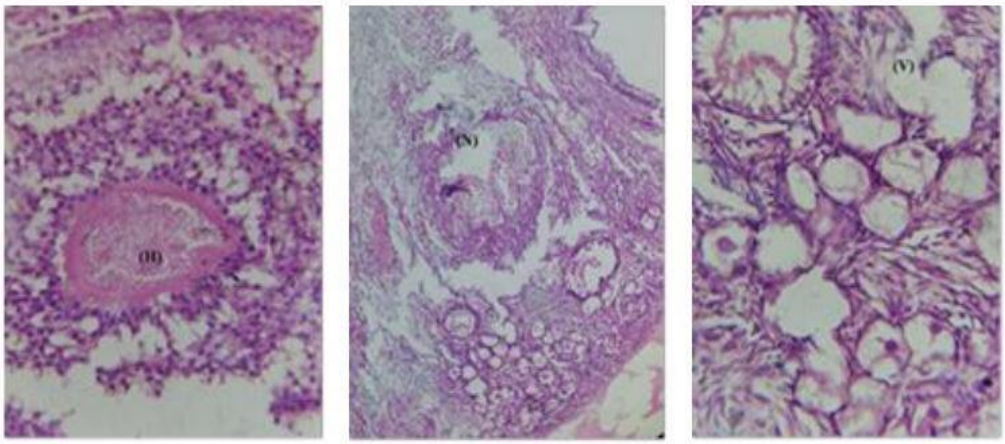


Figure (6): Photomicrograph of ovarian structures (after 30 days) of cauterization in female cats, (H & E). Neutrophilic infiltration (N), eosinophilic infiltration (e), lymphatic infiltration (IF). (X 10 & 40).

Figure (7): Photomicrograph of ovarian structures (after 30 days) of $CaCl_2$ injection in female cats (H & E). Hemorrhage (H), necrosis (N). (X 10 & 40).



4. DISCUSSION

Even though there are many different techniques used for fertility control including surgical and non-surgical, and although ovariectomy is better than ovariohysterectomy in terms of fewer associated complications. Therefore, among this investigation, three techniques for "in situ ovariectomy" are proposed in female cats for induction of direct or indirect ovarian damage, hypothesizing no or minimal complications.

Appearance of crystalized solution in the ovarian tissues following injection of the 20% of $CaCl_2$, indicated slow distribution, and differed than findings of Cavalieri and Hayes (2017) who observed small areas of mineralization in histopathological analysis in cattle and rat ovaries after intra-ovarian administration of $CaCl_2$.

Intra-operative complications showed no significant variation among the operated cats, and the identified complications were those typically associated with Van Goethem et al. (2006) and Dupré et al. (2009). Adhesion formation was not surprising as many authors detected adhesions between ovaries and uterine tubes with surrounding organs. Donesky and Adashi (1995); Amer et al. (2002) reported that the risk of postoperative adhesions cannot be ignored. Hendriks et al. (2010) found that the greater the ovarian surface damage, the higher the risk of peri-ovarian adhesions. There was no encapsulation of calcified material observed periovarian in cats following chemical injection with $CaCl_2$. Meanwhile, Imudia et al. (2008) identified $CaCl_2$ as a foreign body in the abdomen predisposing to the development of adhesions due to leakage of the injected content while using transcutaneous ultrasound-guided approach in female rats. A possible explanation is that the cat ovary is surrounded by ovarian bursa which efficient in restraining the chemosterilant within the ovary compared to the female rat ovary which is encapsulated by a thin membrane that may not be efficient in restraining the chemosterilant within the ovary (Zhang et al., 2013).

The significant difference between the two techniques in the hormonal level indicated that the reduction after ovarian cauterization could be attributed to the extent of ovarian damage coinciding with the findings of Kabakci et al. (2020) who confirmed a reference range for serum estradiol in queen in different estrous phases as; <15 pg/mL for a spayed cat, <20 pg/mL for pregnant queen or queen in diestrus, and 25-50 pg/mL for queen in pro-estrus or estrus. These reference ranges confirm why the estrous signs were variable after injection and cauterization technique.

According to the histological findings, both of cauterization and injection were not satisfactory. They were insufficient to distribute their effects and spread deep into the whole ovary and blood supply to the ovary has not been completely cut off. A somewhat similar findings varied between signs of diminished ovarian reserve after ovarian drilling (Sundus and Tulandi, 1997; Kandil and Selim, 2005; Hendriks et al., 2007; Mrazguia et al., 2007; Weerakiet et al., 2007; Vizer et al., 2007); ovarian atrophy, which was probably related to disruption of the blood supply (Dabirashrafi, 1989); or extensive destruction of the ovary, especially after bipolar electrocoagulation (Hendriks et al., 2010). Vascular congestion with considerable fibrosis in the ovarian cortex, large areas of connective tissue stroma with severe neutrophilic and scattered eosinophilic infiltration may be due to partial damage of ovarian tissue or to degeneration that may have released large amounts of chemotactic factors responsible for the ingression of leucocytes (Heath and Arowolo, 1987). Hemorrhage and areas of necrotic and fibrotic tissue, degenerative changes may be due to osmotic injury from the hypertonic solution. Koger (1976) and McGinnis et al. (1999) reported that the degenerative changes associated with injection of $CaCl_2$ may be due to the necrotizing properties of calcium chloride. The efficacy of calcium chloride in inducing sterilization was supported by the necrosis of the ovarian cells, along with the significant fibrosis (Johnson, 1997 and Immegart et al., 2000).

It is therefore cleared that the insufficient effect of cauterization may be attributed to little number and shallow punctures, not so deep enough to suitable extent. The insufficient effect of $CaCl_2$ injection may be due to unsuitable dose or concentration or less distribution of the chemical. It could conclude that ovarian diathermy using monopolar electro-coagulation seems to be feasible and safe. The ovaries were cauterized without major complications, and 40W thermal energy resulted in incomplete thermal lesions. As compared with the chemical injection technique, the safety and feasibility of cauterization technique proved advantageous.

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