

Reduction of the Incidence of Retained Placenta in Cows Treated with a New Chinese Herbal Medicine *Dang Hong Fu* used as Aqua-acupuncture at GV-1

Chao-ying Luo DVM, Jin-yu Li DVM, Jian-hua Wang DVM, Ji-fang Zheng DVM, Lan-ying Hua DVM, Zhen-ying Hu DVM, Dong-sheng Wang DVM, Yong-jiang Luo DVM, Hai-yan Kou DVM, Rui Wang DVM

ABSTRACT

The objective of this study was to develop a new Chinese herbal aqua-acupuncture formulation *Dang Hong Fu*, test its safety in rabbits and evaluate its efficacy to prevent retained placenta in cows when injected into acupoint GV-1. *Dang Hong Fu* was administered intraocularly, subdermally, intraperitoneally and intravenously into rabbits and no adverse reactions were observed, except transient swelling of the ear flap after subdermal injection. One hundred and twenty four pregnant cows were selected to be in an untreated control group and observed after calving to determine the retained placenta rate for the farm. Fifty pregnant cows from the same farm were selected for the study and randomly assigned to two groups: 30 cows in the *Dang Hong Fu* group and 22 cows in a saline control group. Immediately after calving 40 ml of *Dang Hong Fu* (40 grams of dried herbs) were injected into GV-1 in the herbal group and 40 ml of physiological saline were injected at the same site in the saline control group. Both groups were observed for retained placentas and the time until placental expulsion was recorded in the others. The retained placenta rate for untreated cows that received no treatment was 35.5% (44/124). The incidence of retained placenta in the *Dang Hong Fu* group was 16.7% and in the saline control group 31%. The time for expulsion of placental membranes was a mean of 9 hours (range 3.5-24 hours) in the *Dang Hong Fu* group and a mean of 14.7 hours (range 3.0-24 hours) in the saline control group. When compared to the untreated control group, *Dang Hong Fu* aqua-acupuncture significantly reduced the incidence of retained placentas ($p = 0.047$; < 0.05), but saline aqua-acupuncture did not ($p = 0.740$; > 0.05). Herbal aqua-acupuncture may offer an easy treatment method to reduce the incidence of retained placenta with no adverse side effects.

Key words: *Dang Hong Fu*, herbal aqua-acupuncture, acupoint-injection, acupuncture, GV-1, *Hou-hai*, retained placenta, cows, Chinese herbal formula

When Chinese herbal medicine is injected into acupoints, the herbal dosage may be reduced by 20-50%, the clinic results may be improved by 10%-20%, the treatment time may be shortened and the duration of its effectiveness may be extended.¹⁻³ The injection of Chinese herbal medicine into acupoints provides a new direction for veterinary acupuncture application and has the potential to reduce the side-effects of some Chinese herbal medicines and the chance of medical abuse in clinical practice.

From: Lanzhou Institute of Animal & Pharmaceutics Science, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu, China (Luo C, Li, Zheng, Hua, Hu, Wang, Luo Y) and the Pasturage Company, Xian Modern Agricultural Corporation, Xian, Shaanxi, , PR China (Wang J, Kou, Wang R)

Funded By: The emphases Item of 9th 5-year-programs of the agricultural ministry, P. R. China (9th 5-animal science-02-03-13)

The acupoint GV-1 (*Hou Hai* or *Jiao Cao*) is located in the depression halfway between the anus and the ventral aspect of the caudal (coccygeal) vertebrae. Since GV-1 is one of the standard acupoints used for the treatment of retained placenta, it has been selected as one of the injection sites for Chinese herbal injectable formulations and vaccinations.¹ Using different ingredients and processing methods, seven Chinese herbal injectable preparations were initially developed by the authors for aqua-acupuncture treatment of retained placenta in cows, but only four had acceptable diaphaneity (transparency) and precipitation qualities. When 20 ml of the four preparations were injected into GV-1 of test cows with retained placenta, there was no effect. When 40 ml of the preparations were injected into GV-1, one preparation named *Dang Hong Fu* had the best

effect and it was chosen to be tested for safety and then used in a clinical trial of cows to prevent retained placenta.

Dang Hong Fu is a modification of the classical formula *Sheng Hua Tang*, which was recorded in *Fu Qing Zhu Nü Ke* (Women's Diseases According to *Fu Qingzhu*) written in 1827 and is a very popular postpartum uterine cleansing formula for both women and domestic animals in China.^{4,5}

Dang represents *Dang Gui* (Radix Angelicae Sinensis), *Hong* represents *Hong Hua* (Flos Carthami), and *Fu* means blessing or good fortune. *Dang Gui* can nourish the Blood, stimulate circulation, and regulate menstruation to alleviate pain. *Hong Hua* (Flos Carthami) also has the function of boosting Blood circulation and regulating menstruation. These two primary ingredients are assisted by two secondary ingredients *Yi Mu Cao* (Herba Leonuri Japonici) and *Huang Qi* (Radix Astragali Mongolici). *Yi Mu Cao* (Herba Leonuri Japonici) promotes Blood circulation to restore menstrual flow and *Huang Qi* (Radix Astragali Mongolici), invigorates Qi, strengthens the body, expels pus and releases toxins. These effects are further enhanced by two peripheral herbs *Pao Jiang* (Dried Ginger) which warms the Channels and *Che Qian Zi* (Semen Plantaginis) which controls bleeding. *Che Qian Zi* (Semen Plantaginis) expels Dampness and relieves fever. All these herbs work together to resolve Blood deficiency, Qi Stagnation, Blood Stasis and Cold and Damp coagulation, and thus provide an effective treatment for retained placenta. *Dang Hong Fu* showed promise as a treatment to prevent retained placenta in cows with no evidence of toxicity but, a systematic study was needed.

The objective of this study was to evaluate the safety of the injectable form of *Dang Hong Fu* in rabbits and then inject it into GV-1 in an experimental test group of cows after calving from the same farm and compare the incidence of retained placentas and the time of placental expulsion with a control group receiving physiological saline and a control group of cows receiving no treatment.

MATERIALS AND METHODS

The ingredients of the Chinese herbal formula *Dang Hong Fu* are: *Dang Gui* (Radix Angelicae Sinensis), 30%; *Hong Hua* (Flos

Carthami), 15%; *Yi Mu Cao* (Herba Leonuri Japonici), 20%; *Huang Qi* (Radix Astragali Mongolici), 15%; *Pao Jiang* (Prepared Dried Ginger) 10% and *Che Qian Zi* (Semen Plantaginis) 10%^a. These ingredients were mixed and decocted with water for 30 minutes the first time and 20 mins the second time, and then distilled^{b,c} with alcohol^d. A small amount of Tween-80^d was added to increase solubility. The herbal liquid was bottled in 20 ml ampoules (containing 20 grams of the herbal mixture), sterilized, numbered and labeled (Figure 1). Diaphaneity (transparency) and precipitation of the prepared liquid was examined visually without magnification, under sunlight or lamp light, against a black background and while the ampoules were rotated slowly. This examination was performed once per week for 6 months and then once a month for two years to ensure the solution was stable.

Twelve (6 male and 6 female) pure-bred Japanese White Rabbits, weighing 2-3 kg, were used for the toxicity studies. They were clinically healthy and vaccinated against rabbit hemorrhagic disease

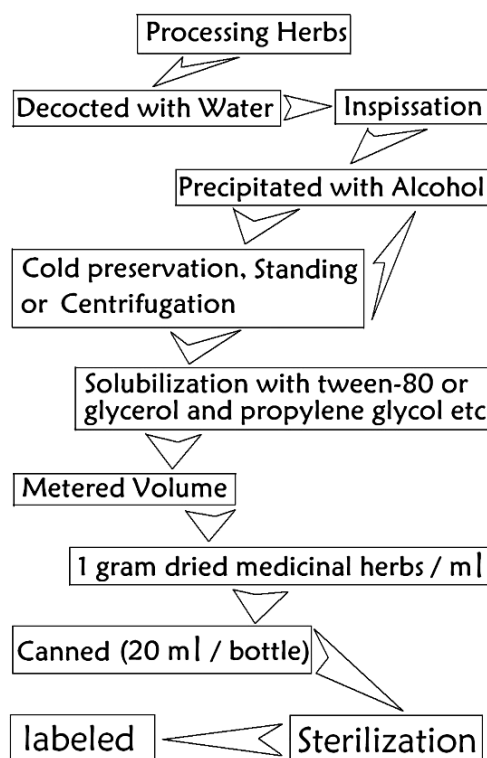


Figure 1: Flow chart of the herbal preparation

and *Pasteurella multocida*. They were housed at 15-18 degrees centigrade with both natural and lamp lighting for illumination. Before the experiments, the rabbits were observed for one week to insure they were being maintained under healthy conditions.

Local sensitivity to the *Dang Hong Fu* injectable solution was evaluated by placing drops on the rabbits' eyes and injecting the solution subdermally in the ear flap and intramuscularly in the quadriceps femoris muscle. Physiological saline was placed onto the right eyes of three rabbits and liquid *Dang Hong Fu* solution was dropped onto the left eyes of the same rabbits and the reactions in the left and right eyes were compared for two days. Three rabbits received 1 ml of saline subdermally in the left ear flap and 1 ml of the injectable solution of *Dang Hong Fu* subdermally in right ear flap and the reaction in the two sides were compared for two days. One ml of physiological saline and one ml of the injectable solution of *Dang Hong Fu* was injected into the left and right quadriceps femoris muscle respectively in the rabbits and local muscle reactions were compared for each site for 2 days.

The other six rabbits were administered 2 ml of the *Dang Hong Fu* injectable solution into the peritoneal cavity every other day 3 times. The rabbits were then divided into two groups, three each. The three rabbits in the first group received 5 ml of the *Dang Hong Fu* injectable solution via jugular vein injection on the 14th day after the intraperitoneal injection. The second group of 3 rabbits received 5 ml of the *Dang Hong Fu* injectable solution via jugular vein injection on the 21st day after the intraperitoneal injection. The rabbits were observed for evidence of anaphylaxis. The rectal temperature of the rabbits was taken 3 hours after the injection to monitor for a pyrogenic effect.

Fifty-two Black-and-White Dairy cows of China, 2-4 years old, 400-800 kg body weight with fairly complete clinical records, were selected for the clinical trial. Cows used in this study were, clinically healthy, and had previously calved with an incidence rate of retained placenta of approximately 35%. They were brought in from the Second, Fourth and Fifth Farms of the Pasturage Company, Xian Modern Agricultural Corporation. Sequenced according to the time they last calved, odd number and even number cows were divided

into two groups, 30 cows in the experimental *Dang Hong Fu* group and 22 cows as the saline control group. To serve as a non-treatment control group, one hundred and twenty four pregnant cows of similar type and ages, from the same farms, were used to determine the number of retained placentas and serve as a non-treatment control group for comparison.

Eighteen gauge 10 cm hypodermic needles were used to inject either the *Dang Hong Fu* solution or physiological saline into GV-1. Individual autoclaved sterilized needles were used for each cow and separate sterile syringes were used for *Dang Hong Fu* or physiological saline solutions so no contamination was possible. The needle was inserted into the depression between the anus and ventral caudal vertebrae and was directed upward and forward 5-7 cm parallel to the dorsal keel. The *Dang Hong Fu* or physiological saline solution was slowly injected while withdrawing the needle.

In the experimental (test) group, 40 ml *Dang Hong Fu* solution (40 grams dried medicinal herbs) was injected into GV-1 immediately after calving. In the control group, 40 ml physiological saline was injected into GV-1 immediately after calving. All of cows were observed for 24 hours. The placenta was considered retained, if it was not expelled or only partially expelled in 24 hours. The incidence rate of retained placenta was recorded for each group. The time it took the placenta to be expelled was also observed and recorded. The time began immediately after calving and ended when the placenta was completely expelled and was recorded in hours. Cows who failed to expel the placenta within 24 hours after calving were treated with other therapies and the time of expulsion was measured by days.

The incidence of retained placenta for *Dang Hong Fu* treated, saline treated and untreated groups was compared using Chi-square tests of crosstabs^d. The *p* value was set at < 0.05 as a significant difference between the groups. The mean time until placental expulsion for the *Dang Hong Fu* and saline GV-1 aqua-acupuncture groups were compared using the T test for independent samples^e. The *p* value was set at < 0.01 as a significant difference between the groups.

RESULTS

Physiological saline resulted in no tearing,

irritation or hyperemia of the conjunctiva in the rabbits. The *Dang Hong Fu* injection solution caused mild tearing (1 drop of tears), but no conjunctival irritation or hyperemia. There was no swelling when saline was injected subdermally in the ear flap, but the *Dang Hong Fu* injection solution caused mild local swelling, which may have been due to the high infiltration pressure, because it was gone in 2 hours (Tables 1 and 2). When injected into the quadriceps femoris muscles of the rabbits, neither the saline nor the *Dang Hong Fu* injection solution caused any local inflammatory reaction within 24 hours.

Within three hours after intravenous injection of the *Dang Hong Fu* injection solution, the maximum increase in body temperature in the three test rabbits was 0.3°C, 0.4°C and 0.5 °C, all below 0.6 °C. These results indicate that the *Dang Hong Fu* injectable solution complies with the pyrogen test standards of safety^f. No anaphylactic reactions were observed in any of the rabbits following intravenous saline or *Dang Hong Fu* injections. No toxicity or reactions at the injection site were observed in the cows in this study.

The results of the study are outlined in

Tables 3 and 4. The incidence of retained placenta in the 124 untreated calved cows was 44/124 (35%). In the group of 30 cows injected with *Dang Hong Fu* solution at GV-1, 5/30 (16%) had a retained placenta and in the group of 22 cows injected with physiological saline at GV-1, 7/22 (31%) had a retained placenta. There was a significant reduction in the incidence of retained placentas in cows receiving *Dang Hong Fu* aqua-acupuncture at GV-1 as compared to cows receiving no treatment (p value was 0.047; < 0.05) (Figure 2). There was no significant reduction in incidence of retained placentas in cows receiving saline aqua-acupuncture at GV-1 as compared to cows receiving no treatment (p value was 0.740; > 0.05) (Figure 2). There was no significant difference in the incidence of retained placenta in cows receiving saline aqua-acupuncture at GV-1 as compared to cows receiving *Dang Hong Fu* aqua-acupuncture at GV-1 (p value was 0.200; > 0.05) (Figure 2).

In the group of 30 cows injected with *Dang Hong Fu* solution at GV-1, the placenta was expelled in a mean time of 9 ± 7.06 hours (range 3.5-24 hours) and for the group of 22 cows injected

Table 1: The local swelling size of the rabbit ear flaps over time after subdermal injection of *Dang Hong Fu* injectable solution

Rabbit	<i>Dang Hong Fu</i> injectable solution (swelling size mm×mm over time)					
	0 min	20 min	40 min	60 min	80 min	120 min
1	16×10	20×15	23×18	20×20	16×10	0
2	15×10	17×15	20×20	21×22	25×10	0
3	15×10	20×20	23×25	15×15	10×10	0
Mean	15×10	18×17	22×21	19×19	15×10	0

Table 2: The local swelling size of the rabbit ear flaps over time after subdermal injection of physiological saline solution

Rabbit	Physiological saline (swelling size mm×mm over time)					
	0 min	20 min	40 min	60 min	80 min	120 min
1	15×10	13×8	11×5	0	0	0
2	15×10	13×10	10×5	0	0	0
3	15×10	13×9	9×4	0	0	0
Mean	15×10	13×9	10×5	0	0	0

Table 3: The number and incidence of retained placentas for untreated cows and cows treated with aqua-acupuncture at GV-1 with either 40 ml *Dang Hong Fu* solution or physiological saline

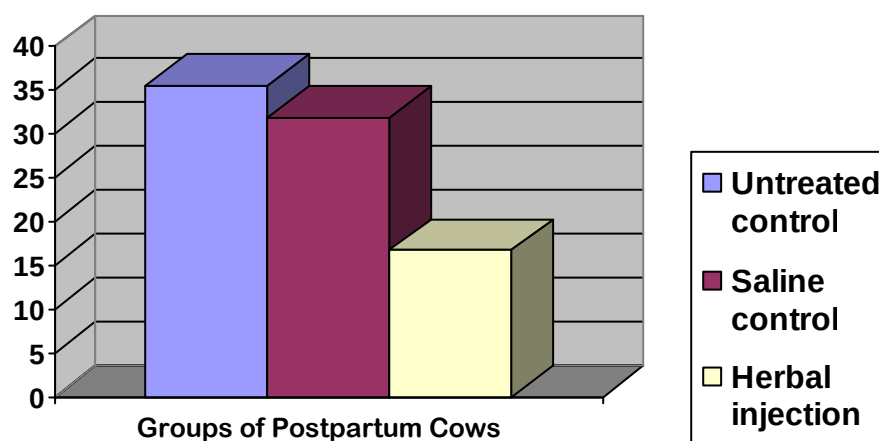
	Untreated Group	<i>Dang Hong Fu</i> GV-1 Aqua-acupuncture Group	Saline GV-1 Aqua-acupuncture Group
Cows (n)	124	30	22
Retained placenta (n)	44	5	7
Retained placenta (%)	35.5% ^{a,b}	16.7% ^{a,c}	31.8% ^{b,c}

^a significantly less in the *Dang Hong Fu* group as compared to the untreated control group (p value 0.047; < 0.05); ^b no significant difference between the saline treated control group and the untreated control group (p value 0.740; > 0.05); ^c no significant difference between the saline treated control group and the *Dang Hong Fu* herbal injection group (p value 0.200; > 0.05).

Table 4: The time of placental expulsion after injection of GV-1 with 40 ml of *Dang Hong Fu* solution versus physiological saline

	<i>Dang Hong Fu</i> GV-1 Aqua-acupuncture Group	Saline GV-1 Aqua-acupuncture Group
Placental expulsion time (range; hours)	3.5-24	3.0-24
Placental expulsion time (mean; hours)	9.0 $\pm$ 7.06 ^a	14.7 $\pm$ 6.97

^a significantly less placental expulsion time in the *Dang Hong Fu* group compared to the saline control group (p value = 0.005; < 0.01)

**Figure 2:** A comparison of the percentage of retained placentas (vertical axis) in the untreated control group, the saline GV-1 aqua-acupuncture control group and the *Dang Hong Fu* GV-1 aqua-acupuncture group.

The percentage of retained placentas was significantly less in the *Dang Hong Fu* herbal injection group as compared to the untreated control group (p value 0.047; < 0.05), but no significant difference was found between the saline treated control group and the untreated control group (p value 0.740; > 0.05) or the saline treated control group and the *Dang Hong Fu* herbal injection group (p value 0.200; > 0.05)

with physiological saline at GV-1, the placenta was expelled in a mean time of 14.7 ± 6.97 hours (range 3.0-24 hours). Compared to saline injected at GV-1, *Dang Hong Fu* solution injected at GV-1 significantly reduced the placental expulsion time (p value was 0.005, < 0.01).

DISCUSSION

Intervet and Schering-Plough Animal Health, Netherlands, defines retained placenta as the disease in which a cow cannot expel the fetal membranes within 12-24 hours after calving.⁶ In this study the placenta was considered retained if it did not completely expel within 24 hours. The incidence of retained placenta ranges from 3 to 39% because of variations in the definition of this disease. In 1987, an incidence rate of 17.8% (1517/8521) was reported in Israel.⁷ In 1996, in a United States Department of Agriculture study, the reported incidence of retained placenta was $7.8 \pm 0.2\%$.⁸ In 2002, the United States National Animal Disease Center in Ames, Iowa reported an incidence rate of 17.9% and the dairy farm at Iowa State University reported an incidence rate of 12.6%.⁸ In 2008, an incident rate of 16.55% (240/1450) was reported in Croatia.⁹ In China, the incidence rate has been reported to be 20-50%.¹⁰⁻¹² Since, the incidence of retained placenta varies by region and also by farm, the incidence of retained placenta from the same farm was used (35.5%) to compare with *Dang Hong Fu* experimental and saline control aqua-acupuncture groups.

It has been well recognized that placental retention can be associated with abortion, stillbirth, twins, dystocia, induction of parturition with PGF2-alpha, metabolic disorders such as milk fever and infections such as brucellosis, leptospirosis, vibriosis, listeriosis, and infectious bovine rhinotracheitis.⁶ In China 88% of the cases lead to endometritis, delayed estrus or permanent sterility of the cows.¹⁰⁻¹² Retained placenta also decreases milk production.¹³ In the United States, the financial loss has been estimated to be \$328/per case.¹⁴ Based on the incidence reports, it is clear that retained placenta is one of the major post partum problems that threatens the health of dairy cows and negatively impacts the milk production industry. Therefore, effective prevention and treatment are essential for both conventional and Traditional Chinese Veterinary Medicine (TCVM).

In this study, it was found that when 40 ml of

Dang Hong Fu solution (40 grams dried herbs) was injected once into the acupoint GV-1 immediately after calving, the incidence of retained placenta was significantly reduced as compared to cows receiving no treatment. Since there was no reduction of the incidence rate of retained placenta in the saline aqua-acupuncture group, compared to the non-treated group, it can be concluded that the decrease in incidence of retained placenta was due to the herbal aqua-acupuncture rather than mere stimulation of the GV-1 acupoint. These clinical experiences of the authors indicate that herbal aqua-acupuncture is superior to saline aqua-acupuncture or no aqua-acupuncture to, not only reduce the incidence of retained placentas in a herd but also decrease the placental expulsion times. The reduction in the rate of retained placenta was clearly shown between the herbal aqua-acupuncture and no treatment experimental groups but not significant between the herbal aqua-acupuncture and saline aqua-acupuncture groups.

Historically, saline aqua-acupuncture has been used for a variety of conditions with therapeutic effects.¹⁸ Since there were a relatively small number of cases in the saline group (22 cows) as compared to the untreated control group (124 cows), this may have negatively skewed the data. If a larger number of cows were treated with GV-1 saline aqua-acupuncture or GV-1 *Dang Hong Fu* aqua-acupuncture and evaluated for retained placenta, there might have been a greater difference between all 3 groups or the GV-1 *Dang Hong Fu* aqua-acupuncture group shown to be superior to saline aqua-acupuncture. However, even with only 30 cows in the GV-1 *Dang Hong Fu* aqua-acupuncture group as compared to 124 in the untreated control group, there was a significant difference between the 2 groups. As well there was a significant reduction in placental expulsion time as compared to the GV-1 saline aqua-acupuncture and both of these findings support the use of GV-1 *Dang Hong Fu* aqua-acupuncture in the clinical setting.

Data was not available regarding the time of placental expulsion after calving for the non-treated control group, so this could not be compared with the treatment groups. The average duration of complete placental expulsion was significantly less in cows with GV-1 *Dang Hong Fu* aqua-acupuncture as compared to GV-1 saline aqua-acupuncture, suggesting that there is a definite

advantage to herbal aquapuncture to ensure earlier placental expulsion. Although there are reports concerning treatment of many diseases with physiological saline aqua-acupuncture, Chinese herbal aqua-acupuncture may prove to be more effective for the treatment of diseases as well. Herbal aqua-acupuncture combines acupoint stimulation and the pharmacologic actions of the herbs.¹⁸ Injection of Chinese herbs into acupoints requires a lower dose of herbs and is easier to administer than oral forms.¹⁵⁻¹⁷

It is impossible to prevent and treat all retained placentas with one formula or one therapy, as the causes of retained placenta vary and are complex and the underlying TCVM patterns differ.¹⁵ Variations of the *Dang Hong Fu* acupoint injection formula may be needed for different disease patterns to improve clinical outcomes. Combining *Dang Hong Fu* acupoint injections with conventional treatments may provide even better results than either treatment alone. Further studies on the effects of Chinese herbal injections used as aqua-acupuncture are needed.

FOOTNOTES

^aHerbs obtained from *Lan Zhou* Medical Materials Company (Western Station), China

^bDistillation equipment: EYELA N-1001 rotary evaporator, EYELA CCA-111 water recirculation chiller and EYELA A-1000S water-jet air ejector, made in Japan

^cGlass instruments for distillation, made by Glass Instrument Manufacturing Factory of Tianjin, China.

^d95% alcohol, Tween-80, glycerol and propylene glycol etc, produced by Tianjin Medical Reagent Company and Shanghai Medical Reagent Company, distributed by Ai Hua Company of Lanzhou University, China.

^eStatistical package for the social science (SPSS) 15 for Windows, IBM

^fAs determined by the Pharmacopoeia of the People's Republic of China, the Second Part, Addendum. China: Chemical Industry Press 2005:85 (in Chinese)

REFERENCES

1. Chu X, Shi KJ. The recent developments on animal acupoint immunity in China. *J Chinese Vet Med* 1996; 22 (5):50-51. (In Chinese)
2. Shao ZY, Ling X, Zhou AL. The experimental studies of pharmacology on a similar character of semiconductor of acupoints. *J Chinese Acupuncture and moxibustion* 1998; (20):121-124. (In Chinese)
3. Luo CY. A New Trend of Acupoint Injection. *J Trad Chinese Vet Med* 2000; (5):16-18. (In Chinese)
4. Luo YK. *Gynaecology of TCM*. Shanghai, China: Shanghai Scientific & Technical Publishers 1986: 135. (in Chinese)
5. Institute of TCVM, CAAS & Lanzhou institute of Vet Med, CAAS. *A New Compilation of TCVM*, Lanzhou, China: People's Publishers of Gansu 1979:193. (in Chinese)
6. Intervet, Schering-Plough Animal Health. Retained placenta in cattle-pathogenesis and consequences. 2009. <http://www.partners-in-reproduction.com/reproduction-cattle/retained-placenta-cattle.asp>.
7. Markusfeld O. Periparturient traits in seven high dairy herds. Incidence rates, association with parity, and interrelationships among traits. *J Dairy Sci* 1987; 70(1):158-166.
8. Kimura K, Goff J P, Kehrl M E. Decreased neutrophil function as a cause of retained placenta in dairy cattle. *J Dairy Sci* 2002; 85 (3):544-550.
9. Krunoslav A, Matija D, Željko P et al. The relationship between diet and retained placenta in cows. *Acta agriculturae Slovenica*, supplement 2; 2008:155-162.
10. Chen ZZ, Shen BQ, Pu YJ. Validation on the curative effect of *Che Qian Zi* (Semen *Plantaginis*) on retained placenta in dairy cows. *J Chinese Vet Med* 1984; 10(8):48-49. (In Chinese)
11. Qu ZM. *A New Edited Therapy of TCVM*. Beijing, China: China Press of Agricultural techniques 1993:201-202. (in Chinese)
12. Zhu JX. Summary on the prevention and cure of retained placenta in dairy cows with TCVM. *J Chinese Vet Med* 1993; (3):19-20. (In Chinese)
13. Lucey S, Rowlands GJ, Russell AM. Short-

- term associations between disease and milk yield of dairy cows. *J Dairy Res* 1986; 53:7–15.
14. Guard C. Set up fresh and milking cows for successful A. I. Hoard's Dairyman. 1999: 8–9.
 15. Yang YC, Zhang YH, Huang GM. Prevention of retained placenta in dairy cows with TCVM. *J. Chinese Vet. Med* 1993; 19 (2):40-41. (In Chinese)
 16. Liu ST, Liu JY, Xu XJ. Prevention and cure on retained placenta with TCVM and its influence on postpartum reproductive functions in dairy cows. *J Chinese Vet Med* 1997; (5):6-8. (In Chinese)
 17. Guo YJ, Guo XP, Liu CS. On the curative effect of *Dang Gui* injection and compound *Danshen* injection injected into acupoint *Bai Hui* and acupoint *Jiao Cao* on retained placenta in dairy cows. *China Dairy Cattle* 2006; (7):33-34. (In Chinese)
 18. Zhang JS. Fifty cases of constipation treated with physiological saline aqua-acupuncture at acupoint *Shen Men* and acupoint *Ke Gang*. *Chinese Journal of Integrated Traditional and Western Medicine*. 1983; (1):28. (In Chinese)

ABSTRACT

Komori M, Takada K, Tomizawa Y, Nishiyama K, Kondo I, Kawamata M, Ozaki M.

Microcirculatory responses to acupuncture stimulation and phototherapy. *Anesth Analg.* 2009 Feb;108 (2):635-40

Department of Anesthesiology, Medical Center East, Tokyo Women's Medical University, 2-1-10, Nishiogu, Arakawa-ku, Tokyo 116-8567, Japan. komorim@nifty.com

Background: Acupuncture stimulation and phototherapy have been reported to have analgesic effects and improve the microcirculation. However, few studies have directly examined changes in peripheral blood vessels, either quantitatively or objectively. We assessed the responses of arteriolar blood flow to acupuncture stimulation and phototherapy under direct vision to examine the effects of these treatments.

Methods: We used 40 rabbits with a rabbit ear chamber attached to the auricle. The rabbit ear chamber was fixed to the auricle under a dissecting microscope. Arterioles were selected and observed with the use of a microscope video camera. Pentobarbital was injected IV. The trachea was intubated and spontaneous respiration was maintained. Rabbits were randomly assigned to receive acupuncture stimulation (acupuncture group, n = 10), near-infrared lamp irradiation (lamp group, n = 10), near-infrared low-powered laser irradiation (laser group, n = 10), or no irradiation (control group, n = 10). In the acupuncture group, an acupuncture needle was placed in the auricle for 20 min. The lamp group repeatedly received 1 s of near infrared irradiation (1540 mW) followed by 4 s of treatment cessation. The laser group continuously received 60 mW of laser irradiation. In the lamp and laser groups, the auricle (same site as that of the acupuncture needles in the acupuncture group) was irradiated for 10 min with a contact probe. Arteriolar diameter and blood flow velocity were measured at baseline and for 60 min after acupuncture or irradiation treatment. Blood flow rate was calculated by multiplying the blood flow velocity by the cross-sectional area of the vessels.

Results: Arteriolar diameter significantly increased to 131% +/- 14% in the acupuncture group ($P < 0.005$), 129% +/- 19% in the lamp group ($P < 0.005$), and 128% +/- 11% in the laser group ($P < 0.005$) when compared with the pretreatment value (100%). Maximum values were reached 20 min after the end of the acupuncture stimulation, and 10 min after the end of lamp and laser irradiation. The three groups showed significant increases in arteriolar diameter when compared with the control group ($P < 0.005$). Blood flow velocity and blood flow rate showed similar trends to arteriolar diameter. Treatment effect persisted for 40-50 min after the end of stimulation and irradiation.

Conclusions: Acupuncture stimulation and phototherapy were directly confirmed to increase the diameter and blood flow velocity of the peripheral arterioles. Acupuncture stimulation and phototherapy, associated with minimal systemic and local side effects, can enhance the microcirculation and may be a useful supportive treatment for diseases caused by poor peripheral blood flow.