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About the Cover

In 2014 the covers of AJTCVM will be blue and the theme water to honor the Water Element.

Min river and Dujiangyan irrigation system

The Dujiangyan is an irrigation system built by the Kingdom of Qin in 256 BC. This famous water way is located in the Min river near Chengdu, the capital of the Sichuan province. Still in use today, the Dujiangyan irrigates over 5,300 square kilometers of land in the region. During the Warring States period (406-221 BC), flooding occurred every spring from melting mountain snow. Li Bing, the Qin Governor at that time, was charged by King Zhao of Qin to find a solution to stop the flooding, yet allow military vessels to pass. It was decided that to achieve this a levee to redirect a portion of the river would be constructed and a channel would be cut through Mount Yulei to redirect excess water to the dry Chengdu plain. The levee was constructed of woven oblong baskets filled with rocks called zhulong and held in place by wooden tripods called macha. The levee was constructed in 4 years. Cutting a channel through the mountain proved more of a challenge, as gunpowder or dynamite had not yet been invented. To penetrate the solid mountain rock a combination of fire and water was used to repeatedly heat and cool the rocks, until they cracked and could be removed. After 8 years, a channel 66 feet wide had been created through the mountain. Not only did the floods stop, but the dry plan became a fertile valley and made Sichuan the most productive agricultural region in China. The people of the region were so happy that they built a shrine on the east side of Dujiangyan to honor Li Bing for eliminating the annual disaster, providing them with a bountiful harvest and allowing them to live a care free life. Unlike modern day dams that are usually constructed with huge walls, the Dujiangyan allows the water, along with the fish, to flow naturally. The Dujiangyan became a United Nations Educational, Scientific and Cultural Organization (UNESCO) World Heritage Site in 2000 and is a popular Chinese tourist attraction.

Errata Corrige

In Table 5 of Wilcox DL, Liu H, Yu Ma et al. Comparison of the Chinese herbal formula Hai Zao Yu Hu Tang and Methimazole for the treatment of feline hyperthyroidism; AJTCVM 4(1) 2009:32, the numbers (n) of the groups were accidentally reversed. The correct number is 11 for the herbal group and 3 for the control (methimazole) group.
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Table of Contents

Errata Corrige ........................................................................................................................................................................... ii

Editorial

Conventional and Traditional Chinese Veterinary Medicine Perspectives of Estrus, Fertility and Infertility ............... 1
Cheryl L Chrisman DVM, MS, EdS, DACVIM, Huisheng Xie DVM, MS, PhD

Traditional Chinese Veterinary Medicine Around the World ............................................................................................................. 7

AAVA Updates ............................................................................................................................................................................ 11

AATCVM Updates ...................................................................................................................................................................... 17

Commentary

Clinical Trials for Acupuncture and Other Traditional Chinese Veterinary Medicine Treatments ............................. 23
Erin Mayo DVM

2014 Reporting Guidelines for Randomized Controlled Blinded Clinical Trials in Traditional Chinese Veterinary Medicine .................................................................................................................................................. 33
Cheryl L Chrisman DVM, MD, EdS, Huisheng Xie DVM, MS, PhD, Aituan Ma PhD, Neal Sivula DVM, PhD, Carolina Medina DVM, Bruce Ferguson DVM, MS

Basic Science Studies

A Randomized, Blinded, Double Controlled Experimental Study of the Effects of Gan Lian Yu Ping Feng on Antibody Titers and Nonspecific Immune Indexes in Chickens Vaccinated Against Infectious Laryngotracheitis Virus ........................................................................................................................................................................ 39
Chunmei Kong DVM, MS, Xiuhui Zhong DVM, PhD, Aituan Ma PhD, Zhujun Zhao DVM

Four Randomized, Controlled and Blinded Studies Comparing the Effects of Cu Yun Guan Zhu Ye and Estradiol on the Vagina, Uterus and Ovaries of Mice .......................................................................................................................................................... 51
Dalu Song DVM, Yuanliang Hu DVM, PhD, Liwen Zhou DVM MS, Baokang Zhang DVM, Xiaohui Chen DVM, Deyun Wang DVM, PhD, Hongxing Wu DVM, Guangliang Cao DVM, Xudong Song DVM, Xiong Shi DVM

Clinical Study

A Randomized and Controlled Study Using an Acupoint Diagnosis and Treatment Instrument to Determine the Phase of Ovarian Follicular Development, Alter Estradiol and Progesterone Release and Improve Pregnancy Rates in Dairy Cows ................................................................................................................................................. 65
Chaoying Luo DVM, Ziwen Lang DVM, Longshan Zhang DVM, Guolin Yang DVM, Jifang Zheng DVM, Jiasheng Xie DVM, Yongjiang Luo DVM, Jinyu Li DVM, Guibo Wang MS, Ruthua Xin MS

Case Series

Treatment of 220 Cases of Bovine Ovarian Inactivity and 209 Cases of Bovine Persistent Corpus Luteum with Cu Yun Guan Zhu Ye .......................................................................................................................................................... 77
Dalu Song DVM, Jingbing Song DVM, Yuanliang Hu DVM, PhD, Baokang Zhang DVM, Liuliang Zhang DVM, Guangliang Cao DVM, Hongxing Wu, DVM Bin Yang DVM, Deyun Wang DVM, PhD
Correlation of Acupuncture Point Sensitivity and Lesion Location in 259 Horses ............................................................ 83

Antonio Alfaro DVM, MSc

Brief Communications

Proliferative Effects of a Polysaccharide Extracted from Xiang Gu (Shiitake) on Cultured Avian Lymphocytes ........... 89
Rongrong Liu MS, Zhenzhen Gao PhD, Yuanliang Hu PhD, Deyun Wang PhD, Baokang Zhang DVM, Yufeng Xu MS, Dong Cai DVM

Pearls from TCVM Practice

Traditional Chinese Veterinary Medicine Diagnosis and Treatment of Acquired Infertility in Female Horses and Dogs ............................................................................................................. 95
Xiuhui Zhong DVM, PhD, Yantao Zhao DVM, PhD

Selected Abstracts

Characteristics of acupuncture treatment associated with outcome: an individual patient meta-analysis of 17,922 patients with chronic pain in randomised controlled trials. ................................................................................................................. 76

Herbal Formula Spotlight

Shen Ling Bai Zhu San ................................................................. 82
Signe E Beebe DVM

Herbal Materia Medica

Chi Shao (Paeonia) ........................................................................ 94
Haleh Siahpolo DVM

Classified Advertisements

AATCVM.......................................................................................... inside front cover
Mayway ........................................................................................... 6
AAVA Meeting.................................................................................. 15
Kan Herb .......................................................................................... 16
Eastern Currents ................................................................................ 21
IVAS .................................................................................................... 22
Chi Institute ....................................................................................... 32, 50
Respond Luminex Vet Laser ................................................................ 38
Golden Flower .................................................................................... 64
PCLAC Book ...................................................................................... 64
Jing Tang ............................................................................................. 88
Job Listings .......................................................................................... 94
AJTCVM Classified Advertising Information and Rates .......................................................... 100
AAVA ................................................................................................. inside back cover

Instructions to Authors – visit www.ajtcvm.org (authors tab)
Each year the American Journal of Traditional Chinese Veterinary Medicine (AJTCVM) focuses on 1 of the Five Elements from traditional Chinese veterinary medicine (TCVM). In 2014 the focus will be on the Water Element and the functions of the associated Zang Fu organs, the Kidney and Bladder. One of the many functions of the TCVM Kidney system is to control the uterus and ovaries and Kidney dysfunction can lead to infertility. The TCVM treatment of female infertility is discussed in this issue of AJTCVM in a basic science study of mice, 2 clinical studies of cows and a “Pearls from Practice” article about small animals and horses. A review of conventional and TCVM female reproductive physiology will be presented here to provide a background for these papers.

Conventional Reproductive Physiology Review

From a conventional perspective, the uterus and ovaries are controlled by the hypothalamus and pituitary of the brain. The hypothalamus secretes gonadotropin releasing-hormone (GnRH) that stimulates the anterior pituitary to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH). In response to increased levels of circulating FSH, the ovaries produce follicles containing oocytes and release estrogens, primarily estradiol. When follicles are mature, a sharp rise in LH levels causes them to rupture and release the ova. The ruptured follicle, under the influence of LH, develops into a corpus luteum that secretes progesterone to support pregnancy.

Stages (phases) of the estrous cycle vary among species and individuals, but most animals have most of the following: 1) proestrus, 2) estrus (female receptive to breeding), 3) interestrous or metestrus, 4) diestrus and 5) anestrus. Proestrus is the time of follicular growth. During proestrus, estrogen levels increase and the endometrial lining of the uterus proliferates. In dogs a serosanguinous vaginal secretion become apparent, associated with diapedesis of red blood cells from capillaries, within the proliferating endometrium. The amount of discharge varies with the breed and individual animal. Estrogen levels usually peak at the end of proestrus in dogs and cows and the beginning of estrus in horses and cats with no obvious proestrus stage.

During estrus the ovarian follicles are mature, estrogen levels fall and the female is sexually receptive (“standing heat”), allowing vaginal penetration by the male. A surge of LH causes spontaneous ovulation in bitches, mares and cows, approximately 24-48 hours later. Cats are induced ovulators. The LH surge necessary for ovulation occurs in response to stimulation of the vagina and cervix during coitus.

During the diestrus stage (pseudo-pregnancy) of bitches and mares, the corpus luteum matures and produces progesterone. During the interestrus and metestrus stages of cats and cows, the corpus luteum begins to form and mature. Some cows may have a small amount of metestral serosanguinous vaginal discharge. Diestrus terminates when the corpus luteum regresses. The uterine lining is not shed in animals, as in humans, but simply resorbs, if conception does not occur. Anestrus is the time of rest during the sexual cycle or occurs during pregnancy.

Estrous cycle stages, length of each stage and numbers per year depend on the species, breed and individual animal and whether the animals are manipulated with artificial light or hormone administration. Dogs typically have an estrous cycle twice yearly, but there may be breed variability. Contrary to popular opinion, large breeds of dogs may have more frequent estrous cycles than small breeds. Although there is variability among individual bitches, proestrus typically lasts 3-21 days, estrus 3-21 days, diestrus 60-90 days and anestrus 90-150 days in dogs (Table 1). If pregnancy occurs, gestation is usually 64-66 days.

Cats can have multiple estrous cycles per year during a breeding season (seasonal polyestrous) or have repeated cycles all year long (polyestrous). Cats living outdoors in natural lighting are more likely to have seasonal polyestrous. Cats are “long day breeders” so in spring and summer, as the daylight reaches 12 hours or more, estrus can recur approximately every 14-21 days. Cats living indoors and in tropical regions often cycle year round. Some cats may not have a proestrus stage, while others have 1-2 days of proestrus (Table 1). Typically estrus lasts 3-16 days, interestrous 1-10 days and then estrus begins again. Ovulation occurs 24-60 hours post coitus and then the Queen will enter diestrus for approximately 40 days. Seasonally polyestrous cats
pregnancy or due to adverse management conditions. \[\text{Gestation is on average 282 days in cows, but varies with individual breeds and animals.}\] 

Traditional Chinese Veterinary Medicine Reproductive Physiology Review

The normal TCVM physiology of female reproduction is a delicate and complex balance primarily between Water (Kidney) and Wood (Liver) Elements, but also affected by Earth (Spleen) and Fire (Heart) Elements (Figure 1). The Uterus (uterus and ovaries) is an Extraordinary Fu organ that is associated with the Wood Element (Liver), but under control of the Water Element (Kidney). The Uterus functions as a Yin organ, when it stores the fetus during pregnancy and as a Yang organ when it expels the fetus during parturition. The Brain is an Extraordinary Fu organ that is associated with the Water Element (Kidney). In conventional physiology the hypothalamus and pituitary orchestrate the normal function of the ovaries and uterus and in TCVM physiology, the Kidney (Brain) orchestrates the normal function of the Uterus (uterus and ovaries).

The Uterus is part of Ming Men (Gate of Life) in female animals. In Chapter 36 of the Huang Di Ba Shi Yi Nan Jing (Yellow Emperor’s Classic of Eighty-One Difficulties), written in the 2nd century AD, it states: “The Gate of Life (Ming Men) is the residence of the

Table 1: Number of annual estrous cycles, duration of estrous cycle stages and gestation period in the canine, feline, equine and bovine

<table>
<thead>
<tr>
<th>Species*</th>
<th>Number of Cycles/ year</th>
<th>Proestrus</th>
<th>Estrus</th>
<th>Diestrus, Interestrus and Metestrus</th>
<th>Anestrus</th>
<th>Gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine</td>
<td>1-3 cycles per year depending on breed; typically twice yearly</td>
<td>3-21 days (9 days average)</td>
<td>3-21 days (9 days average)</td>
<td>Diestrus 60-66 days; Interestrus= anestrus+diestrus</td>
<td>90-150 days</td>
<td>64-66 days</td>
</tr>
<tr>
<td>Feline</td>
<td>Polyestrous or seasonally polyestrous; every 10-20 days year round or in spring and summer</td>
<td>1-2 days, may not be visible</td>
<td>3-16 days</td>
<td>Interestrus 1-10 days, then repeats cycle until ovulates**; If ovulates have diestrus for about 40 days</td>
<td>Only when pregnant or 30-90 days in fall and winter (if seasonal)</td>
<td>63-65 days</td>
</tr>
<tr>
<td>Equine</td>
<td>Multiple cycles; seasonally polyestrous- repeats every 21 days in spring and summer</td>
<td>Usually not visible</td>
<td>2-8 days</td>
<td>Diestrus 14-15 days</td>
<td>Fall and winter</td>
<td>Average 335 days</td>
</tr>
<tr>
<td>Bovine</td>
<td>Multiple cycles; polyestrous- repeats every 18-24 days all year</td>
<td>1-3 days</td>
<td>18-20 hours</td>
<td>Metestrus 1-2 days; Diestrus 5-17 days</td>
<td>When pregnant or adverse management</td>
<td>Average 282 days</td>
</tr>
</tbody>
</table>

*breed and individual variation occurs;  
** cats must be induced to ovulate by vaginal stimulation from coitus

have anestrus in the autumn and late winter, when less than 8 hours of daylight occur, but polyestrous cats may not have anestrus. If pregnancy occurs, the gestation is usually 63-66 days.

Horses are also seasonally polyestrous, having multiple cycles per year, during a breeding season similar to cats (Table 1). Horses are also “long day breeders” and as the day light hours become longer in spring and summer, the mare will have 2-8 days in estrus (follicular phase), then ovulate and have 14-15 days in diestrus (luteal phase). Although there are breed and individual variations, horses repeat estrus and ovulate every 21-22 days throughout the breeding season. Ovulation occurs approximately on the 5th day of estrus. As daylight hours shorten, mares go into anestrus for the fall and winter months. Estrus may be manipulated with artificial light and administration of prostaglandin F₂a, estradiol, progesterone and other hormones to produce foals anytime during the year. If pregnancy occurs the gestation period is on average 335 days, but varies with the size and breed.

Cows are non-seasonally polyestrous and cycle all year round usually every 18-24 days (Table 1). Proestrus typically lasts 1-3 days, estrus 18-20 hours (less in hot climates), metestrus 1-2 days and diestrus 5-17 days (Table 1). If conception does not occur, then proestrus begins again. Anestrus occurs only during pregnancy or due to adverse management conditions. Gestation is on average 282 days in cows, but varies with individual breeds and animals.
Kidney and Uterus. When Kidney Yin functions of the Uterus. Germination and maturation of Kidney transform into estrogen. During the estrus stage, (estrogen) is dominant. During the estrus stage, estrogens are more progesterone is more Yin. As ovarian follicles are developing in proestrus (dogs and cows) or early estrus (cats and horses), as reservoir of Qi and Blood for the 12 Regular Channels. The Chong Channel moves Blood through the Uterus and ensures the Uterus will have sufficient Blood and Qi for optimum function. The Liver, which is the organ that stores Blood, is intimately related to the Chong Channel. The Chong Channel also connects to the CV and GV Channels to create an intricate network of connections to the Kidney and Uterus. The CV Channel receives and regulates the Qi of all the Yin Channels and provides Yin nourishment to the Kidney and Uterus. When Kidney Jing is strong, the Chong Channel and CV Channel bring optimal Kidney Yin and Blood to the Uterus to ensure normal function. When Kidney Jing is weak, infertility can result. The GV Channel connects all Yang Channels and brings Yang Qi to the Uterus. The CV and GV Channels are intimately connected to the Kidney that in turn controls the Uterus. The Uterus is also directly connected to the Kidney via an internal Channel called the Uterus Channel (Bao Luo).

As is well known from Yin Yang Theory, Yin can transform into Yang and vice versa. The proper transformation from Kidney Yin to Kidney Yang is essential for a normal estrous cycle. Compared to each other, estrogens are more Yin and progesterone is more Yang. As ovarian follicles are developing in proestrus (dogs and cows) or early estrus (cats and horses), Yin (estrogen) is dominant. During the estrus stage, Yin transforms into Yang and the Heat of Yang stimulates ovulation. As the corpus luteum matures in diestrus, Yang (progesterone) becomes dominant. The Heart, being the Emperor, controls the transformation of Yang to Yin at the beginning of the estrus cycle and Yin to Yang during ovulation.

Prenatal Qi is the active form of prenatal Jing and consists of prenatal Yin and Yang. The proper balance of Yin and Yang during the estrous cycle is necessary for normal female reproduction and a Deficiency of either can lead to infertility. According to TCVM theory, Kidney Yin gives rise to Liver Yin, Heart Yin, Spleen Yin and Lung Yin. Also according to the Sheng Cycle of the Five Elements Theory, the Kidney creates Liver in a “mother-child” type of relationship (Figure 2). Kidney Yin Deficiency can lead to Liver Yin Deficiency via a Five Elements Theory pathological cycle “sick mother leads to a sick child” that results in infertility among other clinical signs (Figure 3). Kidney Yang is Ming-men Fire (Life Gate of Fire) and Kidney Yang Deficiency can result in Deficient Cold in the Uterus leading to infertility.

The Kidney and Liver are also intimately connected through Jing (Essence) and Blood. Jing (Essence) is stored in the Kidney and Blood is stored in the Liver. Liver Blood nourishes Kidney Jing and Kidney Jing produces Marrow that creates Blood. Kidney Jing Deficiency can lead to Liver Blood Deficiency and infertility can be among the many clinical signs. The Liver also ensures the smooth flow of Qi throughout the body, including the Kidney and Uterus (Figure 1). Liver Qi Stagnation can adversely affect the Kidney via a Five Elements Theory pathological cycle “sick child causes sick mother” and the result is Stagnation of Qi in the Uterus, abnormal estrous cycles and infertility (Figure 3).

According to the Ke (Controlling) Cycle of the Five Elements Theory, Wood (Liver) controls Earth (Spleen) like a grandmother controls her grandchild (Figure 2). When Liver Qi becomes Stagnate, there can be over-control of the Spleen via a Five Elements Theory pathological cycle called the Cheng (Over-controlling) cycle and Spleen Qi can be damaged (Figure 3). Although the Kidney is the source of prenatal Jing, the Spleen is the source of postnatal Jing. Spleen Qi transforms food into Gu Qi, which in turn is transformed into Ying Qi (Nutrient Qi). Ying Qi combines with Body Fluids and circulates within blood vessels as Blood and nourishes the whole body including the Uterus. The Uterus is thus dependent on Spleen Qi to create Blood that is then stored in the Liver and supplied to the Uterus (Figure 1). With chronic Liver Qi Stagnation, Spleen Qi can become damaged and unable to create enough Gu Qi to create Blood. The stores of Blood in the Liver become deficient. Liver Blood Deficiency develops and adversely affects Uterine Blood resulting in infertility.

Infertility from Liver Blood Deficiency and resultant Uterine Blood Deficiency can also occur from malnutrition or over-nutrition (obesity). With malnutrition there is not enough food intake to form Gu Qi to create Blood. Over-nutrition also adversely affects Spleen function and results in the Excess accumulation of Damp and Phlegm. According to the Ke Cycle of the Five Elements Theory, Earth (Spleen) controls Water (Kidney) (Figure 2). An Excess accumulation of Damp and Phlegm in the Spleen can adversely affect the Kidney via a Five Elements Theory pathological cycle, the Cheng (Over-controlling) cycle, and result in Damp and Phlegm accumulation in the Uterus, which interferes with normal function (Figure 3).

Also according to the Ke Cycle of the Five Elements Theory, Water (Kidney) controls Fire (Heart) like a
grandmother controls a grandchild (Figure 2). Bao Mai (Uterus Vessel) is an internal collateral that connects the Uterus and Heart that is especially important in humans. The Heart also functions as the Emperor and promotes the circulation of Blood through the entire body including the Uterus (Figure 1). Heart Qi may also direct the transformation of Yin to Yang in proestrus, estrus and diestrus. The Heart also houses Shen and may also be responsible for some of the behavioral changes that can occur during estrus. When the Heart is too full of emotion and fear, it can insult the Kidney via a Five Elements Theory pathological cycle, the Wu (Insulting or Counter-controlling) cycle, and alter the estrous cycle especially in Fire animals (Figure 3). According to the Sheng cycle of the Five Elements Theory, the Liver creates the Heart in a “mother-child” type of relationship (Figure 2). Liver Qi Stagnation causes Liver Yang rising resulting in Heart Heat,

Figure 1: The primary Zang organs that influence the Uterus and their actions

Figure 2: The Five Elements with associated Zang organ and relationships via the Sheng cycle (creation cycle, mother-child relationship depicted by colored arrows) and Ke cycle (controlling or restraining cycle, grandmother-grandchild relationship depicted by the inner thin blue arrows)
especially in Wood animals. Heart Heat can also adversely affect the Kidney via the Wu (Insulting or Counter-controlling) cycle, and alter the estrous cycle, especially in Wood animals (Figure 3).

In conclusion from an integrated perspective, the normal estrous cycle is dependent on genetics (pre-natal Jing), proper fluctuations of brain and ovarian hormones (Kidney Jing, Yin and Yang), balanced nutrition (Spleen creating post natal Jing, Ying Qi and Blood) and optimal blood circulation (Liver and Heart distributing Qi and Blood respectively). Common conventional causes of infertility are anestrus due to ovarian quiescence with lack of follicular development, anovulatory follicles, a persistent corpus luteum and metritis.2-4,15 Ovarian quiescence may be due to Kidney Qi Deficiency, Liver Blood Deficiency, Kidney Yin Deficiency and Excess Phlegm and Damp.1 Anovulatory follicles may be due to Kidney Qi Deficiency, Kidney Yang Deficiency and Excess Phlegm and Damp. Persistent corpus luteum may be associated with Kidney Yin Deficiency. Metritis is usually associated with Uterine Damp Heat.3 With an understanding of both conventional and TCVM physiology, an integrated approach to the diagnosis and treatment of infertility may result in better outcomes with fewer side effects, than provided by hormone therapy and conventional treatments alone. An accurate Bian Zheng (TCVM pattern identification) is essential for the optimal TCVM treatment of the underlying cause of infertility especially if Chinese herbal medicines are prescribed.4

REFERENCES

Figure 3: The main Five Element Theory pathological cycles that cause abnormal estrous cycles and infertility in domestic animals: 1) Kidney-Liver and Liver-Heart “sick mother causes a sick child” (thin blue arrows), 2) Liver-Kidney “sick child causes a sick mother” (thin green arrow), 3) Liver-Spleen and Spleen-Kidney Cheng (Overcontrolling) cycle (thin black arrows) and 4) Heart-Kidney Wu (Insulting or Counter-controlling) cycle (thin red arrow)


The World Association of Traditional Chinese Veterinary Medicine (WATCVM) held its inaugural meeting September 14, 2013 in conjunction with the 15th Annual International TCVM Conference in, Madrid, Spain.

The mission of the WATCVM is: 1) to unite the global TCVM community through promotion and publication of research on all aspects of TCVM, 2) to develop guidelines for TCVM practice (standardized acupuncture points, Channels and Chinese herbal medicines usage and dosage) 3) To raise funds through the TCVM Foundation to support research and scholarships for veterinary students and faculty to study TCVM and 4) to help establish TCVM curriculums for veterinary medical colleges globally.

WATCVM officers include: Executive Director: Dr. Mushtaq Memon (USA); President: Dr. Qinglan Wang (China); Vice Presidents: Dr. Bruce Ferguson (Australia), Dr Jishu Shi (USA), Dr. Huisheng Xie (USA), Dr. Jianqin Xu (China), Dr. Zhiqiang Yang (China) and Dr. Jose Zilberschtein (Spain); Secretary/Treasurer: Dr. Aituan Ma (China). There are 45 Board members representing 25 countries or world regions.

Dr. Qinglan Wang, the WATCVM president said, “TCVM belongs to China, but also belongs to the world. The WATCVM builds a bridge to connect the China TCVM community to the world, and is the platform to advance TCVM globally with solid scientific research support”.

WATCVM committees currently include: 1) the Education and Curriculum committee, 2) the Standardization of TCVM practice committee, 3) the WATCVM Foundation committee for research and scholarships, 4) the Membership committee and 5) the Out-reach committee. Other committees will be added as needed. The WATCVM website is currently being designed and created.

Individual countries will develop their own branches of WATCVM. Currently the German Association of Traditional Chinese Veterinary Medicine (GATCVM) and the Spain Association of Traditional Chinese Veterinary Medicine (SATCVM) have been established as affiliate organizations of WATCVM. The American Association of Traditional Chinese Veterinary Medicine (AATCVM) has also joined the WATCVM as an affiliate organization. The American Journal of Traditional Chinese Veterinary Medicine (AJTCVM) will be the official journal of the WATCVM.

The South China Agricultural University Vice The
The 15th Annual TCVM Conference held September 12-15, 2013 in Madrid, Spain and sponsored by the Chi Institute of Europe was a big success. Much was learned and a great time was had by all. Pictured below are some of the attendees.

The International Traditional Chinese Veterinary Medicine Institute was co-founded in the summer of 2013 by the South China Agricultural University (SCAU), Guangzhou, China and the Chi Institute of Chinese Medicine, Reddick, FL. The mission of this Institute is to promote TCVM research and education nationally and internationally.

The Chi Institute of Chinese Medicine and the South China Agricultural University offer a Master’s Degree in Traditional Chinese Veterinary Medicine (TCVM). This is an opportunity for veterinarians certified in basic acupuncture, to pursue a deeper understanding of veterinary acupuncture, Chinese herbal medicine, Tui-na, Food Therapy and TCVM clinical trials. For more information about the curriculum and costs visit: www.tcvm.com.

Dr. Huisheng Xie (right) receiving the certificate of Professor of the South China Agricultural University in the College of Veterinary Medicine from Dr. Wu Hong, the Vice President of the South China Agricultural University.

Attendees at the 15th TCVM Annual Conference in Madrid, Spain
The American Academy of Veterinary Acupuncture (AAVA) will hold their annual conference on March 7-9, 2014 in Scottsdale, Arizona (see advertisement in this issue). For more information visit: www.aava.org.


The Chi Institute of the United States will offer an introductory course for veterinarians on the clinical application of veterinary acupuncture and Chinese herbal medicine in Sacramento, California in April and in Houston, Texas in August. For more information visit: www.tcvm.com.

The Association of British Veterinary Acupuncture (ABVA) will offer an acupuncture foundation course at Langford veterinary services in Bristol, UK May 8-11, 2014. Practical information for veterinarians that would like to refresh their knowledge or are new to acupuncture will be presented. For more information visit: www.abva.co.uk.

The Chi Institute of the United States, Europe, Australia and Taiwan will offer a 50-hour canine acupuncture program at the College of Veterinary Medicine, South China Agricultural University, in Guangzhou, China May 19-24, 2014. For more information and to download a flyer visit: www.tcvm.com.

The Schweizerische Tierärztliche Vereinigung für Komplementär- und Alternativmedizin (camvet.ch) will host a conference in English on Advanced diagnostic Techniques and Treatments by Dr. Bruce Ferguson on June 24-27, 2014 at Mühlebachweg 22, 3506 Grosshöchtetten BE, Switzerland. For more information visit: www.camvet.ch.

The Qi Academy of Germany will host an “Advanced Equine Acupuncture” workshop by Dr. Bruce Ferguson July 4-6, 2014 with a focus on classical equine acupoints, balance method with new points and advanced diagnostics. On July 8-9, 2014, Dr. Ferguson will mentor a small group (limited class size) in “Advanced Equine TCVM” with integration of acupuncture, Chinese herbal medicine, Food Therapy and Tui-na. For more information email: Dr. Ferdinand Niessen at: info@qiacademy.eu.

The 2014 China TCVM Annual conference will be held July 28-30, 2014 at Shihezi University, Xinjiang, China (In Chinese). For more information visit: www.atcvm.cn.

National Chia Yi University Veterinary School and Chinese Society of Traditional Veterinary Science will host the 16th Annual International TCVM Conference on August 23-28, 2014 in Taiwan. Dr. Huisheng Xie will be the keynote speaker. Topics will include TCVM for emergencies, TCVM for disorders of the 5 sense organs and evidence-based clinical research. A post-conference tour of Taiwanese national parks, caves, temples and museums will be available. Family and friends welcome. For more information visit: www.tcvm.com, email: taiwantcvm.com or call Dr. Hanwen Cheng at 886-916-908646.

The International Veterinary Acupuncture Society (IVAS) will hold their 39th Annual International Congress September 17-23, 2014 in Bologna, Italy. For more information on registration, speakers and topics visit: www.ivas.org.

The Chi Institute of the United States will present an “Advanced TCVM workshop” October 30-November 2, 2014 in Reddick, Florida. Topics will include advanced herbal medicine and advanced nutrition and Food Therapy. For more information visit: www.tcvm.com.

The 5th Chinese Veterinary Conference and TCVM Conference will be held in October 2014 (English translation available). For more information on dates, location, topics and speakers visit: www.cnvc.org.cn/cn.

The Chi Institute of the United States offers basic certification courses in acupuncture (on-site and on-line). For more information visit: www.tcvm.com.

The International Veterinary Acupuncture Society (IVAS) offers basic certification courses in acupuncture. For more information visit: www.ivas.org.

The Chi Institute of Australia offers basic certification courses in acupuncture. For more information on courses, conferences, topics and speakers visit: www.tcvm.com.

The Australian Veterinary Acupuncture Group (AVAG) and International Veterinary Acupuncture Society (IVAS) along with the College of integrative Veterinary therapies (CIVT) will host an online and onsite acupuncture course in 2014. For more information visit: www.civtedu.org/australian-ivas-veterinary-acupuncture-course.
The Chi Institute of Europe offers basic certification courses in acupuncture. For more information visit: www.tcvm.com.

The German Veterinary Acupuncture Society (GERVAS) offers basic acupuncture courses in English. For more information visit: www.gervas.org/blog/wp-content/uploads/2010/12/Kurrikulum2013Entwurf4-f%C3%BCr-Teilnehmer-Tabelle.pdf.

The Chi Institute of the United States offers veterinary Chinese herbal medicine courses (on site and on-line). For more information visit: www.tcvm.com.

The College of Integrative Veterinary Therapies (CIVT) offers veterinary Chinese herbal medicine programs on-line. For more information visit: www.civtedu.org.

The Belgium Veterinary Acupuncture Society (BEVUS) offers veterinary Chinese herbal medicine courses for veterinarians in English. For more information visit: www.bevas.eu.

The Chi Institute of the United States offers basic and advanced certification courses in Tui-na and Food Therapy. For more information on courses, conferences, topics and speakers visit: www.tcvm.com.

The Chi Institute of the United States offers a course on TCVM for veterinary technicians. The course focuses on TCVM theoretical principles and practical techniques that veterinary technicians need to know, in order to assist TCVM veterinarians. No information about TCVM diagnosis and location of acupoints are presented in this course. For more information visit: www.tcvm.com.

The Qi Academy of Germany offers a variety of basic and advanced courses in acupuncture and Tui-na in German and English by leaders in the field including Dr. Ferdinand Niessen, Dr. Bruce Ferguson and Dr. Are Thoreson. For more information visit: www.qiacademy.eu.

The Pulse Controlled Laser Acupuncture Concept (PCLAC) in horses and dogs Modules 1-4 will be presented by Dr. Uwe Petermann in Germany; language: German; Module I February 8-9, 2014; Module II March 30-31, 2014; Module III July 12-13, 2014; Module IV October 18-19, 2014; For more information and Dr. Petermann’s future courses in the United States visit: www.akupunkturtierarzt.de/Akupunkturtierarzt/Tierarztkurse.html.

The Academy of Veterinary Continuing Education (ATF) of Berlin, Germany offers basic courses in veterinary acupuncture in German. For more information visit: www.bundestieraerztekammer.de/index_atf_termine.php?Year=2014&Month=1&Sel.

If you have any TCVM News Around the World that you would like to share, please contact Dr. Tiffany Rimar at: tcvmvet@gmail.com or Dr. Cheryl Chrisman at: chrismanc@ajtcvm.org.

The Animal Natural Health Care Center (ANHC) will host the ANHC Annual Conference March 13-16, 2014 at Saguaro Lake Ranch, Phoenix Arizona. For more information visit: www.drpitcairn.com.

The American Holistic Veterinary Medical Association (AHVMA) will hold their 2014 annual conference this summer. For more information on the place, dates, topics, speakers and post-conference activities visit: www.ahvma.org.

The Veterinary Botanical Medicine Association (VBMA) offers courses in Chinese and Western herbal medicine and certification as a diplomate in the American College of Veterinary Herbalists. The VBMA also hosts teleseminars and other programs. For more information on available programs visit: www.vbma.org.

The American Veterinary Chiropractic Association (AVCA) certifies veterinarians in veterinary chiropractic and hosts an annual fall continuing education seminar. For more information on available programs visit: www.animalchiropractic.org.

The Healing Oasis Wellness Center LLC offers courses in veterinary spinal manipulation, veterinary massage and rehabilitation therapy and advanced neurology and hosts a summer annual continuing education conference. For more information on available programs visit: www.healingoasis.edu.

The Academy of Veterinary Homeopathy offers courses for basic certification in homeopathy and an annual homeopathy continuing education conference. For more information visit: www.theavh.org.

The Pitcairn Institute of Veterinary Homeopathy will offer a course for basic certification in homeopathy in Portland, Oregon in the summer of 2014. For more information visit: www.drpitcairn.com.

If you have any TCVM News Around the World that you would like to share, please contact Dr. Tiffany Rimar at: tcvmvet@gmail.com or Dr. Cheryl Chrisman at: chrismanc@ajtcvm.org.

Other Complementary and Alternative Veterinary Medicine Conferences and Courses
Updates from the American Academy of Veterinary Acupuncture

MESSAGE FROM THE AAVA PRESIDENT

Ken Ninomiya DVM, CVA (IVAS 91) AAVA President 2013-2014

Dear AAVA Members,

I am writing this in late summer of 2013 and have been president only a few months. Hopefully, the past year has resulted in a measurable dent in the AAVA's 2011, 3-5 year Strategic Plan. As a reminder, the principal goals of the Strategic Plan are:

1. The Member Experience – Providing opportunities and services to support sustainable recruitment and retention.
2. Professional and Public Awareness – Improving veterinary acupuncture awareness by the profession and the public.
3. Unification of Veterinary Acupuncture Associations – Enhance collaboration and understanding to achieve mutual interests and goals.
4. AVMA House of Delegates – Providing a “voice” for veterinary acupuncturists by establishing a seat on the House of Delegates.
5. Specialty Recognition by AVMA – Establishing diplomate status.
6. Academy Structure – Maintaining an effective Academy with adequate resources and a powerful leadership.

I want to thank all those dedicated and hard working committee chairs and members who have helped take us toward these goals in 2013. Dr. Neal Sivula, an Associate Editor of the American Journal of Traditional Chinese Veterinary Medicine (AJTCVM), has taken on many more responsibilities for AAVA in addition to his Associate Editor position for the AJTCVM. He has continued as AAVA "Advanced Certification" (FAAVA) Chair as well as volunteering to Chair AAVA's Conference Committee this year and heading the Organizing Committee for AAVA's spearheaded application to the AVMA American Board of Veterinary Specialties.

Dr. Ron Carsten chaired the AAVA Research and Development Committee (RDC) this year, replacing a position that had been occupied by 2 co-chairs the previous year. In his first couple months as Chair, the RDC developed guidelines and application procedures for research funding, retargeted recipients of funds so as to allow AAVA to contribute $4,000 to the University of Tennessee College of Veterinary Medicine's Small Animal Integrative Medicine Fellowship program, rebranded the AAVA "Pet Memorial Fund" into the "Veterinary Acupuncture Education and Research Fund" (VAERF), and developed a website for the VAERF, and online donation site for the Fund.

Dr. Chris Bessent has been the AAVA Public Relations Committee Chair this past year. During Dr. Bessent's term, AAVA released a new "What is Acupuncture" brochure and launched a goal to supply press releases to regional news agency for veterinarians newly graduated from acupuncture, TCM and Traditional Asian Medical courses.

And finally, I'd like to thank, and acknowledge the Membership Committee Chair, Dr. Leslie Phillips. Dr. Phillips is also AAVA's Vice-President, and therefore responsible for being involved with monitoring all the committees and being the AAVA's liaison to other organizations. I offered Dr. Phillips the option to step down as Chair and find a replacement, but she was intent on heading the efforts that the Membership Committee was assigned to benefit AAVA members.
None of the above Chairs' efforts could be possible without the committee volunteers that do all the work like Drs. Bethany Innis and Shana Berry-Buchanan. AAVA members owe much to fellow members like Drs. Mary Battistella, Kevin May, Christine Eckermann-Ross, and Robert Schwyzer who serve on more than one committee. Drs. Patricia Baley, Tim Holt, Leslie Phillips, Liane Sperlich, and Keum Hwa Choi also serve as Board members and volunteer on one or more committees. I am very grateful to all of you and hope members who see these members at the next meeting will approach them and thank them for their sacrifice.

"Live long and prosper",
Ken Ninomiya DVM, CVA
AAVA President 2013-2014
Oxnard, California

Hello Doctors,
As the incoming 2014-2015 President of the American Academy of Veterinary Acupuncture (AAVA), I hope to engage more members to become active in their Academy. When I was elected to the AAVA Board of Directors, I was honored to sit among our amazing colleagues and AAVA staff. I was fortunate to be a part of some new and improved changes for the AAVA. We implemented a new logo and revisited and revised our strategic goals. We are becoming more technologically advanced and connected thanks to Ken Ninomiya, our wonderful web master and 2013-2014 President.

In 2012 we had a successful membership drive that gave us the membership numbers to then apply to the AVMA House of Delegates. At the time of this writing our application to the AVMA House of Delegates is still in consideration. As Dr. Ken Ninomiya said in his letter for this AJTCVM issue, we have great colleagues helping this organization. Our volunteer Committee Chairs are dedicated and busy doctors and I value their time, energy and expertise. Many of our committees have 3 to 5 members who are doing an excellent job, but we need to share some of the load. We all seek knowledge so please look to the Conference Committee to help get the speakers you want to hear or the topics that most interest you.

We are also looking into more local, advanced continuing education and online CE. But this is where we need you to continue your membership and encourage others to join and support your academy. I want to see a stronger, active and diverse membership. I want to hear your ideas and concerns and your vision to make us a better organization.

Please join me at the Annual AAVA conference March 7-8 2014 in sunny Scottsdale, AZ. I am excited to reach out and connect with all the members of our Academy. Please seek me out and shake my hand.

Hope to see your name on one of our committee's lists. You can join anytime.

With great intention,
Leslie Phillips DVM, MS, CVA
AAVA president 2014-2015
Priscilla T. Limehouse  
DVM, CVA, FAAVA  
Dr. Priscilla Limehouse is a graduate of Colorado State University, College of Veterinary Medicine. Dr. Limehouse practiced conventional veterinary medicine and surgery for 12 years, before becoming certified in veterinary acupuncture. She is a trained massage therapist, schooled in conventional myofacial and deep massage. Dr. Limehouse completed the American Veterinary Chiropractic Association veterinary course in veterinary chiropractic. She moved to California in 1992 to join Limehouse Veterinary Clinic, and continued her study of traditional Chinese medicine and acupuncture at YoSan University of Traditional Chinese Medicine. She was certified in veterinary acupuncture by the International Veterinary Acupuncture Society (IVAS) in 1992 and became a Fellow of the American Academy of Veterinary Acupuncture (AAVA) in 2007. Dr. Limehouse has served as chairperson of the Acupuncture Case Review Committee, a member of the IVAS Examination Committee and is currently a member of the AAVA Advanced Certification Examination Committee.

Mieke Maelfait DVM, CVA  
Dr. Mieke Maelfait is a 1977 graduate of the RUG Rijks University, College of Veterinary Medicine, Gent Belgium. After graduation she practiced in a mixed animal practice in Ramsel Belgium. She was certified in veterinary acupuncture by the International Veterinary Acupuncture Society (IVAS) in 1990. Dr. Maelfait served as a member of the IVAS Examination Committee from 1994-2002. She assisted Dr. Emiel Van den Bosch to prepare Acupuncture Points and Meridians of Horses published in 1999 by Stittsville, Ont.: M.E.D. Servi-Systems Canada Ltd. She has taught veterinary herbal medicine classes in Belgium and veterinary acupuncture classes in Belgium, Italy and Denmark. In 2003 Dr. Maelfait moved to the United States (US) to work with Dr. Marvin Cain, one of the founding fathers of acupuncture in the US. She has been in equine private practice with Dr. Cain since that time.

Carolina Medina, DVM, DACVSMR, CVA, CVCH, CVT, CVFT, CCRT  
Dr. Carolina Medina is originally from Caracas, Venezuela. She received a Doctor of Veterinary Medicine degree from St. George's University in 2005. Between 2005-2006, she became certified in veterinary acupuncture, Chinese herbal medicine, Tui-na and Food Therapy by the Chi Institute of Chinese Medicine and the China National Society of Traditional Chinese Veterinary Medicine. Also in 2005-2006, Dr. Medina completed a 14-month clinical internship in TCVM at the University of Florida College of Veterinary Medicine. In 2010, she became certified in canine rehabilitation therapy through the Canine Rehabilitation Institute. She was a clinical assistant professor and Chief of the Integrative Medicine service at the University of Florida College of Veterinary Medicine from 2008-2013. She was one of the founders of the American Association of Traditional Chinese Veterinary Medicine (AATCVM) in 2006 and served as the secretary and treasurer from 2006-2013. She was also one of the founders of the American Journal of Traditional Chinese Veterinary Medicine (AJTCVM) and has been an Associate Editor and regular contributor since 2006. In 2013 she became a Diplomate of the American College of Veterinary Sports Medicine and Rehabilitation. She is on the Board of Directors for the International Veterinary Academy of Pain Management and the American Association of Rehabilitation Veterinarians. Dr. Medina currently works at a specialty practice in Sacramento, California doing TCVM and rehabilitation therapy. Dr. Medina is an author in the new textbook series “Practical Traditional Chinese Veterinary Medicine”, published by Chi Institute Press, Reddick FL in Spring 2014.

Allen M. Schoen DVM, MS, PhD, CVA  
Dr. Allen Schoen received his DVM from Cornell University College of Veterinary Medicine in 1978 and also holds a Masters Degree in neurophysiology and animal behavior from the University of Illinois. He was certified in
veterinary acupuncture by the International Veterinary Acupuncture Society in 1982 and is a past president of IVAS. Dr. Schoen was one of the first veterinarians to become certified in veterinary chiropractic by the American Veterinary Chiropractic Association. In addition, he has advanced training in botanical medicine and homeopathy. He incorporates the best of all these therapies into his practice of veterinary medicine. He started the Department of Acupuncture at the Animal Medical Center in New York City in 1982. He has been a Clinical Assistant Professor at both Colorado State University College of Veterinary Medicine as well as Tufts University School of Veterinary Medicine. Dr. Schoen has worked diligently to bring the best of the complementary therapies into mainstream acceptance. In 1996, he was appointed to a six-member committee by the American Veterinary Medical Association (AVMA) to develop guidelines for Complementary and Alternative Veterinary Medicine (CAVM). Dr. Schoen is co-editor and author of the textbooks “Complementary and Alternative Medicine” and “Veterinary Acupuncture from Ancient Art to Modern Medicine” published by Mosby, St Louis Mo. He has taught and lectured at veterinary schools and conferences nationally and internationally for over 30 years.

**Don Thompson, DVM, CVA**

Dr. Don Thompson graduated from Cornell University College of Veterinary Medicine in 1984 and has practiced in Northern Vermont in large and small animal private practice since that time. He owns and operates a solo veterinary practice with an even distribution of small animal, equine and food animal patients. Dr. Thompson was certified in acupuncture by the International Veterinary Acupuncture Society (IVAS) in 1993. Since that time acupuncture and Chinese herbal medicine have become integral parts of his daily practice. He has taught basic acupuncture at the Chi Institute and was a lecturer at the 10th Annual Traditional Chinese Veterinary Medicine conference in Chongqing, China. Dr. Thompson is an author in the new textbook series “Practical Traditional Chinese Veterinary Medicine”, published by Chi Institute Press, Reddick FL due to be published in 2014.

**Madeline Yamate, DVM, CVA, MBA**

Dr. Madeline Yamate received a BA degree in Biology from Amherst College in 1982, an MBA degree in Marketing and Organizational Behavior from the J.L. Kellogg Graduate School of Management at Northwestern University in 1991 and DVM from the University of California (UC), Davis, in 2005. Dr. Yamate is certified in veterinary acupuncture, veterinary traditional Chinese herbal medicine, veterinary Tui-na and Chinese Food Therapy by the Chi Institute of Chinese Medicine and the China National Society of Traditional Chinese Veterinary Medicine (TCVM). As a veterinary medical student, Dr. Yamate created and conducted 3 seminar courses on Complementary and Alternative Veterinary Medicine (CAVM) at UC Davis. Following graduation, she was in private practice in northern California, before completing an internship in large and small animal acupuncture at the University of Florida under the supervision of Dr. Huisheng Xie. Dr. Yamate has been a faculty member of the Chi Institute of Traditional Chinese Veterinary Medicine since 2006 and the Chi Institute of Europe since 2008, teaching veterinary acupuncture, Chinese herbal medicine, Tui-na and Chinese Food Therapy. She lectures on CAVM and Traditional Chinese Veterinary Medicine nationally and internationally at colleges of veterinary medicine, veterinary conferences, veterinary clubs and general public events. Dr. Yamate is an author in the new textbook series “Practical Traditional Chinese Veterinary Medicine”, published by Chi Institute Press, Reddick FL due to be published in 2014. She is a founding advisory board member of the American Journal of Traditional Chinese Veterinary Medicine (AJTCVM) and a past board member of the Natura Nutritional Advisory Board. Dr. Yamate currently practices and teaches TCVM at the Center for Integrative Animal Medicine in Davis, CA.
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Updates from the American Association of Traditional Chinese Veterinary Medicine (AATCVM)

Acupuncture, Chinese Herbal Medicine, Tui-na and Food Therapy

MESSAGE FROM THE AATCVM PRESIDENT

Dear AATCVM Members,

Hello to all of my colleagues throughout the world practicing TCVM. I hope that your practices are thriving, and that you and your staff are in good health. Regardless of the first two hopes of mine, I truly wish that you are enjoying the practice of TCVM for both your patients and yourselves. We have so much to learn in this vast field. Even the great naturalist and physician Hippocrates said “Artus longus, vita brevis” or “the art is long (to learn) and life is short (too short to study and understand all the information to become a great doctor)”. So, how do we become and remain good doctors? I believe that there are at least three things for which we must strive.

First and foremost, we must have Passion for our field of endeavor, whether it is conventional medicine or TCVM. It has been said “Reason without passion is sterile, while passion without reason is hysterical”. Thus, a reasoning mind should be brought to energetic fruition through passion for the craft.

Second, I believe that we must be Enthusiastic. I love the word enthusiastic because it is derived from “en theos” meaning to have god within or to be filled by god. I am not religious, but I am spiritual. And to me, to be enthusiastic means to be filled by the Universal Energy (Qi) that animates all things, and to allow this Qi to give power to expression of our TCVM passion. We may use this enthusiasm for many purposes such as to empower our needle techniques, to bring favorable energy into our treatment rooms, and to add clarity and love to our client interactions, particularly when our patients are near the end of their lives.

Lastly, to always learn and grow as TCVM doctors, I believe that we need Continuing Education. I am reminded of a story told to me by Dr. Huisheng Xie, delineating the education of a typical TCM practitioner training in ancient times to be the primary caretaker for a Chinese village. Such an education would consist of some formal and informal training under, hopefully, a master practitioner of TCM. After 1-3 years of training, usually in another city far distant from his or her home, the somewhat young and naïve TCM doctor would walk the long walk back to his or her village, where they would then practice TCM for most if not all of the remainder of their lives. The wonderful opportunity that we call continuing education would be rare, perhaps even non-existent. Occasionally a traveling herbalist might stop in the village for a few days and, if the village TCM practitioner was fortunate, the itinerant TCM herbalist might share some practical information with the local TCM practitioner. So in the past, continuing education was rare and unsystematic. We are so much more fortunate in our TCVM continuing education opportunities now.

Take just a moment to think of all of the opportunities now available to us. We have on-line webinars and seminars, as well as chat groups to put our shared experiences together to increase the probability of positive outcomes. I will participate in human TCM

Bruce Ferguson DVM, MS- President of AATCVM (Photograph depicts the splendor of the Alps)
on-line webinars occasionally to derive information for my more complex cases. We have conferences and seminars (see the “News Around the World” section of the journal for dates this year) at which we may see our colleagues present systematic information concerning a wide range of topics. Sometimes the conferences may focus on TCVM, and sometimes be broader and more integrative. For example, about 40 veterinarians registered for the International Herb Symposium (http://www.internationalherbsymposium.com/) held in Massachusetts at the end of June this year. The entire symposium had roughly 800 registrants, so the veterinarians in attendance were offered all of the lectures from world-renowned herbalists, as well as veterinary tracks sponsored by the Veterinary Botanical Medicine Association. Popular TCVM instructors such as Dr. Connie DiNatale, Dr. Huisheng Xie, Dr. Joyce Harman, myself and others were there to offer and share our knowledge with our colleagues, as well as interested non-veterinarians. Most know that one of the poorly kept “secrets” of such conferences of like-minded individuals is the time between the lectures. The time that we spend moments sharing more personally with our colleagues, can be some of the most enlightening of all.

Many of us have the opportunity to be speakers or teaching assistants at TCVM training programs throughout the world. These opportunities may challenge us to become particularly aware of nuances of TCVM that we may have otherwise not attempted to completely understand, but are forced to by the need to transmit that information to our students. Of course, sitting in on or auditing lectures about topics that we think that we know well, also commonly exposes the deficiencies in our knowledge or at least stimulates us to study further. Lastly, the feeling of contributing to a greater good than just you, your family, or even local community, by helping to educate interested veterinary colleagues from throughout the world, will contribute to the positive effects of being an active part of TCVM continuing education.

The American Journal of TCVM (AJTCVM) is another such resource for continuing your TCVM education. Most of you would agree that you learn something new by reading each of the issues, which seem to continuously improve under the nurturing guidance of our Editor-in-Chief, Dr. Cheryl Chrisman. But another, more rich and deep way to utilize the AJTCVM for your education is to contribute an article or paper to the journal. I believe that it was the great scientist and philosopher, Francis Bacon who said “Reading makeith a full man, writing an exact man, and conversation a ready man”. So when you prepare a submission for the AJTCVM, you are forced to learn more about the subject matter, as well as hone your skills of technical writing and presentation in a rational, logical manner.

Hopefully you will find something in the previous paragraphs that will awaken both your appreciation of and perhaps your future contribution to TCVM continuing education. Please take the opportunity to join like-minded colleagues at continuing education events, discover things that you don’t know, modify what you think that you already do know and sharpen your skills to prepare yourself to be an even better TCVM practitioner. It is then almost an understatement to remind you that doing TCVM continuing education will very likely allow you to become a healthier and better person as well.

Live well, and let the Qi be with you!

Bruce Ferguson, DVM, MS, CVA, CVCH
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WELCOME TO THE NEW AATCVM SECRETARY TREASURER, DR. SUZY BRANNAN

Suzy Brannan DVM (and Guy)- new AATCVM Secretary-Treasurer of AATCVM

First the AATCVM Executive Board would like to whole-heartedly thank Dr. Carolina Medina for 7 years of excellent service managing the finances and other communication activities of AATCVM. She was one of the founding members of the AATCVM and AJTCVM. Dr. Medina has moved from Gainesville, FL to join an integrative veterinary practice in Sacramento, California. We will all miss her wonderful Metal spirit, making her the perfect secretary treasurer. Besides being certified in veterinary acupuncture, Chinese herbal medicine, veterinary Tui-na and Food therapy, in 2013 Carolina
received good feedback. In order to improve the performance and quality of the Forum, we have ordered a new dedicated server for the AATCVM and migrated the site from the shared hosting (where hundreds of websites are running on one server) to the new web server that only hosts the AATCVM website. The net result is that the new web server now provides substantially faster and more stable service than before, speeding up posting and searching times.

The data migration to the new server was successful; however, due to a technical problem, we realized that some members were not able to see new posts after the transition. Our technical support administrator addressed the problem immediately. If you have any problem reading new posts or do not see any new post on the Forum, please do not hesitate to contact our administrator at: ts@ajtcvm.org. We sincerely apologize for the inconvenience this has caused.

In addition, we have recently received several inquiries about the use of the Forum. Below are some suggestions to address the most common problems you might have with the Forum. We hope they can help you better use our case discussion service.

1. Please make sure to post your questions under the proper categories. Case discussion questions shall be posted under "TCVM case discussion" (subcategories including cats, dogs, horses etc.) or "General TCVM discussion" (including acupuncture, herbal medicine etc). Please do not post your case questions in the "Welcome" or the "Announcement" sections of the Forum. Doing so may cause a delay in our response.
If you cannot see the above categories, please click the button next to the "Index" button to switch to the Forum View.

2. If you cannot see any new posts for a while, it might be due to the "cache" on your web browser. We recommend using the latest Firefox as your browser to access the AATCVM Forum.

3. You can also subscribe only to a certain Forum or topic. You will receive an email notification when any new post is made under that forum or topic. To subscribe, go to the Forum or topic you want to subscribe, and then move your mouse on the "Forum Tool" or "Topic Tool" icon, at the right of where the page numbers are displayed. A pull-down menu will be displayed, click the "Subscribe" button.

If, at any time, you have any questions, comments, suggestions or anything else, please feel free to contact me at: tcvmkohr@yahoo.com, or our technical support at: ts@ajtcvm.org. Your feedback and suggestions are very important to us. As always, I thank you for your support and for your contributions to the AATCVM discussion forum.

May your Qi flow smoothly!

Ronald Koh DVM, MS, CVA, CVCH
**AATCVM MEMBERSHIP SERVICES AND AJTCVM WEBSITE NEWS**

Greetings AATCVM members,

Another year has passed, and I sincerely hope that you have had a happy and prosperous one. Thank you for your membership in the AATCVM! Your membership supports TCVM education, professional development and evidence-based research that will help TCVM grow faster and stronger around the world. If you have not reviewed your member profile within the last 6 months, please take this opportunity to review your membership directory and contact information, and update it or confirm it. We ask that you do this to ensure that we have the most complete and up-to-date information about you. The Practitioner Directory will only include you, if you input your data. The AJTCVM journals are mailed to you based on the contact information we have on file. To view your membership profile, you need to login as a member on the AATCVM homepage, and then click on 'My Profile' in the horizontal navigation bar. Please do not hesitate to contact me if you have any questions. Check out the Practitioner Directory. If you cannot find yourself listed, it is because you have not set up your user’s profile on the website or your membership has expired. To add yourself to the Practitioner Directory, please follow the instruction below:

1. Log in onto the website with your username and password.
2. Click “My Profile” shown above the logo.
3. My Profile page will open and display your information as have been provided. Scroll down to the very bottom of this page, and click on “Edit Profile”.
4. Enter or edit any field on the profile page. Please note that everything you enter under the “User Profile” section will be published in the directory and be visible to the public. You should leave a field blank if you don’t want to share certain information.
5. Check the “Agree” button under Term of Service.
6. Click “Submit” and your profile will be saved.
7. The directory is updated every 2 weeks. So after you submitting the profile, it may take up to 2 weeks for your information to be displayed in the directory.

For new members be aware that besides the individual PDF files of articles, you can now read the whole issue of AJTCVM in a flash format in full color with easy turn pages to enjoy on your computer, phone and iPad or other tablet device. We now have our own dedicated server so everything downloads much faster than in the past. If you have any questions, please don’t hesitate to contact our technical support at: ts@ajtcvm.org.

**MESSAGE FROM THE AATCVM RESEARCH COMMITTEE**

Dan Hawkins DVM, MS, DACVS- Chairman of the AATCVM Research Committee

Each spring, AATCVM evaluates research proposals related to Traditional Chinese Veterinary Medicine (TCVM) from individual researchers and those affiliated with any group or country and awards small grants to those that qualify. All research proposals should be well designed, following the outline listed on the AATCVM website (www.aatcvm.org), illustrating that the applicant understands the problem and proposes a sound scientific method of investigation. Completed proposals should be submitted to: HawkinsDL@ajtcvm.org. All proposals must be received at the latest by April 1 of the award year and are reviewed by the AATCVM Research Committee chaired by Dr. Dan Hawkins. The committee may request an external review or consultation by an expert regarding specific issues pertaining to the proposal. The results of the Research Committee evaluation will be sent to the principal investigator by May 1 of the same year. Funding will be awarded to those investigators whose projects have been approved by May 30 of the current year.

The AATCVM Research Committee requests submission of TCVM research proposals as described on the AATCVM website by April 1, 2014.
2010-2014 RESEARCH GRANT

The effects of *Yunnan Baiyao* on tests of platelet activation and coagulability in dogs
Christine M. Egger DVM, MVSc, DACVA; Diana Wray BA, BS, MSc, Julie Wheeler, Debra Voulgaris BA, MA, DVM and Barton Rohrbach VMD, PhD


*Yunnan Baiyao*, administered per os at 1 g/kg to dogs did not alter tests of coagulation in this group of 8 healthy, non-bleeding dogs for 12 hours after per os administration. Neither hypercoagulability nor adverse effects were seen at this dose.

**Phase II, Clinical Study (Ongoing):**
The effects of *Yunnan Baiyao* on thromboelastography and hemostasis in dogs undergoing nasal biopsy

This clinical phase will assess the effects of *Yunnan Baiyao* on TEG measurements, duration of hemorrhage, and the estimated amount of blood loss in actively bleeding clinical canine patients undergoing rhinoscopy and nasal biopsy.

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2011-2012 RESEARCH GRANT

Effect of maropitant, acepromazine and electroacupuncture for the prevention of vomiting associated with administration of morphine in dogs
Ronald Boon Wu Koh, DVM

Maropitant and acepromazine were effective in preventing vomiting/retching (V/R). Electro-acupuncture (EA) at PC-6 alone and a combination of PC-6, BL-20, BL-21, GB-34 and ST-36 did not influence the incidence of V/R, but was significantly effective in reducing V/R events per dog. Both EA groups were also effective in preventing signs of nausea induced by morphine.

The abstract of Dr. Koh’s research was selected for presentation at the 2013 American College of Veterinary Internal Medicine (ACVIM) Forum. Dr. Koh presented his research findings at the ACVIM Forum on June 13, 2013, in Seattle, Washington. The manuscript of his research has been submitted for publication.

Dan Hawkins DVM, MS, DACVS, CVA

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Clinical Trials for Acupuncture and Other Traditional Chinese Veterinary Medicine Treatments

Erin Mayo DVM

ABSTRACT
Recent emphasis on the importance of practicing evidence-based medicine (EBM) can be seen as an opportunity for traditional Chinese veterinary medicine (TCVM) practitioners to participate in the process of validation of acupuncture (AP) and other traditional Chinese veterinary medicine (TCVM) treatments. To do so requires an understanding of how to properly design, conduct and report clinical trials. Of particular importance are a succinct answerable research question, an adequate number of animals, clear inclusion and exclusion criteria, randomization of subjects, blindedness of evaluators, sham treatments, control groups, objective measurements of outcomes as much as possible and statistical analysis of data. Current clinical trial design methods may be well suited to investigate Chinese herbal medicines, but blinding and adequate control groups can be challenging to incorporate into clinical AP trials. Through the use of modifications, such as blinded evaluators and sham AP techniques, these difficulties can be mitigated. Including specific treatments for different TCVM pattern diagnoses, within a biomedical diagnoses, can ensure clinically reliable treatments, transparency and reproducibility of results by other researchers. Reviewing “The Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA)” and adaptations for veterinary TCVM studies, before creating the clinical study design, can ensure that all components are included. Clinical researchers need to be cognizant of the importance of a rigorous study design to ensure high quality results that are clinically relevant, thus improving overall patient care and contributing to the knowledge base of EBM.

Key words: Research, acupuncture, traditional Chinese veterinary medicine, TCVM, Chinese herbal medicine, clinical trials, sham acupuncture, evidence-based medicine, STRICTA

ABBREVIATIONS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>Acupuncture</td>
</tr>
<tr>
<td>CAM</td>
<td>Complementary and alternative medicine</td>
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<tr>
<td>EBM</td>
<td>Evidence-based medicine</td>
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<td>RCT</td>
<td>Randomized controlled trials</td>
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<td>TCVM</td>
<td>Traditional Chinese veterinary medicine</td>
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Recent emphasis on the importance of practicing evidence-based medicine (EBM) has thrust the use of acupuncture (AP), Chinese herbal medicines and other complementary and alternative medicine (CAM) treatments into a negative light by some, with suggestions that there is a paucity of scientific evidence of efficacy. The scientific biomedical community defines “evidence of efficacy” as significant positive effects confirmed by established scientific research methodology. The current scientific methods were originally developed to investigate pharmacological agents and may be poorly suited to evaluate AP and other TCVM and CAM treatments, thus requiring adaptations. The legal and moral imperative to provide safe and effective treatment options to patients is always important for veterinarians to consider. The practice of veterinary medicine may still be as much an art, as it is a science. What constitutes standard of care varies among individuals, but all practitioners are accountable to certain professional standards. Some members of the conventional biomedical community may seem to disparage TCVM and other CAM practices, but others simply want all types of practices held to the same standard. How veterinarians choose to treat a particular patient or diagnosis must always be done with the ability to justify the treatment choice.

In a recent letter to the Journal of the American Veterinary Medical Association, Dr. Richard Palmquist highlights some of the difficulties that are encountered with CAM research, beyond those of conventional research methodology. Financial support of CAM research is limited and often focuses on topics that are important or of interest to specific researchers in academic institutions. Without other clinicians interested in research of AP and other TCVM treatments, little
attention and funding may be directed towards the validation of these procedures. Dr. Palmquist further suggests that “lack of evidence cannot be taken...as evidence of a lack of effectiveness”, and the process of validation “begins by looking”.3

Interest in the addition of new treatment methods often begins with veterinarians in clinical practice, as evidenced by increasing enrollment numbers of private practitioners in TCVM training programs. TCVM practitioners can play a vital role in the validation process of AP and other TCVM treatments. Most TCVM practitioners have experiential evidence of success with AP and other TCVM treatments for a variety of conditions, but this does not satisfy the requirements for EBM. The current pressure from the conventional biomedical community can be viewed as an opportunity to scientifically prove the effectiveness of TCVM treatments. The ability to better understand and improve a chosen treatment method can be achieved through clinical research. As TCVM practitioners provide the medical community with thought-provoking results from pilot clinical studies, increased interest in TCVM treatments will stimulate academic and private institutions to perform larger studies. The end result may not only be evidence for the effectiveness of AP and other TCVM treatments, but integration of TCVM practices into conventional medicine and improved patient outcomes.

The hierarchy of the strength of evidence in research is outlined in Figure 1.4 The opinion of experts based on clinical observations and experience is the first stage of evidence development. Animal research may be more directly applicable to veterinary medicine and higher on the hierarchy, than for humans as shown in Figure 1. Case reports or case series are the next tier from the bottom in the hierarchy and are published to describe clinical phenomena. TCVM practitioners most commonly write case reports and case series. Case series are usually considered stronger evidence than a case report, but neither qualifies as EBM.

Case control studies are observational studies, often used in epidemiology, that have a research question and hypothesis about a clinical phenomenon. Observing and comparing animals with and without a conventional disease or TCVM pattern to identify risk factors that might contribute to the development of the disease is typical for case control studies. Cohort studies are observational longitudinal studies, also often used in epidemiology, to follow 1 or more groups over time to observe some specific difference (e.g. Does a group of animals exposed to a substance have a higher incidence of liver disease compared to a group of animals not exposed to a substance).

Clinical randomized controlled trials (RCT) are used to test the efficacy of medical interventions such as TCVM treatments and may be small pilot studies involving 10-20 animals/group or multicenter studies involving a larger number of animals to ensure significance of results. Large clinical RCT are time consuming and expensive to perform. They can be useful to develop clinical guidelines for EBM, but repeatable results by different researchers are the strongest
The results of systematic reviews and meta-analysis of RCT on a subject form the strongest EBM. Much of what is considered standard of care in conventional veterinary medicine and TCVM is not EBM and more clinical research is needed.

The World Health organization (WHO) "Guidelines for Clinical Research on Acupuncture" states that there are 3 criteria for quality acupuncture studies: validity, reliability and statistical significance. To achieve this, the researcher must be knowledgeable about currently accepted study designs and their challenges and shortcomings, when used to investigate AP and other TCVM treatments. Accepted research design modifications that address the unique aspects of AP are available, but may vary with the specific research question. The essential components of a high quality clinical study must include: (1) a clear, concise research question, hypothesis and objective, (2) an adequate number of study subjects, (3) clear inclusion and exclusion criteria, (4) experimental and control groups, (5) randomization of group allocations, (6) objective outcome criteria and measurements, (7) blindedness and (8) adequate statistical analysis.

The Research Question, Hypothesis and Objective(s) of a Clinical Study

The first step in planning a clinical study is to determine the primary research question. Research questions arise from knowledge about known and unknown aspects of a subject. Initially several research questions may become apparent, but a primary research question should be identified and will determine the basis of the hypothesis and objectives of a clinical study. The breadth of the topic should be considered when formulating the research question. Questions that are too simple may yield inconclusive results and questions that are too broad may be unable to answer with a single study. Breaking a problem down into smaller questions is usually best.

The researcher must determine what clinical unknown can or should be investigated and the need for an investigation. A good research question should be: (1) feasible, (2) interesting, (3) novel, (4) ethical and (5) relevant (FINER criteria). The feasibility of a research question includes consideration of potential patient recruitment problems, the time, effort and costs needed to determine a reliable answer and other practical considerations. If patient recruitment rate or sample size estimates are unknown, a pilot study may be needed to estimate the number of patients and time needed to complete the study. Pilot or feasibility studies are small-scale studies that can provide practical information used to justify a larger scale study that is more likely to be considered EBM. In clinical studies the effects of exclusion criteria on patient numbers and the numbers of clients (in veterinary medicine) that may decline or discontinue involvement and be lost to follow-up are first considered. Medical record reviews of current and past cases can be useful to provide an estimate of potential patient numbers. Another practical consideration is ensuring that the needed skills, equipment and personnel are already available to execute the study. Collaborators may be needed for some aspects of the study (e.g. a statistician to aid with data analysis). Pilot or feasibility studies also offer insights into the commitment and costs needed for a larger project. The results of previous pilot studies serve as justification for further exploration of the research question.

Interest in the research question is dependent on the individual researcher and potential funding agencies. In general, most clinical researchers select a research topic that is personally interesting as well as interesting to peers and the community. Creating a research question that is interesting to specific funding agencies is essential to secure financial support for the study.

Novelty of the research question may be important to research foundations and scientific publications, because of a quest to produce and publish new information. Repeatability of study outcomes by different researchers is an essential part of the scientific process, so not all studies need be original, but should provide additional and novel information. Previous studies may have had design flaws or been performed with too few subjects and these deficiencies can be rectified in a new study. The results of repeated clinical studies can support or call into question the data from previous studies.

Ethics in research is a complicated issue in both human and animal studies. While an in-depth discussion is beyond the scope of this paper, most TCVM practitioners should be able to determine whether a potential research question falls within currently accepted ethical boundaries. One of the most common ethical issues to be considered is the use of placebo treatments or withholding treatments. In cases where there is no accepted treatment, a placebo may be acceptable. In cases of severe illness with a known effective treatment, withholding the treatment is clearly unethical. While this may initially seem straightforward, TCVM practitioners are often faced with situations where it may be unclear whether withholding a conventional treatment would be acceptable. In these cases, consultation with peers, an ethics committee or institutional review board is a viable option to ensure the preservation of ethical standards. If funding is sought, the funding source will likely review the protocol for ethical criteria.

Relevance is essential. A good clinical research question must be answerable and have clinical relevance. Even with a good clinical question, a poorly planned or executed study will yield results that are not relevant or reliable. Statistically significant differences found in a study may not be adequate enough to be clinically relevant or useful in clinical practice.

The PICOT format can be helpful to ensure all pertinent aspects of the research question, hypothesis and objectives have been included. PICOT stands for: 1) population (patients), 2) intervention, 3) comparison
group, 4) outcomes of interest and 5) time. The population is the specific group of patients to be evaluated. The intervention is what will be performed on (or administered to) patients. The comparison group is to whom the experimental group will be compared (e.g. the control group). Outcomes of interest include what are intended to be affected, improved, measured and accomplished. The time is the time period(s) during the study when the outcomes will be assessed.

Once the primary clinical research question has been established, the hypothesis and objective(s) of the clinical study can then be determined. The hypothesis should explain the expected changes in the outcome(s) and is what will be statistically tested. Traditionally the hypothesis is composed of 2 contrasting statements, the null and alternative hypotheses. The null hypothesis (H0) states the outcome the researcher is predicting does not occur (e.g. no significant effect from the intervention). The researcher is actually hoping to reject the null hypothesis through statistical tests. The alternative hypothesis (H1) states what outcome the researcher is predicting to occur (e.g. a significant effect from the intervention). The predicted outcome is thus what is “tested” with the clinical trial and forms the basis for the statistical analysis. The null hypothesis must be disproved in order for the alternative hypothesis to be accepted. If a question does not readily translate into a hypothesis, it may be too broad or complicated and may need to be more specific. Some questions may be descriptive in nature and will not translate into a hypothesis. Questions about prevalence of disease are an example. To answer these questions, one can use other study designs (e.g. observational studies) that provide data that may then generate a different research question and hypotheses for further investigation.

The primary objective of a study is a statement about how the study will answer the research question. Objectives should include key features of the research question, such as the treatments to be used, the target study population and the expected outcome(s). The hypothesis can be similar to the objective, but with one key difference. The hypothesis may change during the design process, but the objectives usually remain established from the start.

An example of a TCVM research question, hypothesis and objective is as follows:

1. **Question:** Is the Chinese herbal medicine *Xiao Ying San* an effective alternative to L-thyroxine supplementation for the treatment of canine hypothyroidism?

2. **Null Hypothesis:** The treatment of hypothyroid dogs with *Xiao Ying San* for 8 weeks will result in no significant improvement of clinical signs, serum free thyroxine (FT3) and serum thyroid-stimulating hormone (TSH) levels, compared to baseline levels and is an effective alternative to L-thyroxine supplementation.

3. **Alternative Hypothesis:** The treatment of hypothyroid dogs with *Xiao Ying San* for 8 weeks will result in significant improvements of clinical signs, serum free thyroxine (FT3) and serum thyroid-stimulating hormone (TSH) levels, compared to baseline levels and is an effective alternative to L-thyroxine supplementation.

4. **Objective:** The primary objective of the study is to evaluate the improvement in clinical signs, increase of total serum free thyroxine (FT3) levels and reduction of serum thyroid stimulating hormone (TSH) levels, after 8 weeks of *Xiao Ying San* administration, as compared to pre-treatment measures and 8 weeks of L-thyroxine supplementation in hypothyroid dogs.

Creation of the hypothesis and objective may seem simple at first, but in TCVM studies, problems arise because different AP and Chinese herbal medicine treatments are required for different TCVM patterns associated with a conventional diagnosis, such as canine hypothyroidism. Canine hypothyroidism can be due to Liver Qi Stagnation, Yang Qi Deficiency and Qi-Yin Deficiency. *Xiao Ying San* is contraindicated in animals with Yin Deficiency. To accurately evaluate the effectiveness of *Xiao Ying San* and do no harm to the animal, the research question must be modified to state: “Is the Chinese herbal medicine *Xiao Ying San* an effective treatment for canine hypothyroidism associated with Yang/Qi Deficiency?” and the hypotheses and objectives must then be modified accordingly. The criteria for diagnosis of Yang/Qi Deficiency and evidence for clinical improvement of the TCVM examination and clinical laboratory tests must be clearly outlined in the outcome measures portion of the study design discussed below.

**Adequate Animal Numbers**

Adequate animal numbers is essential for meaningful statistical analysis and reliability of results. Patient recruitment will partly depend on the prevalence of the problem to be studied. Conditions with low prevalence may require a long study period to enlist a sufficient number of study subjects. Besides the frequency of occurrence of the specific disease, another potential obstacle is client consent. The caretakers of animals to be included in a study must clearly understand that they will not have a choice of treatment (e.g. the animals will be randomly assigned to a group). Animals may be more difficult to recruit, when caretakers are not allowed the freedom to choose a treatment. Recruitment problems can be overcome with proper client communications and having all clients sign a release form stating their understanding and acceptance of the terms. A financial incentive may also be necessary for recruitment. In pilot studies the number of animals is determined by the researcher, based on their experience and personal judgment, and are usually small (e.g. 10-20 animals/group). The results of pilot studies are not
considered EBM, as larger clinical trials by more than 1 research center will then be needed for EBM. In larger clinical trials, the number of animals needed to achieve significance between groups can be determined by preliminary data from a pilot study or a statistical power analysis.

Inclusion and Exclusion Criteria

Another important aspect of the clinical trial design is the process of determining specific inclusion and exclusion criteria for subjects. These criteria will identify the target study population (e.g. hypothyroid dogs). The inclusion criteria will specify which dogs are to be included in the study. The exclusion criteria will be what factors will exclude them from the study, such as co-morbidities that might affect the results.

Since it not possible to recruit every possible animal that fits the criteria, a sample population can be studied based on the inclusion and exclusion recruitment criteria. A sample population is a small number of animals selected from a larger population that is then used to make estimations or predictions about specific traits in the larger population. The sample population must be appropriately representative of the study population for the estimations to be accurate. If the sampling process is flawed, bias will be introduced and the conclusions will not be generalizable to the study population. Recruiting a sufficiently large number of animals and using inclusion and exclusion criteria to narrowly define the target study population will increase the possibility that the sample population will be representative of the target population. Using specific selection criteria will ensure less variation in the sample population, especially when patients are to be recruited from multiple sources. Reporting the selection criteria and process will also allow clinicians to apply the study findings to specific patients in their own practice.

Inclusion and exclusion criteria define the target study population and can include age, weight, breed or diagnosis requirements. They may also include what diagnostic procedures will be used to establish the before and after clinical status of the patient. While not considered the standard approach, it is becoming more commonplace for researchers to include both conventional biomedical and traditional Chinese medicine (TCM) and TCVM diagnostic techniques, along with the previously discussed pattern diagnoses. Inclusion criteria for a study population of hypothyroid dogs for a TCVM study could include clinical signs of hypothyroidism and Yang Qi Deficiency, serum free (FT$_4$) levels below 0.8µg/dl and serum TSH levels above 40 µU/L. Including both the conventional and TCVM diagnoses will provide an integrated screening method of patient selection, when TCVM treatments are being studied.

It is important to ensure a clear and consistent approach to diagnosis, including standard questionnaires or other methods, and strive for consistency between practitioners in the process of diagnosis and treatment formulation. Including both conventional and TCVM methods can offer opportunities to explore differences in patient responses, when they may have the same conventional diagnosis, but different TCVM pattern diagnosis or vice versa. There is an inherent variability between how TCVM practitioners evaluate patients, such as interpretation of tongue and pulse changes. Developing systematic and standardized approaches to the TCVM evaluation of patients will keep the diagnostic and treatment formulation process consistent and transparent. Additionally, conventional diagnostic tests will offer a set of standardized data.

Control Groups

Animals are divided into experimental and control groups. Having 1 or more adequate control groups is essential. The control group of animals can receive standard conventional treatments and comparison groups of animals can receive various combinations of AP and/or other TCVM treatments alone or combined with conventional treatments. If the control group receives an accepted conventional treatment, the treatment protocol needs to be appropriate and adequate for the condition, as per current literature and clinical trials. The control group may also receive a placebo or sham treatment in which patients receive no treatment or all aspects of the treatment except the “active ingredient”. Placebos or sham procedures are inert or ineffective treatments that simulate the actual treatment. They are useful to reduce the non-specific positive or negative effects of treatment administration that may affect the outcome. The placebo or sham procedure must only produce minor effects (not enough to be therapeutic) to allow comparison of outcomes between an investigational treatment and no treatment. They also allow investigators to demonstrate that the investigational treatment offers effects beyond that of the natural course of disease. As previously discussed, having a control group that receives a placebo, sham procedure or no treatment must be ethical. In TCVM clinical RCT, the control group often receives the conventional standard of care.

Randomization to Eliminate Selection Bias

Randomization of the allocation of patients to treatment and control groups is essential to eliminate selection bias and minimize the effect of known and unknown confounding variables. Since it is almost impossible to anticipate and control every variable and difference in a test subject population, randomization can evenly distribute any potential confounding variables among all the groups, thus cancelling their effect on the final outcomes. Free research randomizer programs are available to randomly assign patients to treatment and control groups.

Outcome Measures and Criteria

Another important step is the determination of outcome measures, the parameters evaluated to demonstrate an effect. Effect is the overall change
from a baseline value (e.g. a 50% reduction in pain) and is independent of other factors (e.g. the control results). The effects are changes that occur in any circumstance, not just in comparison to a control group. The effects to be measured will depend on the condition being investigated, though they generally are a combination of objective and subjective parameters. Developing objective outcome criteria is a challenge in clinical research.Clinicopathological test results are objective, but some treatments may be effective (e.g. result in improvement of clinical signs) and not change clinicopathological tests. For clinical examination findings, a scale of numbers with specific well-defined criteria (e.g. criteria for the comparative pain scale from 1-10) can help establish some objective guidelines and provide data that can be statistically analyzed.

When determining what outcome measures to monitor during a trial, it is important to determine how much effect is expected, otherwise known as the power of the study.4,8,10 This can be estimated from preliminary data of a pilot study. In some situations the demonstration of a large effect can be accomplished with a small sample number. In AP and other TCVM studies, when the effect may be small, large sample numbers are needed. This is especially true when using sham AP techniques as the control or comparison group.10,11 Sham AP techniques have been shown to have up to 50% of the effect of true treatment instead of the expected 30% associated with a pharmaceutical placebo.9 This can lead to vast differences in sample size calculations. Acupuncture treatment is commonly expected to yield a 70% treatment effect, so if the difference in treatment effect between AP and sham AP is only 20% and not 40%, this will vastly change the number of samples needed to demonstrate the difference.

In some situations the ultimate effect of AP and other TCVM treatments may be difficult to measure in the short term. The final clinical outcome of TCVM treatments may not be short-term changes in biomarkers, but in the over-all course of the disease. It may be better to define the TCVM effect as the ability to produce subtle overall changes in the internal environment to allow the body’s natural healing to take place, rather than producing dramatic changes in a handful of enzymes or specific tissues.12 This may require a long follow-up period. In general, follow-up monitoring periods should be a minimum of 3 months and preferably a year.9

**Blinding to Eliminate Bias**

Blinding (masking) is needed to eliminate investigator, evaluator and client bias.4,9,10 The currently accepted double-blind design in clinical RCT involves blinding the client (patient in human medicine) and either the practitioner or an evaluator or both. The TCVM practitioner can be blinded in studies of Chinese herbal medicines, as treatments and placebos can be prepared to appear the same by a 3rd party and unknown until after the evaluation process. Blinding presents problems in AP and Tui-na, since an acupuncturist will know if a sham treatment has been administered. A 3rd party must be used for the outcomes evaluation in AP and Tui-na research.9 If clients’ opinions are included as an outcome measure, then client blindedness is important, as bias for or against AP or other TCVM treatments can affect outcomes. Designing objective methods of outcomes assessment from blinded evaluators is the best option. When the acupuncturist is not responsible for evaluating treatment outcomes, systematic bias associated with lack of blinding is minimized.

**Statistical Analysis**

A well stated null hypothesis (or alternative hypothesis) can help justify the statistical analysis used to compare outcomes between experimental groups.10 For clinical RCT, statistical comparison of differences between groups is essential. Probability values (p-value) indicate statistical difference. The lower the p-value, the stronger the evidence; p-values below (<) 0.05 are usually considered significant, while p-values <0.01 are considered very significant. Statistical significance may not always equate to clinical relevance as previously discussed.

Baseline comparisons between study groups are also important. The purpose of comparing groups before treatment initiation is to demonstrate that there is no group differences (beyond random chance) and selection bias has not been introduced. This is especially important if the study sample numbers are low and attrition is high. If the initial comparison shows no significant differences in the groups, there should be no significant bias introduced if several participants discontinue participation.

The appropriate statistical test will depend on the study design. Statistical methods to analyze the data are decided during the planning phase. Though it may be tempting, doing post-hoc analysis is generally discouraged.4 Statistical analysis software programs are available, but novice researchers are encouraged to consult with a statistician to ensure the appropriate statistical tests are selected and the calculations are correctly performed.

**Special Considerations for Acupuncture Research**

A placebo should confer no specific treatment effect.7 Sham AP procedures do not qualify as placebos, because they can confer significant physiologic effects, much greater than pharmacologic placebos.9 There are many varieties of sham AP that include using: 1) acupoints different from the prescribed acupoints for the diagnosis, 2) non-acupoints, 3) non-penetrating devices at acupoints and 4) other pseudo-interventions such as inactivated lasers at acupoints. One AP sham method in humans involves using non-acupoints with needle penetration less than 4 mm.10 Another sham AP technique is to tap toothpicks or other blunt devices on the skin at acupoints, but not penetrate the skin.10
Sham procedures in human clinical RCT are designed to eliminate bias by preventing the patient from knowing whether they received an active treatment or not, especially when the patient’s opinion of the treatment outcome is requested. In both humans and animals, sham procedures may be used to reduce the chance of unknown factors affecting the outcome. When doing research on AP treatments, it may be difficult to determine if the difference seen in the groups is due to the AP needles in acupoints or other non-specific and effects associated with the AP treatment.

Sham AP is done to control for any possible “non-specific” effects that may occur during treatment. These are the non-therapeutic effects that may occur from interaction of patient and practitioner, environmental effects or another unknown effect of AP administration. The “specific” effect that is being investigated is the one that results in a change in the patient’s clinical condition. Unfortunately, sham AP techniques appear to have more non-specific effects than pharmacological or other physical placebos, making it difficult to demonstrate significant differences between sham and true AP. Studies that compare the effect of AP to standard conventional treatments often show greater differences, than those comparing AP to sham AP. In one study, the effects from sham AP (using a non-penetrating device) showed greater physiologic effects (less pain and severity of symptoms) than an inert pill. Therefore, it is not appropriate to label AP sham procedures as “placebo”, since all AP sham procedures have been shown to produce significant non-placebo and non-specific effects.

What constitutes “non-specific” and “specific” effects depends on the research question being asked. If the hypothesis is that a quiet environment results in a more effective treatment, the research question will focus on the specific effect of the environment and not on the non-specific effects of the acupoint prescription. Some practitioners and researchers suggest that the “non-specific” effects are not really “non-specific”. These researchers argue that the attempt to break down AP treatment (and all the associated ritual and interaction that accompanies it) into constituent parts is impossible, due to the complex nature of the interplay between the patient, practitioner, needle and environment. Some would also say that it is the interplay that produces the results and removal or alteration of one of these aspects would generate altered results. This is similar to the idea of the sum being greater than the parts.

Another source of controversy in AP clinical trial design is whether to use an individualized or predetermined acupoint prescription protocol. Some authors contend that “there is little evidence that individualized treatment strategies are superior to more standardized approaches…equally there is no evidence to suggest that there is any superiority in terms of effect between a Westernized and a traditional Chinese medicine format.” Standardized point selections help to increase internal validity (the ability to reproduce results in a given set of circumstances). The goal is to control all other variables except the independent variable (e.g. treatment). This makes it easier to establish a stronger relationship between cause (treatment) and effect (outcome). If the acupoint selection is tightly controlled, the results can be more easily compared.

The use of standard protocols (e.g. 1 treatment for every case with a specific conventional diagnosis) does not reflect the way TCVM is practiced by most veterinarians. The individualization of acupoint prescription to each patient’s specific presentation and underlying TCVM patterns is a hallmark of many AP treatments. This may produce better outcomes, but individualized treatments cannot be easily compared, especially if other treatments like Chinese herbal medicine, Tui-na and Food therapy are also included. Negative results with standardized protocols could be due to: 1) lack of specificity of the treatment for the patient (e.g. treating for a conventional diagnosis and not the TCVM pattern), 2) over-simplification of the treatment or 3) patient unresponsiveness to AP.

When deciding on acupoint selection, one does not have to simply choose either standardization or individualization, as there may be a spectrum of options. One option is a set of standard points with optional points that can be chosen by the practitioner for the specific case. Manualization is a technique in which a set of diagnostic guidelines and treatment designs are predetermined. This allows freedom to individualize within a predetermined framework. Further allowance for individualization is to have no specified acupoint protocol except to exclude other treatments or have no acupoint protocol or exclusions of other treatment modalities. In general, when determining a protocol, simplification may be useful for “simple” problems (e.g. PC-6 for nausea or LI-4 for dental pain). When investigating more complicated clinical issues, however more complicated treatments protocols are usually necessary.

One of the difficulties associated with AP research is the ability to guarantee the adequacy of the treatment being tested. In pharmaceutical research, a dose of a chemical can be rationalized on the basis of pharmacokinetic data. Validity of acupoint prescriptions is not so easily proven, thus making it even more difficult to apply current standards of clinical research methodology. One proposed option of ensuring an appropriate treatment protocol is the Birch Relevant and Irrelevant Treatment Selection method. This involves literature review, practitioner survey and consultation with expert panels to develop a consensus on appropriate acupoints for a particular condition or presentation, thus providing justification for the tested treatment beyond the investigator’s personal opinion.

Internal versus External Validity

How a trial is designed will ultimately dictate its internal and external validity. Internal validity is the ability of the trial results to demonstrate the treatment
effect within a tightly defined population. The inclusion criteria of these trials are very strict and the procedures are standardized. This eliminates bias and confounding results associated with unknown factors. The results should also be reproducible in the same set of circumstances, by other researchers. External validity is the ability of the trial results to translate to the general population. In these trials, the sampling criteria are much less rigorous to reflect the patient pool of the average practitioner. The treatment procedures may also be less standardized to reflect daily practice. These trials are more representative of actual clinical practice. However the results are more difficult to interpret because of the greater potential for confounding results and lack of reproducibility.

An example of this concept is efficacy and effectiveness trials. Efficacy is the comparison of the treatment effects to placebo effects, thus demonstrating the difference between an intervention and a control in an ideal set of circumstances. Efficacy trials demonstrate therapeutic effects in an ideal setting with a homogenous sample and standard protocol of treatment with a “sham” or placebo comparison group. As discussed, the results should show a therapeutic effect from the AP needle and not that of some other component. This has good internal validity, but does not represent the general population that may ultimately receive the treatment or how the treatment may be administered. Effectiveness trials demonstrate therapeutic effects in actual clinical settings, as a practitioner would normally administer treatment and therefore are a more realistic approach. There are few exclusion criteria and no sham control/comparison group. With high external validity, these trial results may be more easily translated to daily practice, but may be marred by unknown confounding variables and bias and thus not be considered EBM. Therefore if TCVM practitioners are performing clinical research that may be time consuming and costly, studies must be well designed so the results are considered EBM and the effectiveness of TCVM treatments critically evaluated. Consultation with others experienced in designing clinical trials may be necessary to ensure a research plan that will produce valid results. Groups supporting TCVM research may offer guidance and usually have an outline of information to include when preparing a grant.

**Reporting and Publication**

Once the data have been analyzed and conclusions drawn, the final step is reporting the information. The Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA) were designed to provide researchers with guidelines to improve the reporting of clinical trial methods and results, but are also useful when designing a clinical trial. These instructions are an extension of the Consolidated Standards of Reporting Trials (CONSORT), which are guidelines for reporting all types of clinical RCT. The STRICTA standards provide additional information specific to AP trials, including many of the CONSORT guidelines for non-pharmacological treatments and pragmatic trials. The purpose of these recommendations is to ensure researchers are reporting information fully to improve transparency, reduce ambiguity and allow for successful replication of the study. The guidelines are applicable across a wide range of trial designs in recognition of the variety of study types and level of individualization of treatments.

**Getting Started**

One may begin planning a clinical trial by carefully reviewing STRICTA and TCVM Clinical Trial Guidelines to ensure that the design contains all elements necessary to result in a high quality publishable study that will contribute to the EBM knowledge base. The steps involved in conceiving, designing and conducting clinical research are outlined in Figure 2. The initial steps are the most critical. If the study is designed poorly, the results may be non-publishable and a waste of time and money. However, anyone who endeavors to conduct rigorous research must also realize that no study design is perfect. Compromises may have to be made due to money or time restrictions. Bias may be introduced because of an inability to recruit an adequately diverse study sample. Researchers must strive to follow the above methods of randomization, blinding and controlling to minimize factors that can produce inaccurate or questionable results. When compromises must be made, an alteration in the study plan may be necessary. Describing the limitations of a study identifies the flaws and potential alterations that could affect the results. Even when the results may not

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**Figure 2: Process for designing clinical trials**
be generalizable, they may provide an interesting starting point for another researcher that may have resources to conduct a more rigorously designed trial.

An important part of conducting effective clinical research is to establish a competent team to design and execute the study. This may involve consulting or corroborating with fellow clinicians and researchers to perform multi-center clinical trials or serve as blinded evaluators of outcomes. Continuing education meetings offer opportunities to network and find other TCVM veterinarians with similar clinical research interests. If there are particular areas in which one does not feel adequately skilled (e.g. study design, statistics or scientific writing), consulting experts in these areas may be needed. A common problem for inexperienced clinical researchers is to start with a project that is too large or time consuming to ever complete. A pilot study that can be performed with less time and expense might be the best way to start. The data from the pilot study may provide justification for further studies or new research questions. With a commitment to learn how to effectively design, execute and publish the results of high quality clinical studies, TCVM practitioners can elevate AP and other TCVM practices from testimonials of success to EBM status.

REFERENCES
Advanced TCVM Workshop

This program builds upon the material covered in the Veterinary Herbal Medicine, Food Therapy and Acupuncture courses. It is divided into two sessions: Advanced Herbal Medicine and Advanced Food Therapy. It is the perfect opportunity for those who desire to improve their advanced TCVM knowledge and techniques using these modalities!

Program Features
- How to create your own herbal formulas
- How to make capsules and powder
- Food and Nutrition Balance
- Feline Food Therapy
- Up to 28 cr. hours in most states

Advanced Herbal Medicine Session
Oct 30, 2014
8:30 - 12:30: Top 20 Single Herb Application
1:30 - 5:30: Advanced Herbal Medicine Wet Lab
  - How to make custom herbal decoction based on pattern diagnosis
  - How to make capsules
  - How to make powder
  - Quality Control and Safety

Advanced Food Therapy Session
Nov 1, 2014:
8:30 - 12:30: Advanced Food Therapy
12:30 - 5:30: Class A: Advanced Food Therapy Wet Lab
  Class B: Feline Food Therapy Food and Nutrition Balance

Nov 2, 2014:
8:30 - 12:30:
  Class A: Feline Food Therapy Food and Nutrition Balance
  Class B: Advanced Food Therapy Wet Lab

Main Speakers and Instructors
- Huisheng Xie, DVM, PhD
- Aituan Ma, DVM, PhD
- Constance DiNatale, DVM, CVA, CVFT
- Justin Shmalberg, DVM, CVA, CVFT, CVCH
- Xiaolin Deng, OMD, AP, MS

Tuition and Fees:
- Advanced Herbal Medicine: $600
- Advanced Food Therapy: $600
- Both Sessions: $1,100
2014 Reporting Guidelines for Randomized Controlled Blinded Clinical Trials in Traditional Chinese Veterinary Medicine*

Cheryl L Chrisman DVM, MS, EdS, DACVIM, Huisheng Xie DVM, MS, PhD, Aituan Ma PhD, Neal Sivula DVM, PhD, Carolina Medina DVM, DACVSMR, Bruce Ferguson DVM, MS, Mushtaq Memon BVSc, MSc, PhD, DACT

PURPOSE OF THE REPORTING GUIDELINES
- To improve the quality of experimental designs, execution and reporting of clinical trials and other studies described in articles published by the American Journal of Traditional Chinese Veterinary Medicine (AJTCVM)
- To provide a standard format for authors to follow, when composing reports of clinical trial findings
- To achieve complete and transparent reporting so all study findings can be duplicated, critically appraised and accurately interpreted
- To promote evidence-based Traditional Chinese Veterinary Medical (TCVM) principles and treatments

USE OF THE REPORTING GUIDELINES
- The reporting guidelines will be used for authors and editors and reviewers evaluating manuscripts submitted to AJTCVM.
- Authors will be asked to comment on the items from the list outlined below, not present in a manuscript, during the editing and reviewing processes.
- The long-term goal of the AJTCVM is to primarily publish reports of randomized, blinded and controlled clinical trials that conform to these guidelines to support the practice of TCVM evidence-based medicine.

CHECKLIST FOR REPORTING GUIDELINES

TITLE AND ABSTRACT
1. Title and Abstract
   - Indicate if this is a prophylactic or therapeutic clinical trial, treatments used, outcome(s) of interest, and animal species used in the title.
   - If there was randomized assignment of subjects in the study, state “randomized” in the title and abstract.
   - If the study was controlled or placebo controlled, state “controlled” in the title and abstract.
   - If the study was blinded, state “blinded” in the title and abstract.
   - Clearly state whether the problem occurred naturally or was induced (e.g. “A randomized, controlled, blinded clinical trial of the effects of electro-acupuncture for the treatment of naturally occurring back pain in sport horses”).
   - No new information should be presented in the abstract, only information found in the paper.
   - The abstract should contain a brief summary of the problem, experimental design, pertinent findings, statistical analysis, adverse effects and conclusions in 250 words.

INTRODUCTION
2. Background Information and Objectives
   - Begin with a brief description and importance of the problem to be studied.
   - Provide the scientific background of the problem with references to previous pertinent studies and an explanation of study rationale.
   - Include an explanation of the benefits and possible adverse reactions of the interventions proposed.
   - A specific research question can be included (e.g. “Will rest and electro-acupuncture reduce thoracolumbar pain in sport horses better than rest alone and be an effective alternative to phenylbutazone?”)
   - The introduction should end with a clear statement of the research hypothesis and objectives of the study.
   - The hypothesis can then be stated. (e.g. “Electro-acupuncture and rest will reduce thoracolumbar pain in sport horses better than rest alone and provide a safe alternative to phenylbutazone”)
   - Indicate if this is a prophylactic or therapeutic clinical trial, treatments used, outcome(s) of interest, and animal species used in the title.
   - Objectives are what the research trial was designed to determine (e.g. “In the current study, sport horses with thoracolumbar pain were treated with rest and 3 electro-acupuncture
treatments 1 week apart, rest and phenylbutazone 4.4 mg/kg of body weight every 12 hours initially, followed by 2.2 mg/kg of body weight every 12 hours for 5 days or rest alone to determine: 1) if post treatment pain scores reduced significantly compared to pre-treatment pain scores in each group, 2) if pain scores were significantly reduced in the electro-acupuncture and phenylbutazone group compared to horses receiving rest alone, 3) if pain scores of horses receiving electro-acupuncture were the same or significantly greater or less than those receiving phenylbutazone.

METHODS
3. Identify the IACUC protocol or equivalent for animal use approval.

4. Trial design
• Indicate the type of trial (e.g. double blind, placebo controlled prospective study).

5. Participants- Study setting
• Define the study Population (not to be confused with the study sample)
• Clearly explain inclusion and exclusion criteria for case selection.
  ♦ Provide age, sex, breed and body weight of animals.
  ♦ Provide use of animals and color (if important) (e.g. sport horses, agility dogs).
• Explain where the study took place, how the animals were housed, type of caregivers. If housed in a facility, indicate the standard of care, according to the established standards of a specific group (e.g. “The trial was performed at the University of Florida Veterinary Medical Center (UFVMC), on client owned dogs, housed with the respective client. The study was approved by the UFVMC Clinical Care and Use committee and consent to study forms were signed by the client for each dog included in the study”).

6. Interventions
• Describe each intervention thoroughly including control interventions.
• A researcher reading the report should know exactly how to repeat the study, using the same methods as reported.
• A clinician reading a trial report should know exactly how to perform the intervention, if they choose to use it on a patient.
• Acupuncture:
  ♦ List all acupoints treated and their indications and actions and depth of needle insertion (usually in a Table form).
  ♦ Provide needle gauge, length and type (indicate manufacturer as a foot note).
  ♦ Indicate the technique used (e.g. dry needles non-manipulated, dry needles manipulated, electro-acupuncture, aqua-acupuncture, moxibustion, laser and other).
  ♦ If dry needles were manipulated, provide a detailed description of the manipulation technique, so others can accurately repeat.
  ♦ If electro-acupuncture was performed, provide equipment information (indicate manufacturer as a foot note) and the frequencies used including duration of treatment at each frequency.
  ♦ If aqua-acupuncture was performed, provide details of the hypodermic needle size, depth inserted, primary substance (indicate manufacturer as a foot note), substance concentration, dilution substance (indicate manufacturer as a foot note) and amount injected at each site; if different amounts were used for different acupoints, then list in Table form.
  ♦ If moxibustion was performed, provide a complete description of the herb (indicate manufacturer as a foot note), technique and duration at each acupoint; if different durations were used for different acupoints, then list in Table form.
  ♦ If laser acupuncture was performed, provide equipment information (indicate manufacturer as a foot note), the frequencies used, the duration of each frequency and total duration of each treatment; if different durations were used for different acupoints, then list in Table form.
  ♦ If some other treatment of acupuncture points was performed provide a detailed description of the technique.
• Clearly indicate duration of each treatment, frequency of treatment, total numbers of treatments and total time period over which treatments were administered; may list in a Table for clarity.
• Herbal medicine:
  ♦ Provide details of all herbal medicines and individual herbs used in the study.
  ♦ Indicate Pin-yin and English names.
  ♦ Provide manufacturer as a superscript letter and a footnote.
  ♦ State the dose in g/kg body weight.
  ♦ Indicate if given before, after or with meals.
  ♦ Indicate form of herbal medicine: tea pill, granule, capsule, powder etc.
  ♦ Indicate the frequency of dosing per day.
  ♦ Indicate total duration of treatment.
  ♦ Indicate the ingredients in each Chinese herbal medicine in a Table form and include Pin-yin and English names, grams or percent of each herb in the formula and actions of each ingredient.
7. Outcomes
- Identify and completely define all primary (pre-specified) outcomes or end points by which groups will be compared.
- When available, use previously developed and validated scales or consensus guidelines (e.g. a previously published and accepted pain scale).
- For clinical outcomes (e.g. use of clinical scores for lameness or pain) clearly define each score and state if sensitivity and specificity of observer(s) is known.
- A description of secondary outcomes is optional, except when an adverse occurred (see 19. below).
- Data of secondary outcomes are used to evaluate additional effects of the intervention, anticipated or not, but are usually not considered in the statistical analysis of the primary outcome.
- Author-generated unpublished scales can contain bias and should be avoided if possible.

8. Sample size, interim analyses and rules for early discontinuation
- A study should be large enough to have a high probability (power) of detecting, as statistically significant a clinically important difference of a given size, if such a difference exists.
- Consultation with a biostatistician is often needed during the planning stages to determine the sample size necessary to ensure significance at least p<0.5 and a 95% confidence level.
- Indicate how the sample size was determined (explain and justify assumptions used).
- A clear explanation should be provided, if the actual sample size differed from the originally intended sample size.
- Describe the rationale for other data collection points besides the end point.
- Outline the rules for early discontinuation of the study (e.g. un-anticipated untoward effects).
- Clearly explain the rationale for early discontinuation of a study, in the event this occurs.

Randomization
- Randomization has three major advantages:
  1) Eliminates selection bias by balancing both known and unknown prognostic factors, in the assignment of treatments. Without randomization, treatment comparisons may be prejudiced, whether consciously or not, by selection of participants of a particular kind to receive a particular treatment.
  2) Permits the use of probability theory to express the likelihood that any difference in outcome between intervention groups merely reflects chance.
  3) May facilitate blinding the identity of treatments to the investigators, animal caretakers and outcome evaluators, possibly by use of a placebo, which reduces bias after assignment of treatments.
- Successful randomization in practice depends upon adequate generation of an unpredictable allocation sequence and concealment of that sequence until assignment occurs.
- Randomized assignment of subjects into groups has three steps: sequence generation (see 8. below), allocation concealment (see 9. below) and implementation (see 10. below).

9. Randomization - sequence generation and type
- Describe the technique used for random assignment to test and control groups (e.g. random number table, computerized generated
randomized name or number list).

10. Randomization - mechanism of allocation concealment
- Provide a statement of how the allocation method was concealed from the investigator, project coordinator and client.
- Concealment of the allocated intervention at the time of enrollment reduces bias.
- Blindedness (see 11., below) is different as it is concealing group assignment - not method of assignment.

11. Randomization - implementation
- Provide a statement of who generated the allocation sequence, enrolled participants and assigned participants to interventions.
- It is best to have an uninvolved party generate the group assignment, which remains unknown to the investigators or project coordinators until time of admission into the group.
- Examples: animal numbered and randomly assigned by a technician not involved in the study using a computer generated program; assignment is concealed in a sealed envelope with the animal number on the front and opened only by the investigator when the intervention is to be performed.

12. Blinding (masking)
- Blinding is an important safeguard against bias, particularly when assessing subjective outcomes.
- Blinding is the withholding of information about the assigned interventions from people involved in the trial, such as investigators and clients who may potentially be influenced by this knowledge.
- Provide a statement of whether or not those administering the interventions, investigators, and caretakers evaluating the outcomes were knowledgeable about which intervention an animal received.
- Obviously those administering acupuncture and sham acupuncture treatments cannot be blinded, so the outcome of the intervention needs to be accessed by a blinded evaluator and caretaker.
- Report how the success of blinding was evaluated (e.g. ask the clients or evaluators whether they think a patient received the experimental or placebo or sham treatment and compare with actual treatment).
- Report any known compromises in blinding.
- State the similarity of characteristics of the interventions (e.g. appearance, taste, smell and method of administration; easier to make herbal medicine, placebo and conventional medication have a similar appearance than acupuncture and sham treatment).
- Herbal medicine must be compared with an inert substance of similar appearance in the same capsule at the same dosing frequency or with a conventional standard of care medication (compounded in a form to appear similar to the herbal medicine); then can be “double blinded” from investigators, evaluators and clients.
- In studies utilizing client feedback, investigators may have to separate the client from the animal during acupuncture or sham treatments, so they will not know which was performed.
- If a study was not blinded, provide a justification for not blinding.

13. Statistical methods and additional analyses
- Specify which statistical procedure was used for each analysis; further clarification may be necessary in the results section of the report.
- Study findings are often assessed in terms of their statistical significance, using a p-value.
- The p-value represents the probability that the observed data (or a more extreme result) could have arisen by chance, when the interventions did not truly differ.
- The mean plus or minus standard deviation with specific p-values should be supplied on all data where indicated and are best clearly displayed in Table form.
- Data analyses should be based on counting each participant once for any given outcome.
- Authors should provide a confidence interval for the estimated effect, which may be interpreted as the range of values for the treatment effect that is compatible with the observed data.
- A 95% confidence interval is customary, which gives the range expected to include the true value in 95 of 100 similar studies.
- Subgroup analyses are usually discouraged, as they may confound the overall conclusion.
- If additional analyses were performed between subgroups, authors should clarify the choice of variables that were adjusted for, how continuous variables were handled and whether the analysis was planned or suggested by the data.

RESULTS

14. Participants - flow, losses and exclusions
- Indicate the number of animals that were assessed for eligibility and not included in the study, did not receive the intervention as allocated, did not complete treatment or were not included in the final analysis.
- This information permits the reader to assess to what extent the estimated efficacy of therapy might be underestimated in comparison with ideal circumstances.
- State the reasons for lack of complete treatment, follow-up or inclusion in the analysis.

15. Recruitment and reason for discontinuation of a study
- Provide dates defining recruitment and follow-up periods.
- If follow-up times were determined by a specific
outcome, then indicate the minimum, maximum and mean duration of follow-up periods.

- If the study was discontinued before originally planned, the reasons should be fully disclosed, including intrinsic and extrinsic factors and who made the decision to stop the trial.
- Indicate the role the funding agency played in the deliberations and decision to stop the study.

16. Baseline data
- Include baseline data such as clinicopathological test results of study animals before the test or control interventions (see 4. above).
- Baseline data can include other differences in study animals not previously described (above in section 4.).
- Baseline data is often put in Table form in a column before the data collected during and after test or control interventions.
- Comparisons at baseline should be based on consideration of the prognostic strength of the variables measured and the size of any chance imbalances that have occurred.

17. Numbers analyzed
- The number of participants per group should be given for all analyses.
- Give exact numbers of animals with a specific outcome out of the total number of animals evaluated with the percent in parentheses (e.g. 87/100 [87%]).

18. Outcomes and estimations
- Trial results are often more clearly displayed in a table but should be described in the text.
- Study results can be reported as a summary of the outcome in each group.
- The number of animals with or without the outcome out of the total or the mean and standard deviation of measurements, together with the difference between the groups is known as the effect size.
- The estimated effect size and its precision (such as 95% confidence interval) should be stated.
- Results should be reported for all planned primary and secondary end points, not just for analyses that were statistically significant or “interesting”.
- Selective reporting within a study is a widespread and serious problem.
- Interpretation should focus on the final results at the close of the trial, not the interim results.

19. Ancillary analyses
- Multiple analyses of the same data create a risk for false positive findings and should be avoided.
- Report all analyses performed and clarify which were originally planned (see 12. above) and which were not planned.
- Analyses that were pre-specified in the trial protocol are less biased than those later suggested by the data.

- Adjustment for variables because they differ significantly at baseline is likely to bias the estimated treatment effect.
- If an adjustment was made for baseline variables, both unadjusted and adjusted analyses should be reported.

20. Adverse events
- Report all adverse events or side effects observed in all groups.
- Randomized trials offer the best approach for providing safety data as well as efficacy data.

DISCUSSION

21. Limitations
- The discussion should include:
  - a brief synopsis of the key findings
  - a discussion of key findings presented in the results section
  - do not offer new results in this section
  - consideration of possible mechanisms and explanations
  - comparison with relevant findings from other published studies (whenever possible including a systematic review combining the results of the current study with the results of all previous relevant studies)
  - limitations of the present study (and methods used to minimize and compensate for those limitations)
  - a brief section that summarizes the clinical and research implications
  - a concluding paragraph to summarize findings and recommendations based on the results
- Trial limitations should be discussed and include sources of potential bias, imprecision and multiplicity of analysis, if relevant.
- Internal validity is the extent to which the design and conduct of the study eliminate the possibility of bias.

22. Generalization of study results
- External validity is the extent to which the results of a study can be generalized to other patients and circumstances.
- If internal validity of the study is poor there can be no external validity.
- Applicability of the study findings to similar and other problems should be clearly stated.
- Although some variation in treatment response between an individual patient and the patients in a trial or systematic review is to be expected, the differences tend to be in magnitude rather than direction.

23. Interpretation- overall evidence
- Interpretation of findings should be consistent with results, balancing benefit and risks and considering other relevant evidence.
• Interpret the results in the context of current evidence.
• Bayesian methods can be used to statistically combine the trial data with previous evidence.

**OTHER INFORMATION**

24. Registration
• If a trial is registered, provide the number.

25. Protocol
• If the complete study protocol is available, provide the web location for interested readers.

26. Funding source
• State the source of funding (e.g. “This study was supported by a grant from the American Association of Traditional Chinese Veterinary Medicine Research Foundation”).

* Note: Adapted for reporting of clinical trials in traditional Chinese veterinary medicine from STRICTA, CONSORT, TCM CONSORT and REFLECT Statements.

**REFERENCES**


A Randomized, Blinded, Double Controlled Experimental Study of the Effects of Gan Lian Yu Ping Feng on Antibody Titers and Nonspecific Immune Indexes in Chickens Vaccinated Against Infectious Laryngotracheitis Virus

Chunmei Kong DVM, MS, Xiuhui Zhong DVM, PhD, Aituan Ma PhD, Zhujun Zhao DVM

ABSTRACT
Vaccine failures cause economic losses in the poultry industry. The hypothesis of this randomized, blinded and double controlled study was that 1 or more dosages of Gan Lian Yu Ping Feng (GLYPF; a modified Yu Ping Feng San) would have immune-enhancing effects. Serum antibody, interferon gamma (IFN-γ) and interleukin 4 (IL-4) levels and immune organ indexes were evaluated at 7, 14, 28 and 42 days after infectious laryngotracheitis (ILT) virus vaccination. Three hundred and thirty six, 45-day-old chickens were divided into 8 groups of 42 chickens each. In Groups I-III and IV-VI, 0.25 g/ml, 0.5 g/ml or 1 g/ml respectively of GLYPF were added to the drinking water for 3 days, beginning before or after vaccination respectively. Group VII received 0.0004 g/ml Wen Du Qing (positive control). Group VIII (negative control) was vaccinated, but received no herbal medicine. The serum antibody titers of Groups III and VI (GLYPF 1 g/ml) were very significantly higher ($p<0.01$), than all the other groups at 14, 28 and 42 days. Serum IFN-γ levels were also significantly higher ($p<0.05$) and serum IL-4 levels significantly lower ($p<0.05$) in Groups III and VI compared to all other groups at 42 days. The organ indexes of Groups III and VI were also significantly higher than Group VIII negative control.

In conclusion, 1 g/ml GLYPF, administered in the drinking water 1 day before and 2 days after or 3 days after vaccination, can improve immune responses to ILT vaccination in chickens and may be useful to reduce vaccine failures on poultry farms.

Key words: Chinese herbal medicine, Gan Lian Yu Ping Feng, Wen Du Qing, chickens, poultry, infectious laryngotracheitis virus, vaccination, immune function

ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>g</td>
<td>Grams</td>
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<tr>
<td>GLYPF</td>
<td>Gan Lian Yu Ping Feng</td>
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<tr>
<td>IFN-γ</td>
<td>Interferon gamma</td>
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<tr>
<td>IL-4</td>
<td>Interleukin-4</td>
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<tr>
<td>ILT</td>
<td>Infectious laryngotracheitis</td>
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<tr>
<td>TCVM</td>
<td>Traditional Chinese veterinary medicine</td>
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As the poultry farming industry has grown, infectious disease outbreaks have increased. Vaccinations against infectious diseases are usually effective, but vaccine failures have also been increasing, due to changes in the pathogenic organisms, reduced duration of antibody protection and poor cellular immunity. Therefore, it is important to investigate alternative treatments to enhance the innate immunity and defense functions of poultry and animal. Current conventional immunological principles and methodology have been applied to the study of the immune enhancing effects of tonic herbs, known to replenish the vital energy and nourish Qi, as well as other Chinese herbal medicines. In traditional Chinese veterinary medicine (TCVM), Zheng Qi is considered the disease-resistant Qi and analogous to the functions of the conventional immune system. Several Chinese herbal medicines have been shown to possess immune-enhancing properties and play a novel role in the prevention and control of various infectious diseases in domestic animals and poultry. Administering Chinese herbal medicine to enhance disease control in animal production has become a focus of veterinary research in China.

Yu Ping Feng San (Jade-screen powder) is a classical Chinese herbal medicine, documented in the ancient medical text Danxi Xinfa (Danxi’s Understanding of Chinese Medicine). Yu Ping Feng San is typically composed of 30 g Huang Qi (Astragalus), 60 g Bai Zhu (Atractylodes) and 60 g Fang Feng.
The Chinese herbs in *Yu Ping Feng San* have been used to nourish *Qi* and strengthen the immune system for thousands of years and treat influenza, tracheobronchitis and asthma.\textsuperscript{11} Though there are reports of the immune-enhancing effects of *Yu Ping Feng San* in humans and other mammals, there have only been a few reports of the immunomodulating effects in poultry.\textsuperscript{11-14} The authors have modified *Yu Ping Feng San* by adding *Chuan Xin Lian* (Andrographis) and *Gan Cao* (Glycyrrhiza) to form *Gan Lian Yu Ping Feng* (GLYPF) (Table 1).

The hypothesis of this randomized, positive and negative controlled, blinded study was that 1 or more dosages of GLYPF would enhance immune responses of chickens vaccinated against infectious laryngotracheitis (ILT) virus. The objectives of the study were to evaluate the immunomodulatory effects of 3 doses of GLYPF administered 3 days before and after or only 3 days after vaccination against ILT virus by measuring serum antibody, interferon gamma (IFN-\( \gamma \)) and LI-4 levels and organ indexes for thymus, spleen and bursa of Fabricius at 7, 14, 28 and 42 days after vaccination. The study aim was to develop a protocol for a high quality immune-enhancing Chinese herbal medicine that might be clinically useful to boost the immune response and prevent ILT infections on poultry farms, with minimal side effects.

**MATERIALS AND METHODS**

After comparing the immune-enhancing properties of 3 different Chinese herbal medicines in an unpublished study by the authors, *Gan Lian Yu Ping Feng* (GLYPF) was formulated for the current study. The ingredients and actions of GLYPF are outlined in Table 1.\textsuperscript{15} The 5 Chinese herbs needed to formulate GLYPF were purchased, authenticated and mixed in a container at the ratios listed in Table 1. The herb mixture was then mixed with water (2-3 times more than the herb volume), boiled for 20-30 minutes and strained to collect the herbal liquid. More water was added to the herbal remains, boiled again for 20-30 minutes and again strained and the liquid collected. The liquids from each collection were combined and boiled to form a decoction consisting of 1 g/ml GLYPF. The decoction was stored at 4°C to later be added to the drinking water of the chickens, in 3 different dilutions. Freeze dried chicken ILT vaccine\textsuperscript{9} was purchased for use in the study. *Wen Du Qing*, a Chinese herbal medicine used to treat ILT in chickens, was also purchased for use in the study, for the positive control group (Table 2).

The experimental birds consisted of 336, day-old *Jing Bai* (Beijing white) male chickens.\textsuperscript{4} The birds were housed fed and watered conventionally. All care and experimental procedures were performed in accordance with the guidelines of the Chinese Council for Animal Care. At 44 days of age, the chickens were randomly divided into 8 groups of 42 birds each. All chickens were vaccinated with the ILT vaccine at 45 days of age.

Chickens in Groups I, II and III were given free choice drinking water, containing 0.25 g/ml, 0.5 g/ml or 1 g/ml of GLYPF respectively for 3 consecutive days, beginning 1 day prior to immunization and 2 days afterward (Table 3). Chickens in Groups IV, V and VI were given free choice drinking water containing 0.25 g/ml, 0.5 g/ml or 1 g/ml of GLYPF respectively for 3 consecutive days, beginning on the day of vaccination (Table 3). To serve as a vaccinated positive herbal control group, Group VII received 0.0004 g/ml of *Wen Du Qing* in the drinking water for 3 consecutive days, beginning on the day the birds were vaccinated (Table 3). To serve as a vaccinated negative control group, Group VIII was immunized on the same day as the

<table>
<thead>
<tr>
<th>Pin Yin Name</th>
<th>English Name</th>
<th>Ratio</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Huang Qi</em></td>
<td>Astragalus</td>
<td>3</td>
<td>Tonifies <em>Wei Qi</em>, stabilizes the Exterior, replenishes <em>Qi</em></td>
</tr>
<tr>
<td><em>Bai Zhu</em></td>
<td>Atractylodes</td>
<td>2</td>
<td>Replenishes Spleen <em>Qi</em>, stabilizes the Exterior</td>
</tr>
<tr>
<td><em>Fang Feng</em></td>
<td>Ledebouriella</td>
<td>2</td>
<td>Clears the surface</td>
</tr>
<tr>
<td><em>Chuan Xin Lian</em></td>
<td>Andrographis</td>
<td>2</td>
<td>Clears Heat, releases Toxins, dries Damp</td>
</tr>
<tr>
<td><em>Gan Cao</em></td>
<td>Glycyrrhiza</td>
<td>1</td>
<td>Tonifies Spleen and <em>Qi</em>, clears Heat, harmonizes the formula</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pin Yin Name</th>
<th>English Name</th>
<th>Amount (g)</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Yin Yang Huo</em></td>
<td>Epimedium</td>
<td>62.5</td>
<td>Tonifies Kidney <em>Yang</em>, strengthens <em>Yang Qi</em></td>
</tr>
<tr>
<td><em>Huang Qi</em></td>
<td>Astragalus</td>
<td>62.5</td>
<td>Tonifies <em>Wei Qi</em>, stabilizes the Exterior, replenishes <em>Qi</em></td>
</tr>
</tbody>
</table>
chickens in the other groups, but received no Chinese herbal medicine (Table 3).

At 7, 14, 28 and 42 days after vaccination (52, 59, 73 and 87 days of age respectively), 8 chickens were randomly selected from each group and blood was collected via cardiopuncture. The serum was separated and ILT antibody titers and IFN-\(\gamma\) and IL-4 cytokines were analyzed, according to the manufacturer's instructions. Following blood collection, the chickens were euthanized, weighed and the thymus, spleen and bursa of Fabricius were removed and weighed. The organ index for each was calculated using the formula: organ index = organ weight /body weight.

Data were expressed as mean plus or minus the standard deviation (Mean±SD). The one-way analysis of variance (ANOVA) was used to compare the differences between groups. A \(p\)-value of less than \(p<0.05\) was considered significant and \(p<0.01\) was considered very significant.

RESULTS

The changes in ILT virus antibody titers 7-42 days after vaccination for all groups are outlined in Table 4 and Figure 1. By 14, 28 and 42 days after immunization, the antibody titers of Groups III and VI (GLYPF 1 g/ml) were very significantly higher \((p<0.01)\), than all the other groups. Groups I and II, receiving 0.25 g/ml and 0.5 g/ml GLYPF respectively 1 day before and 2 days after vaccination, had significantly lower \((p<0.05)\) ILT antibody titers at 14, 28 and 42 days than all the others, including the negative control group.

By 42 days after immunization, the serum IFN-\(\gamma\) levels of Groups III and VI, receiving 1 g/ml GLYPF, were significantly higher \((p<0.05)\), than all the other groups (Table 5, Figure 2). Groups I and II, receiving 0.25 g/ml and 0.5 g/ml GLYPF respectively 1 day before and 2 days after vaccination, had lower serum IFN-\(\gamma\) levels than all the others, including the negative control group.

By 28 and 42 days after vaccination, the serum IL-4 levels were significantly lower \((p<0.05)\) in Groups III and VI, compared to all the other groups (Table 6, Figure 3). Serum IL-4 levels were significantly lower \((p<0.05)\) in Group VII (Wen Du Qing) compared to Group VIII (negative control). By 28 and 42 days after vaccination, Groups I and II, receiving 0.25 g/ml and 0.5 g/ml GLYPF respectively 1 day before and 2 days after vaccination, had higher serum IL-4 levels than all the others, including the negative control group.

The results of the organ index calculations for the thymus, spleen and bursa of Fabricius at 7, 14, 28 and 42 days after vaccination are outlined in Tables 7-9 and illustrated in Figures 4-6. No gross pathological changes were found in these organs in any of the groups. By 28 and 42 days after vaccination, the thymus organ indexes of Groups III and VI, receiving 1 g/ml GLYPF, were very significantly higher \((p<0.01)\) than Group VIII.

### Table 3: Treatments, dosing schedules and analysis days of the chickens in Groups I-VIII of the study

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Chickens</th>
<th>Gan Lian Yu Ping Feng (gm/ml)</th>
<th>Dosing Schedule</th>
<th>Vaccination age (days)</th>
<th>Analysis Post Vaccination*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>42</td>
<td>0.25</td>
<td>1 day prior to vaccination, 2 days after</td>
<td>45</td>
<td>7, 14, 28 and 42 days</td>
</tr>
<tr>
<td>II</td>
<td>42</td>
<td>0.5</td>
<td>1 day prior to vaccination, 2 days after</td>
<td>45</td>
<td>7, 14, 28 and 42 days</td>
</tr>
<tr>
<td>III</td>
<td>42</td>
<td>1</td>
<td>1 day prior to vaccination, 2 days after</td>
<td>45</td>
<td>7, 14, 28 and 42 days</td>
</tr>
<tr>
<td>IV</td>
<td>42</td>
<td>0.25</td>
<td>3 days beginning on day vaccinated</td>
<td>45</td>
<td>7, 14, 28 and 42 days</td>
</tr>
<tr>
<td>V</td>
<td>42</td>
<td>0.5</td>
<td>3 days beginning on day vaccinated</td>
<td>45</td>
<td>7, 14, 28 and 42 days</td>
</tr>
<tr>
<td>VI</td>
<td>42</td>
<td>1</td>
<td>3 days beginning on day vaccinated</td>
<td>45</td>
<td>7, 14, 28 and 42 days</td>
</tr>
<tr>
<td>VII**</td>
<td>42</td>
<td>0**</td>
<td>3 days beginning on day vaccinated</td>
<td>45</td>
<td>7, 14, 28 and 42 days</td>
</tr>
<tr>
<td>VIII***</td>
<td>42</td>
<td>0***</td>
<td>NA</td>
<td>45</td>
<td>7, 14, 28 and 42 days</td>
</tr>
</tbody>
</table>

* Days after vaccination that serum analyzed for antibody titers and cytokines and organs harvested to calculate organ indexes on 8 randomly selected chickens/time period; **Positive control group, received 0.0004 g/ml Wen Du Qing instead of Gan Lian Yu Ping Feng in the drinking water ***Vaccinated negative control group (no herbal medicine in drinking water)
Table 4: Serum infectious laryngotracheitis (ILT) virus antibody titers (log^2) of experimental Groups I-VIII at 4 time periods after vaccination

<table>
<thead>
<tr>
<th>Groups</th>
<th>M±SD on Days after Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
</tr>
<tr>
<td>I</td>
<td>4.56±0.008 \textsuperscript{a}</td>
</tr>
<tr>
<td>II</td>
<td>4.67±0.012 \textsuperscript{b}</td>
</tr>
<tr>
<td>III</td>
<td>4.86±0.057 \textsuperscript{B}</td>
</tr>
<tr>
<td>IV</td>
<td>4.81±0.012 \textsuperscript{B}</td>
</tr>
<tr>
<td>V</td>
<td>4.69±0.019 \textsuperscript{b}</td>
</tr>
<tr>
<td>VI</td>
<td>4.85±0.015 \textsuperscript{B}</td>
</tr>
<tr>
<td>VII</td>
<td>4.75±0.001 \textsuperscript{b}</td>
</tr>
<tr>
<td>VIII</td>
<td>4.67±0.007 \textsuperscript{B}</td>
</tr>
</tbody>
</table>

M±SD = mean plus or minus the standard deviation of 8 chickens at each time period; values in the same column with different letters show significant differences, lowercase letters means difference is significant \(p<0.05\), capital letters means difference is very significant \(p<0.01\).

Figure 1: From Table 4, the M±SD serum ILT antibody titers (log^2) at 4 time periods after vaccination against infectious laryngotracheitis virus; Groups I, II and III had 0.25 g/ml, 0.5 g/ml or 1 g/ml of *Gan Lian Yu Ping Feng* (GLYPF) respectively in the drinking water for 3 consecutive days, beginning 1 day prior to vaccination; Groups IV, V and VI had 0.25 g/ml, 0.5 g/ml or 1 g/ml of GLYPF respectively in the drinking water for 3 consecutive days, beginning on the day of vaccination; Group VII received 0.0004 g/ml of *Wen Du Qing* in the drinking water for 3 consecutive days beginning on the day of vaccination; Group VIII was vaccinated, but received no herbal medicine; different letters show significant difference, lowercase letters means difference is significant \(p<0.05\), capital letters means difference is very significant \(p<0.01\); **positive control group
**Table 5:** Serum IFN-γ levels of experimental Groups I-VIII at 4 time periods after vaccination against infectious laryngotracheitis virus

<table>
<thead>
<tr>
<th>Groups</th>
<th>M±SD on Days after Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
</tr>
<tr>
<td>I</td>
<td>5.04±0.011&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>5.09±0.007&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>5.26±0.089&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>5.13±0.010&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>5.11±0.011&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VI</td>
<td>5.27±0.092&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VII</td>
<td>5.17±0.012&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VIII</td>
<td>5.12±0.086&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

M±SD = mean plus or minus the standard deviation of 8 chickens at each time period; values in the same column with different letters show significant differences, lowercase letters means difference is significant \(p<0.05\), capital letters means difference is very significant \(p<0.01\)

**Figure 2:** From Table 5, the M±SD serum IFN-γ values at 4 time periods after vaccination against infectious laryngotracheitis virus; Groups I, II and III had 0.25 g/ml, 0.5 g/ml or 1 g/ml of Gan Lian Yu Ping Feng (GLYPF) respectively in the drinking water for 3 consecutive days, beginning 1 day prior to vaccination; Groups IV, V and VI had 0.25 g/ml, 0.5 g/ml or 1 g/ml of GLYPF respectively in the drinking water for 3 consecutive days, beginning on the day of vaccination; Group VII received 0.0004 g/ml of Wen Du Qing in the drinking water for 3 consecutive days beginning on the day of vaccination; Group VIII was vaccinated, but received no herbal medicine; different letters show significant difference, lowercase letters means difference is significant \(p<0.05\), capital letters means difference is very significant \(p<0.01\); **positive control group
Table 6: Serum IL-4 values of experimental Groups I-VIII at 4 time periods after vaccination against infectious laryngotracheitis virus

<table>
<thead>
<tr>
<th>Groups</th>
<th>M±SD on Days after Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
</tr>
<tr>
<td>I</td>
<td>5.45±0.025 &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>5.36±0.023 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>5.12±0.010 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>5.27±0.023 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>5.35±0.021 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VI</td>
<td>5.14±0.008 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VII</td>
<td>5.29±0.035 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VIII</td>
<td>5.37±0.027 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

M±SD = mean plus or minus the standard deviation of 8 chickens at each time period; values in the same column with different letters show significant difference, lowercase letters means difference is significant \( p<0.05 \), capital letters means difference is very significant \( p<0.01 \);

Figure 3: From Table 6, the M±SD serum IL-4 values (pg/ml) of experimental Groups I-VIII at 4 time periods after vaccination against infectious laryngotracheitis virus; Groups I, II and III had 0.25 g/ml, 0.5 g/ml or 1 g/ml of *Gan Lian Yu Ping Feng* (GLYPF) respectively in the drinking water for 3 consecutive days, beginning 1 day prior to vaccination; Groups IV, V and VI had 0.25 g/ml, 0.5 g/ml or 1 g/ml of GLYPF respectively in the drinking water for 3 consecutive days, beginning on the day of vaccination; Group VII received 0.0004 g/ml of *Wen Du Qing* in the drinking water for 3 consecutive days beginning on the day of vaccination; Group VIII was vaccinated, but received no herbal medicine; different letters show significant difference, lowercase letters means difference is significant \( p<0.05 \), capital letters means difference is very significant \( p<0.01 \); **positive control group
Table 7: Thymus indexes of experimental Groups I-VIII at 4 time periods after vaccination against infectious laryngotracheitis virus

<table>
<thead>
<tr>
<th>Groups</th>
<th>M±SD on Days after Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
</tr>
<tr>
<td>I</td>
<td>5.26±0.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>5.32±0.48&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>5.34±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>5.34±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>5.30±0.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VI</td>
<td>5.33±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VII</td>
<td>5.32±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VIII</td>
<td>5.28±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

M±SD = mean plus or minus the standard deviation of 8 chickens at each time period; values in the same column with different letters show significant difference, lowercase letters means difference is significant p<0.05, capital letters means difference is very significant p<0.01

Figure 4: From Table 7, the M±SD serum thymus organ index at 4 time periods after vaccination against infectious laryngotracheitis virus; Groups I, II and III had 0.25 g/ml, 0.5 g/ml or 1 g/ml of Gan Lian Yu Ping Feng (GLYPF) respectively in the drinking water for 3 consecutive days, beginning 1 day prior to vaccination; Groups IV, V and VI had 0.25 g/ml, 0.5 g/ml or 1 g/ml of GLYPF respectively in the drinking water for 3 consecutive days, beginning on the day of vaccination; Group VII received 0.0004 g/ml of Wen Du Qing in the drinking water for 3 consecutive days beginning on the day of vaccination; Group VIII was vaccinated, but received no herbal medicine; different letters show significant difference, lowercase letters means difference is significant p<0.05, capital letters means difference is very significant p<0.01; **positive control group
Table 8: Spleen indexes of experimental Groups I-VIII at 4 time periods after vaccination against infectious laryngotraheitis virus

<table>
<thead>
<tr>
<th>Groups</th>
<th>M±SD on Days after Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
</tr>
<tr>
<td>I</td>
<td>3.09±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>3.15±0.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>3.18±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>3.15±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>3.11±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>VI</td>
<td>3.16±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VII</td>
<td>3.14±0.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VIII</td>
<td>3.11±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

M±SD = mean plus or minus the standard deviation of 8 chickens at each time period; values in the same column with different letters show significant difference, lowercase letters means difference is significant (p<0.05), capital letters means difference is very significant (p<0.01).

Figure 5: From Table 8, the M±SD spleen organ index at 4 time periods after vaccination against infectious laryngotraheitis virus; Groups I, II and III had 0.25 g/ml, 0.5 g/ml or 1 g/ml of Gan Lian Yu Ping Feng (GLYPF) respectively in the drinking water for 3 consecutive days, beginning 1 day prior to vaccination; Groups IV, V and VI had 0.25 g/ml, 0.5 g/ml or 1 g/ml of GLYPF respectively in the drinking water for 3 consecutive days, beginning on the day of vaccination; Group VII received 0.0004 g/ml of Wen Du Qing in the drinking water for 3 consecutive days beginning on the day of vaccination; Group VIII was vaccinated, but received no herbal medicine; different letters show significant difference, lowercase letters means difference is significant p<0.05, capital letters means difference is very significant p<0.01; **positive control group.
Table 9: Bursa of Fabricius indexes of experimental Groups I-VIII at 4 time periods after vaccination against infectious laryngotracheitis virus

<table>
<thead>
<tr>
<th>Groups</th>
<th>M±SD on Days after Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
</tr>
<tr>
<td>I</td>
<td>1.62±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>1.68±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>1.73±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>1.69±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>1.66±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>VI</td>
<td>1.72±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VII</td>
<td>1.68±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VIII</td>
<td>1.65±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

M±SD = mean plus or minus the standard deviation of 8 chickens at each time period; values in the same column with different letters show significant difference, lowercase letters means difference is significant \( p<0.05 \), capital letters means difference is very significant \( p<0.01 \).

Figure 6: From Table 9, the M±SD bursa of Fabricius organ index at 4 time periods after vaccination against infectious laryngotracheitis virus; Groups I, II and III had 0.25 g/ml, 0.5 g/ml or 1 g/ml of *Gan Lian Yu Ping Feng* (GLYPF) respectively in the drinking water for 3 consecutive days, beginning 1 day prior to vaccination; Groups IV, V and VI had 0.25 g/ml, 0.5 g/ml or 1 g/ml of GLYPF respectively in the drinking water for 3 consecutive days, beginning on the day of vaccination; Group VII received 0.0004 g/ml of *Wen Du Qing* in the drinking water for 3 consecutive days beginning on the day of vaccination; Group VIII was vaccinated, but received no herbal medicine; different letters show significant difference, lowercase letters means difference is significant \( p<0.05 \), capital letters means difference is very significant \( p<0.01 \); **positive control group.
Interferon-γ and IL-4 are typical cytokines representing the Th1 and Th2 cellular immune respectively. Interferon-γ is a kind of cytokine possessing various modulating functions and plays a crucial role in immunomodulating Th1 and Th2 responses. Under normal conditions IFN-γ gene expression is inhibited, but stimulation by factors such as virus, bacteria and certain Chinese herbal medicines, expression of IFN-γ gene become activated and expresses itself. The IFN-γ gene is disinhibited, when virus or herbal medicines contact the cell membranes and then mRNA is synthesized and IFN-γ is released outside the cells.

High levels of serum IFN-γ indicate increased Th1 cell activity and activation of cellular immunity. In addition, IFN-γ is a typical cytokine secreted by Th1 cells and an indicator of Th1 cell-mediated immunity. In the present study by 42 days, chickens receiving 1 g/ml GLYPF had significantly elevated serum IFN-γ levels, indicating increased cellular immunity (Table 5, Figure 2).

Interleukin-4 is a typical cytokine secreted by Th2 cells. Elevation of IL-4 contents results in an imbalance of Th1/Th2, leading eventually to Th1 or Th2 type diseases. In the present study at 42 days, chickens receiving 1 g/ml GLYPF had significantly reduced IL-4 levels compared to the other groups (Table 6, Figures 3). The GLYPF significantly elevated the IFN-γ levels and decreased IL-4 levels indicating that the herbal medicine induced a Th1 response and brought the Th2 biased response to a normal Th1/Th2 balance.

The immune organs are those structures carrying on immunological functions in poultry and mammalian animals. The development of immune organs corresponds with the ability of the body to respond and combat the invasion of pathogenic organisms. The important immune organs in poultry defense systems include the thymus, the spleen and the bursa of Fabricius. Rivas et al reported that the organ weights of the thymus, spleen, and bursa of Fabricius divided by the body weight could be used as indexes to evaluate the immunological status in chickens. The increase in relative or absolute weights of these organs indicate increases in both cellular and humoral immunity. In the present study by 42 days, Groups III and VI receiving 1 g/ml GLYPF had significant elevations of organ indexes compared to the negative control, indicating increases in both cellular and humoral immunity. The authors can not explain why Groups I and II receiving 0.25 g/ml and 0.5 g/ml GLYPF respectively 1 day before and 2 days after vaccination had very significantly lower organ indexes (p<0.01) than all the others including Group VIII, the negative control group.

**DISCUSSION**

Antibody titers are an index for humoral immunity, a very important specific immunological response mediated by B-lymphocytes. Humoral immunity plays a vital role in combating infectious factors and is the first line of defense against the invasion of exogenous pathogens and the inactivation and eradication of pathogenic organisms. The antibodies produced by B-lymphocytes will trap or catch the pathogens directly to explain why Groups I and II receiving 0.25 g/ml and 0.5 g/ml GLYPF respectively 1 day before and 2 days after vaccination had no different than treatment for 3 days beginning on the day of vaccination. These results confirmed that GLYPF had humoral immune-enhancing properties (Table 4, Figure 1).

Antibodies are produced against ILT virus were significantly elevated in chickens treated with GLYPF at dosages of 1 g/ml in the drinking water for 3 days. Treating with GLYPF 1 day before and 2 days after vaccination was no different than treatment for 3 days beginning on the day of vaccination. The GLYPF significantly elevated the IFN-γ levels and organ indexes and higher serum IL-4 levels than all the others including the negative control group. The same dosages given for 3 days beginning on the day of vaccination did not result in the same effect. Further studies need to determine if this is a repeatable effect at low doses or represent some type of negative effect. No adverse side effects were seen in any of the other birds.

In conclusion, based on the results of this study, the hypothesis that 1 or more dosages of GLYPF would enhance immune responses of chickens, vaccinated against infectious laryngotracheitis (ILT) virus could be accepted. The results were that 1 g/ml GLYPF, administered in the drinking water 1 day before and 2 days after or 3 days after vaccination improved immune responses to chickens following vaccination against ILT virus. Randomized controlled clinical trials are needed on poultry farms, but 1 g/ml GLYPF in the drinking water may be clinically useful to boost the immune response to ILT virus vaccinations and prevent ILT infections on poultry farms.

**Acknowledgements**

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**FOOTNOTES**

- Tong Ren Tang Herbal pharmacy; Beijing, China
- Hayao Group Biological Vaccine Company Ltd. Lot Number 201101, Ha’erbin, China
REFERENCES


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16th Annual International TCVM conference and post-conference tour in Taiwan 2014

16th Annual International TCVM Conference

Dates: August 19-22, 2014 Location: Chia Yi University, Taiwan
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- TCVM for disorders of 5 sense organs including otitis, uveitis, sinusitis/nasal congestion, nasal tumor, gingivitis/stomatitis in dogs, cats and horses
- Evidence-based clinical research

Hosted by: Chi Institute Taiwan
Chinese Society of Traditional Veterinary Science (Taiwan)
National Chia Yi University (Taiwan)

Post-conference Tour: August 23-28, 2014
- Chiayi City, Fenchi Lake, Alishan Mountain
- Tainan City, Fort Zeelandia, Eternal Golden Castle, Confucian’s Temple, Sakam Tower
- Kaoshiung City, Fo Gunag Mountain Monastery, Sizihwan Bay, Love River
- Kenting National Park, Eluanbi Lighthouse, Taitung County
- Hualien County, Sanxiantai, Pahsientung Cave, Taroko National Park
- Taipei City, National Palace Museum, Taipei Martyrs’ Shrine, Taipei 101 Observatory

Conference/Tour Fee (early registration rate, save $400 by 3/20/2014)

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<th>Share Room</th>
<th>Conference</th>
<th>Tour</th>
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<td>Family/Friend</td>
<td>No</td>
<td>$1,630</td>
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</tr>
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</table>

What’s Included
The airport pick-up and drop-off, transportation in Taiwan, accommodations (4 or 5 star hotels), 3 meals per day, conference and proceedings, tours, TCM Hospital Visit, Food Therapy Experience, Foot Massage, Tai-ji Practice, and taxes and tips.

Registration or Questions
Please visit www.tcvm.com, email taiwan@tcvm.com or call Chi Institute Taiwan Director, Dr. Hanwen Cheng, at 886-916-908646.

Alishan Mountain Fo Gunag Mountain Monastery Taroko National Park Sanxiantai
Four Randomized, Controlled and Blinded Studies Comparing the Effects of Cu Yun Guan Zhu Ye and Estradiol on the Vagina, Uterus and Ovaries of Mice

Dalu Song DVM, Yuanliang Hu DVM, PhD, Liwen Zhou DVM MS, Baokang Zhang DVM, Xiaohui Chen DVM, Deyun Wang DVM, PhD, Hongxing Wu DVM, Guangliang Cao DVM, Xudong Song DVM, Xiong Shi DVM

ABSTRACT

Estradiol is used to treat ovarian inactivity and persistent corpus luteum, common problems causing economic losses in animal industries. Estradiol can cause untoward side effects and hormone residues in milk and meat. Chinese herbal medicine may have similar effects to estradiol, without the adverse side effects. The authors created a Chinese herbal medicine, 

Cu Yun Guan Zhu Ye (CYGZY),

by combining 0.2 g/ml Hong Hua (Carthamus), 0.4 g/ml Yin Yang Huo (Epimedium) and 0.4 g/ml Yi Mu Cao (Leonurus) suspected to have mild estrogen-like effects. The objectives of 4 randomized, controlled and blinded experiments were to compare the light and electron microscopic effects of CYGZY and estradiol on: 1) vaginal and uterine epithelium, 2) ovarian follicular development, 3) endometrial alkaline phosphatase (AP) and 4) uterine and ovarian blood vessels in murine models. The hypothesis of the studies was that CYGZY would have effects on the vagina, uterus and ovaries that were similar to, but milder than, estradiol. The CYGZY increased vaginal keratinization and thickness, uterine thickness and glandular development, promoted ovarian follicular development and maturation and endometrial AP activity in the same way, but significantly less than estradiol and significantly more than normal saline. The CYGZY also significantly increased arteriole and venule diameters and the number of connections to capillary beds compared to normal saline. Overall CYGZY had a stronger effect than each component herb alone. The CYGZY may provide a novel, effective treatment for ovarian inactivity and persistent corpus luteum, with less untoward side effects than estradiol.

Key words: Chinese herbal medicine, Cu Yun Guan Zhu Ye, fertility-promoting intrauterine infusion liquid, Hong Hua (Carthamus), Yin Yang Huo (Epimedium), Yi Mu Cao (Leonurus), Chinese medicinal herbs, infertility, reproduction, inactive ovaries, ovarian inactivity, retained corpus luteum

ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>CYGZY</td>
<td>Cu Yun Guan Zhu Ye</td>
</tr>
<tr>
<td>IP</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>LSD</td>
<td>Least significant differences</td>
</tr>
<tr>
<td>LHRH</td>
<td>Luteinizing hormone-releasing hormone</td>
</tr>
<tr>
<td>M±SD</td>
<td>Mean plus or minus the standard deviation</td>
</tr>
</tbody>
</table>

The incidence of infertility in livestock due to ovarian inactivity and persistent corpus luteum has been increasing and results in huge economic losses in animal industries. Estradiol is generally used for the treatment of these disorders, but results in untoward side effects (e.g. ovarian cysts and reduced milk production) and hormone residues in milk and meat. Chinese herbal medicine may provide an effective safe alternative to estradiol therapy. The authors combined 3 Chinese herbs, Hong Hua (Carthamus), Yin Yang Huo (Epimedium) and Yi Mu Cao (Leonurus) and created a decocted and alcohol extracted preparation named Cu Yun Guan Zhu Ye (CYGZY) (Table 1). Although suspected to have estrogen-like effects on the female reproductive tract of mice, the conventional mechanisms of action of CYGZY are unknown. The hypothesis of the study was that CYGZY would have effects on the vagina, uterus and ovaries that were similar to, but milder than, estradiol. The objective of 4 randomized, controlled and blinded experiments was to compare the light and electron microscopic effects of CYGZY and estradiol on: 1) vaginal and uterine epithelium, 2) ovarian follicular development, 3) endometrial alkaline phosphatase and 4) uterine and ovarian blood vessels in vivo in murine models. The aim of the study was to evaluate CYGZY as a potential alternative therapy to estradiol for ovarian inactivity and persistent corpus luteum in animals.

MATERIALS AND METHODS

Animal Care and Use, Randomization, Blinding and
Statistical Analysis

The housing, handling, treatment and studies of the animals in all experiments were in accordance with the guidelines of the Chinese Council for Animal Care and the ethics committee of the Nanjing Agricultural University, Nanjing, China. The lottery method of randomization was used to allocate animals to respective groups and select animals for examination at each time period. All people evaluating the outcomes at each time period were blinded to the treatment group. Three different people, who equally shared the work, evaluated the outcomes of each experiment, for a total of 12 people. Data were expressed as mean +/- the standard deviation (M±SD). Duncan’s multiple range and least significant difference (LSD) tests were used to determine the difference among groups. A p-value of less than (<) 0.05 was considered significant and p<0.01 was considered very significant.

Solution Preparations

Hong Hua (Carthamus), Yin Yang Huo (Epimedium) and Yi Mu Cao (Leonurus) were purchaseda and verified by Professor Jin Bin Song from Nanjing University of Chinese Medicine. A sterile solution of each individual herb was prepared via water decoction and alcohol extraction and each herbal solution contained 1 g/ml of the respective Chinese herb. Then 0.4 g/ml of Yin Yang Huo (Epimedium), 0.4 g/ml Yi Mu Cao (Leonurus) and 0.2 g/ml Hong Hua (Carthamus) were combined, decocted and alcohol extracted to form a 1 g/ml CYGZY solution (Table 1). Sterile estradiol benzoateb liquid was diluted with premium quality mineral oil to 50 µg/ml to use as a positive control solution. Standard 0.9% saline (NaCl) was used as a negative control solution.

Experiment 1 Materials and Methods: The effects of CYGZY and component Chinese herbs on vaginal and uterine epithelium of ovariectomized mice

The ovaries of adult female Kunming micec were removed as previously described.3 After a 20 day recovery period, vaginal smears were evaluated for 5 consecutive days to ensure no activity and 60 mice were then randomly divided into 6 groups of 10 mice each (Table 2). Respective solutions assigned to each group were administered intraperitoneally (IP). Group I was a positive control group and Group II was a negative control group and mice received 0.5 ml (50µg/ml) estradiol benzoate and 0.5 ml 0.9% NaCl respectively IP.

Table 1: Ingredients of the Chinese herbal medicine Cu Yun Guan Zhu Ye and their actions12

<table>
<thead>
<tr>
<th>Pin Yin Name</th>
<th>English Name</th>
<th>Amount g/ml</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hong Hua</td>
<td>Carthamus</td>
<td>0.2</td>
<td>Invigorates Blood, removes Stasis, unblocks the Channels, relieves pain</td>
</tr>
<tr>
<td>Yin Yang Huo</td>
<td>Epimedium</td>
<td>0.4</td>
<td>Tonifies Kidney Yang, strengthens Yang Qi, eliminates Dampness, expels Wind</td>
</tr>
<tr>
<td>Yi Mu Cao</td>
<td>Leonurus</td>
<td>0.4</td>
<td>Invigorates Blood, resolves Stagnation, promotes urination, reduces edema, clears Heat, detoxifies</td>
</tr>
</tbody>
</table>

Table 2: Experiment 1: Materials and methods applied to study the effects of the Chinese herbal medicine Cu Yun Guan Zhu Ye and individual component Chinese herbs on vaginal epithelium and morphological structure of the uteri of ovariectomized mice, compared to estradiol (positive control) and saline (negative control)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Mice</th>
<th>IP Solution</th>
<th>Amount</th>
<th>Treatment Days</th>
<th>Evaluation Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>Estradiol benzoate 50µg/ml</td>
<td>0.5 ml</td>
<td>Day 3-6</td>
<td>7</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>0.9% NaCl</td>
<td>0.5 ml</td>
<td>Day 1-6</td>
<td>7</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>Cu Yun Guan Zhu Ye</td>
<td>0.5 ml</td>
<td>Day 1-6</td>
<td>7</td>
</tr>
<tr>
<td>IV</td>
<td>10</td>
<td>Hong Hua (Carthamus)</td>
<td>0.5 ml</td>
<td>Day 1-6</td>
<td>7</td>
</tr>
<tr>
<td>V</td>
<td>10</td>
<td>Yin Yang Huo (Epimedium)</td>
<td>0.5 ml</td>
<td>Day 1-6</td>
<td>7</td>
</tr>
<tr>
<td>VI</td>
<td>10</td>
<td>Yi Mu Cao (Leonurus)</td>
<td>0.5 ml</td>
<td>Day 1-6</td>
<td>7</td>
</tr>
</tbody>
</table>

IP = All solutions were administered intraperitoneally; all herbal solutions were 1 g/ml; On day 7, vaginal smears were obtained and vaginal sections and uteri were removed and examined with light and electron microscopy.
Groups III, IV, V and VI received 0.5 ml (1 g/ml) of CYGZY, *Hong Hua* (Carthamus), *Yin Yang Huo* (Epimedium) and *Yi Mu Cao* (Leonurus) respectively IP (Table 2). Groups II-VI (saline and Chinese herbal medicine groups) received IP injections daily for 6 consecutive days. Due to its marked and rapid action, Group I mice were given IP estradiol on days 3-6 so all groups ended treatment on the same day. On day 7 of the experiment (24 hours after the last solution injection) vaginal smears were examined to calculate the keratinization rate of the vaginal epithelium. The keratinization rate was the number of keratinized cells in 100 epithelial cells. The mice were then euthanized, and the cranial segments of the vagina and complete uterus were removed from each mouse and grossly examined. The uteri were weighed and vaginal and uterine samples were prepared for routine microscopic and electron microscopic examination. Histological characteristics were described for the vaginal and uterine epithelial cells and glands. The thickness of the vaginal epithelium was measured and recorded. The thickness of the uterine epithelium was measured in μm and recorded. The numbers of endothelial glandular cells per light microscopic field (1000X magnification) were counted for 30 microscopic fields. The cross sectional endometrial area with the greatest number of secretory glands was measured (μm²). Under electron microscopy (10,000X magnification) the diameters of the 30 largest secretory glands were measured (μm).

**Experiment 2 Materials and Methods: The effects of CYGZY and component Chinese herbs on ovarian follicular development of mice**

Sixty, 21-day-old female Kunming mice were randomly divided into 6 groups of 10 animals each. As in experiment 1, Group I was a positive control group and Group II was a negative control group and mice received 0.5 ml (50μg/ml) estradiol benzoate and 0.5 ml 0.9% NaCl respectively IP. Groups III, IV and VI received 0.5 ml (1 g/ml) of CYGZY, *Hong Hua* (Carthamus), *Yin Yang Huo* (Epimedium) and *Yi Mu Cao* (Leonurus) respectively IP (Table 3). The mice were euthanized 24 hours after the last injection and their ovaries removed and tissue sections prepared for light and electron microscopy. Ovarian follicles were counted and classified in 30 light microscopy (1000X magnification) fields. Follicles were classified as: 1) mature follicles (>100 μm), 2) secondary follicles (<100 μm with follicular cavities) and 3) primordial follicles (<100 μm with no follicular cavities). With the assistance of electron microscopy (10,000X) the diameters of 30 of the largest mature follicles was measured.

**Experiment 3 Materials and Methods: The effects of CYGZY on endometrial alkaline phosphatase (AP) activity in ovariectomized mice**

The ovaries of adult female Kunming mice were removed as previously described. After a 20 day recovery period and inactive vaginal smears, 30 mice were then randomly divided into 3 groups of 10 mice each (Table 4). Group I received 0.5 ml (50µg/ml) estradiol benzoate IP on days 3-6, Group II received 0.5 ml 0.9% NaCl IP on days 1-6 and Group III received 0.5 ml (1 g/ml) of CYGZY IP on days 1-6. On day 7, the mice were euthanized and the middle portion of the uterus of each was removed and fixed in cold butanone. Paraffin sections were prepared and a calcium-cobalt staining method was applied. Under a light microscope (1000X magnification), the surface of the endometrial epithelial cells and glands and longitudinal muscle layers were observed for the deposition of dark particles, indicating alkaline phosphatase (AP) activity. The amount of black stain in the endometrium of Group I

<p>| Table 3: Experiment 2: Materials and methods applied to study the effects of the Chinese herbal medicine <em>Cu Yun Guan Zhu Ye</em> (FPL) and individual component Chinese herbs on ovarian follicular development of mice, compared to estradiol and saline |</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Mice</th>
<th>IP Solution</th>
<th>Amount</th>
<th>Treatment Days</th>
<th>Evaluation Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>Estradiol benzoate 50μg/ml</td>
<td>0.5 ml</td>
<td>Day 3-6</td>
<td>7</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>0.9% NaCl</td>
<td>0.5 ml</td>
<td>Day 1-6</td>
<td>7</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td><em>Cu Yun Guan Zhu Ye</em></td>
<td>0.5 ml</td>
<td>Day 1-6</td>
<td>7</td>
</tr>
<tr>
<td>IV</td>
<td>10</td>
<td><em>Hong Hua</em> (Carthamus)</td>
<td>0.5 ml</td>
<td>Day 1-6</td>
<td>7</td>
</tr>
<tr>
<td>V</td>
<td>10</td>
<td><em>Yin Yang Huo</em> (Epimedium)</td>
<td>0.5 ml</td>
<td>Day 1-6</td>
<td>7</td>
</tr>
<tr>
<td>VI</td>
<td>10</td>
<td><em>Yi Mu Cao</em> (Leonurus)</td>
<td>0.5 ml</td>
<td>Day 1-6</td>
<td>7</td>
</tr>
</tbody>
</table>

IP = All solutions were administered intraperitoneally; all herbal solutions were 1 g/ml; on day 7, mice were euthanized and ovaries removed and examined with light and electron microscopy
(estradiol) was the standard and Groups II and III were compared to this.

Experiment 4 Materials and Methods: The effects of CYGZY and component Chinese herbs on uterine and ovarian blood vessels of mice

Thirty healthy non-pregnant adult female mice were randomly selected, fasted for 14 hours and divided into 5 groups of 6 mice each. The mice were anesthetized with 20% urethane (dose 0.5ml/g) IP and the broad ligament (uterine and ovarian mesentery) was surgically isolated for microscopic observation. A 38°C (100.4°F) Locke solution was administered via intravenous drip and mice were stabilized for 10 minutes. After stabilization and 15 minutes before administration of herbal and control solutions, the diameters of 30 arterioles and venules were measured and the numbers of arteriole and venule connections to the capillary networks were counted in 30 light microscopic fields (1000X magnification). Fifteen minutes later solutions were administered via esophageal intubation at a dose of 0.01ml/g of body weight as follows: Group II 0.9% NaCl (blank control), Group III CYGZY 1g/ml, Group IV Hong Hua (Carthamus) 1g/ml, Group V Yi Mu Cao (Leonurus) 1g/ml and Group VI Yin Yang Huo (Epimedium) 1g/ml (Table 5). A Group I estradiol was not included in this study. The diameter of 30 arterioles and venules were again measured and the numbers of intersections with capillary networks in 30 light microscopic fields (1000X magnification) were again counted at 15 and 30 minutes after administration of the respective solutions.

RESULTS

Experiment 1 Results: The effects of CYGZY and component Chinese herbs on vaginal and uterine epithelium of ovariectomized mice

The keratinization rates of vaginal smears and vaginal epithelial thickness of the groups are outlined in Table 6 and Figure 1. The keratinization rate of exfoliated vaginal epithelium was significantly higher

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Mice</th>
<th>IP Solution</th>
<th>Amount</th>
<th>Treatment Days**</th>
<th>Evaluation Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>Estradiol benzoate 50µg/ml</td>
<td>0.5 ml</td>
<td>Day 3-6</td>
<td>7</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>0.9% NaCl</td>
<td>0.5 ml</td>
<td>Day 1-6</td>
<td>7</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>Cu Yun Guan Zhu Ye</td>
<td>0.5 ml</td>
<td>Day 1-6</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 5: Experiment 4: Materials and methods applied to study the effects of the Chinese herbal medicine Cu Yun Guan Zhu Ye and individual component Chinese herbs on the uterine and ovarian blood vessels of adult female mice, compared to saline

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Mice</th>
<th>Solution via Esophageal Intubation</th>
<th>Dose</th>
<th>Pre-solution evaluation time**</th>
<th>Post-solution evaluation time**</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>6</td>
<td>0.9% NaCl</td>
<td>0.1ml/g</td>
<td>15 minutes</td>
<td>15 and 30 minutes</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>Cu Yun Guan Zhu Ye</td>
<td>0.1ml/g</td>
<td>15 minutes</td>
<td>15 and 30 minutes</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>Hong Hua (Carthamus)</td>
<td>0.1ml/g</td>
<td>15 minutes</td>
<td>15 and 30 minutes</td>
</tr>
<tr>
<td>V</td>
<td>6</td>
<td>Yi Mu Cao (Leonurus)</td>
<td>0.1ml/g</td>
<td>15 minutes</td>
<td>15 and 30 minutes</td>
</tr>
<tr>
<td>VI</td>
<td>6</td>
<td>Yin Yang Huo (Epimedium)</td>
<td>0.1ml/g</td>
<td>15 minutes</td>
<td>15 and 30 minutes</td>
</tr>
</tbody>
</table>

* No Group I estradiol; **Diameter of arterioles and venules were measured and the numbers of capillary intersections were counted within the uterine and ovarian mesentery (broad ligament), 15 minute before and 15 and 30 minutes after administration of the respective solutions; all herbal solutions were 1 g/ml


(p<0.05) in Group I (estradiol) compared to Group III (CYGZY), Group IV (Hong Hua [Carthamus]) and Group V (Yin Yang Huo [Epimedium]). The keratinization rate of exfoliated vaginal epithelium was very significantly higher (p<0.01) in Group I (estradiol) compared to Group II (NaCl, negative control) and Group VI (Yi Mu Cao [Leonurus]). Chinese herbal medicine Group III (CYGZY), Group IV (Hong Hua [Carthamus]) and Group V (Yin Yang Huo [Epimedium]) had a significantly higher keratinization rate (p<0.05).

Table 6: Experiment 1 Results: Vaginal keratinization rate and epithelial thickness of mice after intraperitoneal administration of the Chinese herbal medicine Cu Yun Guan Zhu Ye or individual component Chinese herbs, compared to estradiol and saline

<table>
<thead>
<tr>
<th>Groups</th>
<th>IP Solution</th>
<th>Keratinization rate*</th>
<th>Thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Estradiol benzoate 50μg/ml</td>
<td>95.2±2.23aA</td>
<td>88.89±38.53aA</td>
</tr>
<tr>
<td>II</td>
<td>0.9% NaCl</td>
<td>20.1±3.88cB</td>
<td>24.51±6.52cB</td>
</tr>
<tr>
<td>III</td>
<td>Cu Yun Guan Zhu Ye</td>
<td>57.4±4.67b</td>
<td>69.77±29.54a</td>
</tr>
<tr>
<td>IV</td>
<td>Hong Hua (Carthamus)</td>
<td>46.3±3.95b</td>
<td>24.27±6.17cB</td>
</tr>
<tr>
<td>V</td>
<td>Yin Yang Huo (Epimedium)</td>
<td>54.4±6.43b</td>
<td>45.50±16.42b</td>
</tr>
<tr>
<td>VI</td>
<td>Yi Mu Cao (Leonurus)</td>
<td>23.9±3.88c</td>
<td>39.87±9.73b</td>
</tr>
</tbody>
</table>

IP = intraperitoneal administration; all herbal solutions were 1 g/ml; *keratinization rate was the number of keratinized cells/100 epithelial cells; data expressed in M±SD; different lower case letters in a column indicate significant difference (p<0.05); different upper case letters indicate a very significant difference (p<0.01)

Figure 1: From Table 6, the M±SD vaginal keratinization rate and epithelial thickness of mice after intraperitoneal administration of the following: Group I Estradiol, Group II NaCl, Group III Cu Yun Guan Zhu Ye, Group IV Hong Hua (Carthamus), Group V Yi Mu Cao (Leonurus) and Group VI Yin Yang Huo (Epimedium); different lower case letters in a column indicate significant difference (p<0.05); different upper case letters indicate a very significant difference (p<0.01)
compared to Group II (NaCl, negative control) and Group VI (Yi Mu Cao [Leonurus]).

There was no significant difference (p<0.05) in the vaginal epithelial thickness of Group I (estradiol, positive control) and Group III (CYGZY). The vaginal epithelium of Groups V (Yin Yang Huo [Epimedium]) and VI (Yi Mu Cao [Leonurus]) were significantly thicker (p<0.05) than Group II (NaCl, negative control), but significantly thinner (p<0.05) than Groups I or III. There was no significant difference in the vaginal thickness of Group IV (Hong Hua [Carthamus]) and Group II (NaCl). The vaginal epithelium in Group I (estradiol) was very significantly thicker (p<0.01) than in Group II (NaCl) and Group IV (Hong Hua [Carthamus]).

The uterine epithelial and glandular changes are outlined in Table 7 and Figure 2. The uterine bodies in Group I (estradiol) were extremely thickened and had marked edema and the weight ratio was 0.492% ±0.106%, which was very significantly higher (p<0.01), than all other groups. Groups III-IV treated with Chinese herbal medicine had thicker uterine epithelium and significantly higher weight ratios (p<0.05) than Group II (NaCl). The uterine epithelium was significantly thicker (p<0.05) in Group I (estradiol) than all the other groups (Table 7). The uterine epithelium was significantly thicker (p<0.05) in Group III (CYGZY), than the other individual herbal medicine groups (Group IV-VI) and Group II (NaCl) (Table 7). Groups IV-VI (individual herbal groups) had significantly thicker (p<0.05) uterine epithelium than Group II (NaCl). The total number of endometrial secretory glands of Group I and the Chinese herbal medicine groups (Groups II-VI) were the same and significantly higher (p<0.5) than Group II (NaCl) (Table 7). The cross sectional area of with the greatest number of secretory glands was significantly higher in Group I compared to all other groups and Group III (CYGZY) was significantly higher than Groups II, IV and VI. The M±SD diameter of the 30 largest secretory glands was not significantly different in Group III (CYGZY), and Group I (estradiol) but significantly greater than all other groups (p<0.05).

On electron microscopy the microvilli of uterine epithelial cells were short and scarce in Group II (NaCl). In Group I (estradiol) and all the groups receiving Chinese herbal medicine, the microvilli were dense and long and there were glycogen secretions among them. In Group I (estradiol) and all groups receiving Chinese herbal medicines, there were also more Golgi complexes and mitochondria than in Group II (NaCl). The tubules were dilated and the organelles were more developed in Groups I and III-VI compared to Group II (NaCl). Further in Group II (NaCl), endometrial glands were minimal and glandular atrophy was common. Endometrial blood vessels were also more dilated in Groups I and III-VI compared to Group II (NaCl). All these proliferative changes observed on electron microscopy were less for the Chinese herbal medicine groups, than the estradiol group, but obviously more than the saline group.

**Experiment 2 Results: The effects of CYGZY and component Chinese herbs on ovarian follicular development of mice**

The numbers of the different types of ovarian follicles in each group are outlined in Table 8 and Figure 3. Group I (estradiol) had significantly more mature follicles (p<0.05) than the groups receiving Chinese herbal medicine (Groups III-VI) and very significantly more mature follicles (p<0.01) than Group II (NaCl). All the Chinese herbal medicine groups had significantly more mature follicles (p<0.05) than Group II (NaCl) and Group III (CYGZY) had significantly more mature follicles than the individual component herb groups (Groups IV-VI). Group II (NaCl) had significantly more secondary follicles (p<0.05) than any of the other groups. The numbers of secondary follicles were not significantly different in Group I (estradiol) and any of the Chinese herbal medicine groups (Groups III-VI). Group II (NaCl) had significantly more primordial follicles than any of the other groups. Group I (estradiol) and Group III (CYGZY) had no significant difference in the number of primordial follicles, which were significantly less than the other Chinese herbal medicine groups.

The size range and mean diameters of the mature ovarian follicles in each group are outlined in Table 9 and Figure 4. There was no significant difference in size range between the groups. There was no significant difference between follicular diameters between Group I (estradiol) and any Chinese medicine group. The follicular diameters of Group I (estradiol) and Group III (CYGZY) were very significantly larger (p<0.01) than Group II (NaCl). The follicular diameters of the other Chinese herbal medicine groups (Groups IV-VI) were significantly larger (p<0.05) than Group II (NaCl).

**Experiment 3 Results: The effects of CYGZY and component Chinese herbs on endometrial alkaline phosphatase (AP) activity in ovariectomized mice**

Deposition of black cobalt sulfide in the top regions of the endometrial epithelial cells and glandular epithelial cells and the longitudinal muscle layer was present in Group I (estradiol) and Group III (CYGZY), indicating AP activity, but none in Group II (NaCl). There was darker and more intense staining observed in Group I (estradiol) than Group III (CYGZY).

**Experiment 4 Results: The effects of CYGZY and component Chinese herbs on uterine and ovarian microcirculation of mice**

Changes in the diameter of the arterioles and venules in the ovarian and uterine mesentery before and after intraesophageal administration of the respective solutions for each group are outlined in Table 10 and Figure 5 and Table 11 and Figure 6 respectively. There
Table 7: **Experiment 1 Results:** Uterine epithelial thickness, number of secretory glands and diameter of the largest glands of mice after intraperitoneal administration of the Chinese herbal medicine *Cu Yun Guan Zhu Ye* or individual component Chinese herbs, compared to estradiol and saline

<table>
<thead>
<tr>
<th>Groups</th>
<th>IP Solution</th>
<th>Epithelial thickness (μm)</th>
<th>Secretory glands*</th>
<th>Cross sectional area of most glands (μm²)</th>
<th>Diameter of largest glands** (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Estradiol benzoate 50µg/ml</td>
<td>50.14±29.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.00±6.66&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>10740.29±6562.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.80±16.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>0.9% NaCl</td>
<td>5.72±1.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.71±4.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>549.81±158.44&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.61±1.84&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td><em>Cu Yun Guan Zhu Ye</em> (FPL)</td>
<td>22.22±15.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.00±5.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3588.59±3381.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.95±11.53&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td><em>Hong Hua</em> (Carthamus) 1g/ml</td>
<td>9.69±3.69&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.40±4.90&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>997.50±462.62&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>5.44±3.76&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td><em>Yin Yang Huo</em> (Epimedium) 1g/ml</td>
<td>12.42±3.63&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.56±7.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1338.48±478.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.38±2.84&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VI</td>
<td><em>Yi Mu Cao</em> (Leonurus) 1g/ml</td>
<td>10.40±2.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.42±4.96&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1243.84±549.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.24±2.94&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

IP = intraperitoneal administration; all herbal solutions are 1 g/ml; *number of glands in 30 light microscopic fields (1000X magnification); **30 glands/mouse examined; data expressed in M±SD; different lower case letters in a column indicate significant difference (p<0.05)

**Figure 2:** From Table 7, the M±SD uterine epithelial thickness, number of secretory glands and diameter of the largest glands of mice after intraperitoneal administration of the following: Group I Estradiol, Group II NaCl, Group III *Cu Yun Guan Zhu Ye*, Group IV *Hong Hua* (Carthamus,) Group V *Yi Mu Cao* (Leonurus) and Group VI *Yin Yang Huo* (Epimedium); different lower case letters in a column indicate significant difference (p<0.05)
Table 8: Experiment 2 Results: Number of mature, secondary and primordial ovarian follicles in 30 light microscopic fields (1000X magnification) of mice after intraperitoneal administration of the Chinese herbal medicine Cu Yun Guan Zhu Ye or individual component Chinese herbs, compared to estradiol and saline

Table 8:

<table>
<thead>
<tr>
<th>Groups</th>
<th>IP Solution</th>
<th>Number of ovaries*</th>
<th>Mature follicles</th>
<th>Secondary follicles</th>
<th>Primordial follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Estradiol benzoate 50µg/ml</td>
<td>9</td>
<td>45.00±17.52</td>
<td>38.89±19.12</td>
<td>15.67±11.73</td>
</tr>
<tr>
<td>II</td>
<td>0.9% NaCl</td>
<td>9</td>
<td>4.44±2.96</td>
<td>61.22±9.27</td>
<td>34.44±9.34</td>
</tr>
<tr>
<td>III</td>
<td>Cu Yun Guan Zhu Ye</td>
<td>12</td>
<td>36.83±9.96</td>
<td>47.50±10.68</td>
<td>14.00±7.78</td>
</tr>
<tr>
<td>IV</td>
<td>Hong Hua (Carthamus)</td>
<td>13</td>
<td>26.00±7.72</td>
<td>49.15±10.68</td>
<td>24.69±8.02</td>
</tr>
<tr>
<td>V</td>
<td>Yin Yang Huo (Epimedium)</td>
<td>10</td>
<td>27.30±9.75</td>
<td>45.90±7.39</td>
<td>26.50±6.19</td>
</tr>
<tr>
<td>VI</td>
<td>Yi Mu Cao (Leonurus)</td>
<td>10</td>
<td>28.90±9.17</td>
<td>49.00±9.73</td>
<td>22.10±7.91</td>
</tr>
</tbody>
</table>

IP = intraperitoneal administration; all herbal solutions are 1 g/ml; *Total number of ovaries examined for each group; data expressed in M±SD; different lower case letters in a column indicate significant difference (p<0.05) and different upper case letters indicate a very significant difference (p<0.01)

Figure 3: From Table 8, the M±SD number of mature, secondary and primordial ovarian follicles of mice after intraperitoneal administration of the following: Group I Estradiol, Group II NaCl, Group III Cu Yun Guan Zhu Ye, Group IV Hong Hua (Carthamus,) Group V Yi Mu Cao (Leonurus) and Group VI Yin Yang Huo (Epimedium); different lower case letters in a column indicate significant difference (p<0.05)
was no significant difference in the diameter of the arterioles or venules in any group prior to solution administration. At 15 and 30 minutes after solution administration, the diameters of the arterioles and venules in Group III (CYGZY), Group IV (Hong Hua [Carthamus]) and Group V (Yi Mu Cao [Leonurus]) were significantly greater \((p<0.05)\) than the arterioles and venules in Group II (NaCl) and Group VI (Yin Yang Huo [Epimedium]).

The numbers of arteriole and venule connections to the capillary network in the ovarian and uterine mesentery before and after intraesophageal administration of the respective solutions for each group are outlined in Table 12 and Figure 7. Like the diameters of the arterioles and venules, there was no significant difference in the numbers of connections to the capillary networks in any group prior to solution administration. At 15 and 30 minutes after solution administration, the number of capillary connections in Group III (CYGZY), Group IV (Hong Hua [Carthamus]) and Group V (Yi Mu Cao [Leonurus]) had significantly increased \((p<0.05)\) compared to the connections in Group II (NaCl) and Group VI (Yin Yang Huo [Epimedium]).

### Table 9: Experiment 2 Results: Size range and mean diameter of mature ovarian follicles of mice after intraperitoneal administration of the Chinese herbal medicine Cu Yun Guan Zhu Ye or individual component Chinese herbs, compared to estradiol and saline

<table>
<thead>
<tr>
<th>Groups</th>
<th>IP Solution</th>
<th>Number of follicles*</th>
<th>Size range (μm)</th>
<th>Diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Estradiol benzoate 50μg/ml</td>
<td>12</td>
<td>156-260</td>
<td>216.92±33.03^AA</td>
</tr>
<tr>
<td>II</td>
<td>0.9% NaCl</td>
<td>12</td>
<td>94-218</td>
<td>126.73±40.18^AB</td>
</tr>
<tr>
<td>III</td>
<td>Cu Yun Guan Zhu Ye</td>
<td>16</td>
<td>182-312</td>
<td>226.50±37.58^AA</td>
</tr>
<tr>
<td>IV</td>
<td>Hong Hua (Carthamus)</td>
<td>18</td>
<td>156-260</td>
<td>205.72±25.74^a</td>
</tr>
<tr>
<td>V</td>
<td>Yin Yang Huo (Epimedium)</td>
<td>14</td>
<td>166-250</td>
<td>211.71±25.93^a</td>
</tr>
<tr>
<td>VI</td>
<td>Yi Mu Cao (Leonurus)</td>
<td>16</td>
<td>109-260</td>
<td>192.13±37.52^a</td>
</tr>
</tbody>
</table>

IP = intraperitoneal administration; all herbal solutions are 1 g/ml; *total number of the largest follicles measured; data expressed in M±SD; different lower case letters in a column indicate significant difference \((p<0.05)\) and different upper case letters indicate a very significant difference \((p<0.01)\)
Figure 5: From Table 10, the M±SD diameter (μm) of 30 ovarian and uterine mesentery arterioles before and after intraesophageal administration of the Chinese herbal medicine Cu Yun Guan Zhu Ye or individual component Chinese herbs, compared to saline.

Table 10: Experiment 4 Results: Diameter (μm) of 30 ovarian and uterine mesentery arterioles before and after intraesophageal administration of the Chinese herbal medicine Cu Yun Guan Zhu Ye or individual component Chinese herbs, compared to saline.

<table>
<thead>
<tr>
<th>Groups*</th>
<th>Solution via Esophageal Intubation</th>
<th>15 minutes before solution</th>
<th>15 minutes after solution</th>
<th>30 minutes after solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>0.9% NaCl</td>
<td>68.8±13.1a</td>
<td>66.7±10.2b</td>
<td>66.7±12.9b</td>
</tr>
<tr>
<td>III</td>
<td>Cu Yun Guan Zhu Ye</td>
<td>66.7±15.1a</td>
<td>106.3±15.3a</td>
<td>108.3±18.8a</td>
</tr>
<tr>
<td>IV</td>
<td>Hong Hua (Carthamus)</td>
<td>66.7±10.2a</td>
<td>116.7±17.1a</td>
<td>116.7±12.9a</td>
</tr>
<tr>
<td>V</td>
<td>Yi Mu Cao (Leonurus)</td>
<td>56.3±10.5a</td>
<td>108.3±15.1a</td>
<td>102.1±9.4a</td>
</tr>
<tr>
<td>VI</td>
<td>Yin Yang Huo (Epimedium)</td>
<td>66.7±12.9a</td>
<td>77.1±18.4b</td>
<td>77.1±18.4b</td>
</tr>
</tbody>
</table>

* No Group I estradiol; All herbal solutions were 1 g/ml; data expressed in M±SD; different lower case letters in a column indicate significant difference (p<0.05)

Table 11: Experiment 4 Results: Diameter (μm) of 30 ovarian and uterine mesentery venules before and after intraesophageal administration of the Chinese herbal medicine Cu Yun Guan Zhu Ye or individual component Chinese herbs, compared to saline.

<table>
<thead>
<tr>
<th>Groups*</th>
<th>Solution via Esophageal Intubation</th>
<th>15 minutes before solution</th>
<th>15 minutes after solution</th>
<th>30 minutes after solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>0.9% NaCl</td>
<td>122.8±16.6a</td>
<td>117.7±17.0b</td>
<td>119.8±15.0b</td>
</tr>
<tr>
<td>III</td>
<td>Cu Yun Guan Zhu Ye</td>
<td>135.4±39.1a</td>
<td>181.3±30.4a</td>
<td>183.3±30.3a</td>
</tr>
<tr>
<td>IV</td>
<td>Hong Hua (Carthamus)</td>
<td>129.2±33.2a</td>
<td>183.3±31.3a</td>
<td>181.3±25.9a</td>
</tr>
<tr>
<td>V</td>
<td>Yi Mu Cao (Leonurus)</td>
<td>112.5±15.8a</td>
<td>175.0±26.2a</td>
<td>168.8±19.0a</td>
</tr>
<tr>
<td>VI</td>
<td>Yin Yang Huo (Epimedium)</td>
<td>135.4±9.4a</td>
<td>127.1±12.3b</td>
<td>129.2±10.2b</td>
</tr>
</tbody>
</table>

* No Group I estradiol; All herbal solutions were 1 g/ml; data expressed in M±SD; different lower case letters in a column indicate significant difference (p<0.05)
Figure 6: From Table 11, the M±SD diameter (μm) of 30 ovarian and uterine mesentery venules before and after intraesophageal administration of the following: Group II NaCl, Group III Cu Yun Guan Zhu Ye, Group IV Hong Hua (Carthamus,) Group V Yi Mu Cao (Leonurus) and Group VI Yin Yang Huo (Epimedium); different lower case letters in a column indicate significant difference ($p<0.05$); no Group I estradiol in this study

Table 12: Experiment 4 Results: Numbers of arteriole and venule connections to the capillary network of the ovarian and uterine mesentery in 30 light microscopic fields (1000X magnification) before and after intraesophageal administration of the Chinese herbal medicine *Cu Yun Guan Zhu Ye* or individual component Chinese herbs, compared to saline

<table>
<thead>
<tr>
<th>Groups*</th>
<th>Solution via Esophageal Intubation</th>
<th>15 minutes before solution</th>
<th>15 minutes after solution</th>
<th>30 minutes after solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>0.9% NaCl</td>
<td>6.6±1.5$^a$</td>
<td>5.8±1.7$^b$</td>
<td>6.2±1.7$^b$</td>
</tr>
<tr>
<td>III</td>
<td>Cu Yun Guan Zhu Ye</td>
<td>7.0±1.8$^a$</td>
<td>11.0±2.6$^a$</td>
<td>10.7±2.2$^a$</td>
</tr>
<tr>
<td>IV</td>
<td>Hong Hua (Carthamus)</td>
<td>7.2±2.1$^a$</td>
<td>10.8±2.9$^a$</td>
<td>11.3±2.8$^a$</td>
</tr>
<tr>
<td>V</td>
<td>Yi Mu Cao (Leonurus)</td>
<td>6.7±1.4$^a$</td>
<td>10.5±2.1$^a$</td>
<td>11.2±2.3$^a$</td>
</tr>
<tr>
<td>VI</td>
<td>Yin Yang Huo (Epimedium)</td>
<td>5.7±1.0$^a$</td>
<td>7.0±0.9$^b$</td>
<td>7.3±0.8$^b$</td>
</tr>
</tbody>
</table>

* No Group I estradiol; All herbal solutions were 1 g/ml; data expressed in M±SD; different lower case letters in a column indicate significant difference ($p<0.05$)

Figure 7: From Table 12, the M±SD number of arteriole and venule connections to the capillary network of the ovarian and uterine mesentery in 30 light microscopic fields (1000X magnification) before and after intraesophageal administration of the following: Group II NaCl, Group III Cu Yun Guan Zhu Ye, Group IV Hong Hua (Carthamus,) Group V Yi Mu Cao (Leonurus) and Group VI Yin Yang Huo (Epimedium); different lower case letters in a column indicate significant difference ($p<0.05$); no Group I estradiol in this study
DISCUSSION

After the ovariectomy due to loss of estrogen, the vaginal epithelium becomes thin and the surface cells have no keratinization, as demonstrated in Group II (NaCl) of experiment 1. After the mice are given adequate estradiol, the vaginal epithelium becomes thick and keratinization of the surface cells occurs as seen in Group I (estradiol) of experiment 1. In Group 1 (estradiol) of experiment 1, keratinization of almost all of the epithelial cells had occurred and the vaginal was significantly (p<0.05) or very significantly (p<0.01) thicker than any other group. In the group that received normal saline, the vaginal epithelium was significantly or very significantly thinner and the keratinization rate of the epithelial cells significantly or very significantly lower than all other groups. In the group receiving CYGZY, the degree of cell keratinization and vaginal epithelial proliferation was significantly greater than the group receiving normal saline, but significantly less than the group receiving estradiol. The CYGZY had estrogen-like effects on the vagina, although weaker than estradiol. According to the results of the study, the Hong Hua (Carthamus) and Yin Yang Huo (Epimedium) are most likely the ingredients responsible for the estrogen-like effects, as mice in these groups exhibited the same effects, as those receiving CYGZY. In a study of another follicle-stimulating decoction containing Yin Yang Huo (Epimedium) in ovariectomized mice, keratinization of vaginal epithelium also occurred consistent with the results of experiment 1 reported here.5

The morphology of the uterus is also closely related to the secretion of estrogen as demonstrated in experiment 1. After mice are ovariectomized, atrophy of the uterine horns occurs, but after adequate estrogen is restored, the uterus returns to normal, as confirmed by the results of experiment 1 where Group I (estradiol) and Group II (NaCl) of experiment 1, as the uterine epithelial thickness in Group I (estradiol) was very significantly greater (p<0.01) than that of Group II (NaCl). All the groups treated with traditional Chinese medicines were also significantly greater (p<0.05) than the NaCl group, but significantly less than the group receiving estradiol. The uterine epithelial thickness was significantly greater in the group receiving CYGZY, than groups receiving any individual Chinese herbal ingredient. The conclusion was that CYGZY and to a lesser extent the individual Chinese herbal ingredients have estrogen-like effects on the uterus, although weaker than estradiol. In another study, Yin Yang Huo (Epimedium) was shown to increase the weight of the uterus of normal rats, consistent with the finding of the current experiment 1.6 The electron microscopic changes of the uteri further support the conclusion that CYGZY has estrogen-like effects, but less than estradiol.

In a study by Alyer et al a small dose of estrogen promoted the luteinizing hormone-releasing hormone (LHRH) receptor response and the pituitary response to LHRH, promoting follicle development and ovulation in rats.7 Small doses of estrogen cause secretion of LHRH via a feedback mechanism on the hypothalamus to stimulate the anterior pituitary gland to release luteinizing hormone and promote follicular development and ovulation in cattle.8 As can be seen from the results of experiment 2, CYGZY stimulates primordial and secondary ovarian follicular development of young mice to the same degree and mature ovarian follicular development only slightly less than estradiol. The CYGZY was shown to have estrogen-like effects on the ovaries, although slightly weaker than estradiol in experiment 2. In a study of female rats, Yin Yang Huo (Epimedium) decoction administered at a dose of 1ml/100g weight increased the pituitary response to LHRH, 5 days after drug administration.9 The estrogen-like effects of CYGZY and Yin Yang Huo (Epimedium) are most likely achieved through feedback to the hypothalamus similar to estradiol.

The CYGZY may adjust the dynamic equilibrium of the sexual cycle and enhance the function of the hypothalamus-pituitary-ovarian axis, as described in traditional Chinese veterinary medicine theory of Kidney-Chong and Ren Tian Gui (Uterus).9 Li Chaojing et al presented a discussion of this, when they explored the mechanism of the Kidney taking charge of reproduction and ovulation an provided important insights into the mechanism of using CYGZY to treat infertility due to ovarian inactivity.

High levels of uterine AP in cattle are related to sperm motility and fertility, while low levels of AP have been correlated with infertility.10 Studies have shown that AP can cause hydrolysis of β-monoglyceride and hence increase energy in epithelial cells and glands of the endometrium.11 Evaluation of the degree of deposition of black cobalt sulfide in the top regions of the endometrial epithelial cells and glands and the longitudinal muscle layer in experiment 3 showed CYGZY increased endometrial AP of the mice in the same way as the estradiol, but to a lesser degree. CYGZY may be useful to increase the activity of the uterine AP to promote estrus and increase impregnation rates in other species. Although the estrus inducing effects of CYGZY are slower than estradiol, cattle treated with CYGZY have a higher pregnancy rate in the first estrus, which may be related to increased AP.2

In experiment 4, CYGZY was shown to dilate ovarian and uterine arterioles and venules and increase their connections to the capillary network, thus promoting uterine-ovarian microcirculation. The ingredients Hong Hua (Carthamus) and Yi Mu Cao (Leonurus) were primarily responsible for the increased blood flow from CYGZY administration. Increased blood flow to the uterus and the ovaries can enhance the activity of endometrial secretory cells. Luteolytic substances released by the uterus reach the ovaries via the uterine-ovarian veins and because of its ability to dilate blood vessels CYGZY may be an effective treatment for persistent corpus luteum.11 In the author'
experience in cattle with infertility due to retained corpus luteum, 5-7 days after treatment with CYGZY the serum progesterone levels gradually decrease and the level of the serum estradiol levels increase producing estrus.

In another in-vitro and in-vivo uterine motility study of rabbits, the authors found that CYGZY increased uterine contraction amplitudes compared to saline. Hong Hua (Carthamus) alone produced a stronger simulating effect even resulting in tonic uterine contractions Yin Yang Huo (Epimedium) alone reduced uterine contraction amplitude. The conclusion was that CYGZY produces a mild increase in uterine motility resulting from a balance between the strong excitatory effects from Hong Hua (Carthamus) and moderate inhibitory effects from Yin Yang Huo (Epimedium).

In conclusion, from the results of these 4 studies it can be seen that CYGZY has a mild estrogen-like effect on vaginal and uterine epithelium, ovarian follicle production and endometrial AP levels and improves the uterine and ovarian microcirculation in mice. Because CYGZY has a milder effect than estradiol, it may provide a novel and effective treatment for infertility in other species due to ovarian inactivity and persistent corpus luteum of other species with less untoward side effects and residues in meat and milk.

Acknowledgement: The authors wish to thank Professors Xu Funan, Chen Wanfang, Hua Xingbang for their guidance and Zhang Liuliang, Xu Liren, Dai Xingting, Xu Kuiwu, Jin Hui for their participation in these studies.

FOOTNOTES

a Dahua Pharmacy of Chinese Medicine in Nanjing, Jiangsu province, China
b Shanghai 9th Pharmaceutical Factory, Shanghai, China
c Experimental animals were obtained from Nanjing Medical University experimental animal center

REFERENCES
Dr. Uwe Petermann DVM

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A Randomized and Controlled Study Using an Acupoint Diagnosis and Treatment Instrument to Determine the Phase of Ovarian Follicular Development, Alter Estradiol and Progesterone Release and Improve Pregnancy Rates in Dairy Cows

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ABSTRACT

Bovine pregnancy rates have been decreasing, causing economic hardship in the dairy industry. A simple, quick and inexpensive diagnostic method to determine ovarian follicular phase and optimal breeding times is needed. The hypothesis of this randomized controlled study was that the XXH-IIA Acupoint Diagnosis and Therapeutic Instrument (developed by the Lanzhou Institute of Husbandry and Pharmaceutics Science) would be useful to diagnose phases of follicular development and treat acupoints, altering hormone levels and improving pregnancy rates in cows. The resistance was measured bilaterally at acupoints Shen-pang and Yan-pang (new bovine acupoints described by the authors) and the classical acupoint Luan-chao. Very significant unbalanced resistance between left and right acupoints ($p<0.01$) were found for Yang-pang during phase 2, Luan-chao during phase 3 and Shen-pang during phase 4, but not in cows with inactive ovaries. Electro-stimulation of Shen-pang and Yan-pang produced a significant increase in serum 17β-estradiol levels ($p<0.05$) 20 minutes after electro-stimulation in 14/25 cows (56%) and 13/42 cows (31%) respectively. Electro-stimulation of Shen-pang and Luan-chao produced a significant increase in serum progesterone levels ($p<0.05$) in 7/20 cows (35%) and 9/13 cows (69.2%), 20 minutes after electro-stimulation, with a trend to develop peak levels in 3-7 hours. Unilateral electro-stimulation of Shen-pang or Yan-pang (cows in phase 2 or 4 respectively) very significant increased the pregnancy rate ($p=0.001$) in 200 cows compared to 130 controls. Evaluation of bilateral low resistance characteristics and electro-stimulation of Shen-pang, Luan-chao or Yan-pang may be an effective diagnostic and treatment method to improve fertility in cows.

Key words: acupoint low resistance, acupuncture, fertility of dairy cows, reproductive hormones, acupoint electro-stimulation, traditional Chinese veterinary medicine

ABBREVIATIONS

| AC | Alternating current |
| B  | Balanced value (electrical resistance between left and right bilateral acupoints) |
| DC | Direct current |
| Hz | Hertz or cycles per second |
| M±SD | Means plus or minus the standard deviation |
| P4 | Progesterone |
| TCM | Traditional Chinese medicine |

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During each estrous cycle of dairy cows, 2-4 phases (waves) of follicular growth occur. In each phase of growth a dominant follicle emerges and matures to a pre-ovulatory stage, while the others undergo atresia. Pregnancy rate directly affects the economic benefits of dairy cow production and can be influenced by many factors, such as semen quality, breeding technology, feeding, reproductive diseases and the estrus condition of cows. Bovine pregnancy rates have been reducing in China and are causing economic hardship in the dairy industry of China. Insemination at the correct time within an ovarian follicular phase can increase pregnancy rates in dairy cows and thus milk production in the dairy industry. Clinical observations and rectal palpation of ovaries are routinely performed to estimate...
ovarian follicular phases. More accurate methods to determine follicular phases (e.g. ultrasonography and reproductive hormone measurements) are time consuming and expensive. A simple, quick and inexpensive diagnostic method to determine follicular phase and optimal breeding times is needed.3

In traditional Chinese medicine (TCM) theory, acupuncture points (acupoints) and Channels have been suggested to have unique electrical properties.4 Applying an external electrical source to the acupoint, results in increased conductance, reduced resistance and impedance, increased capacitance and elevated electrical potentials, compared to adjacent non-acupoint skin areas.4 The electrical resistance (opposition to the flow of applied direct current [DC]) of acupoints is often measured with an ohmmeter and is the basis of many acupoint locator devices. Electrical impedance (the opposition to the flow of applied alternating current [AC]) of acupoints is more challenging to measure, as a source of AC electrical stimulation and an instrument to measure AC voltage and current are needed.4 Electrodermal alterations of acupoints, compared to surrounding tissue, have been an important biophysical measurement, supporting the objective reality of the presence of acupoints.5 However, skin resistance and impedance vary with the acupoint evaluated and the amount of current applied for the measurement and has resulted in conflicting results from different studies.5,6 The final electrodermal reading is dependent on electrode polarizability, stratum corneum resistance, presence of sweat glands, choice of contact medium, electrode geometry and other factors.4 Details of the electrical basis of acupoint detecting, monitoring and diagnosis can be found elsewhere.4 The electrical current applied to acupoints for diagnosis is much less than that typically applied for treatment of an acupoint.

Over the past 60 years various electrical devices have been developed to detect and monitor acupoints and to identify imbalances between acupoints associated with different diseases in the body.4 Many popular acupoint diagnostic devices are DC-resistance devices, using a monopolar arrangement prone to electrode polarizing effects that can interfere with resistance measurements. An instrument called the XXH-IIA Acupoint Diagnosis and Therapeutic Instrument was developed by the Lanzhou Institute of Husbandry and Pharmaceutics Science7 and has been used clinically for acupoint diagnosis and treatment for over 30 years.7,8 The XXH-IIA Acupoint Diagnosis and Therapeutic Instrument has a multipolar composite surface electrode probe that delivers a 20 µA and 20 Hz DC-current and records the resistance at 9 sites within a 1 cm² area over the acupoint when in diagnostic mode (Figure 1). The acupoint site of lowest resistance, out of the 9 sites evaluated, can then be electrically stimulated with a 40 Hz and 6-8 volt pulse DC current using the same instrument in treatment mode. In this study, the XXH-IIA Acupoint Diagnosis and Therapeutic Instrument was

![Figure 1: XXH-IIA Acupoint Diagnosis and Therapeutic Instrument used in this study; the white arrow points from the 1.5 cm diameter surface electrode probe to a diagram of the 9 sub-electrodes embedded in the probe head used to detect the area of lowest resistance within the acupoint; the probe on the left is the reference electrode; in this study an alligator clip electrode was used at the reference acupoint Wei-ben](image-url)
used as a diagnostic and therapeutic instrument.

The hypothesis of this randomized controlled clinical study was that the XXH-IIA Acupoint Diagnosis and Therapeutic Instrument would be useful to diagnose stages of ovarian follicular development and treat acupoints, resulting in alterations of hormone levels and improved pregnancy rates in dairy cows. The objectives of the diagnostic part of the study were to: 1) measure resistance at left and right bilateral acupoints Shen-pang, Luan-chao and Yan-pang and identify resistance imbalances between the 2 sides in cows in the 2nd, 3rd and 4th phases of follicular development 2) determine if resistance imbalances between left and right bilateral acupoints could be correlated with the phase of follicular development, 3) compare the percentage of acupoint resistance imbalances of cows with ovarian activity to cows with inactive ovaries. The objectives of the treatment part of the study were to: 1) measure serum 17β-estradiol and progesterone (P4) levels in cows in estrus before and at several time periods after electro-stimulation of acupoints Shen-pang, Luan-chao and Yan-pang and 2) compare pregnancy rates of cows that received unilateral electro-stimulation of Yan-pang (phase 2 cows) and Shen-pang (phase 4 cows) before and after insemination to un-stimulated inseminated control cows. The aim of the study was to evaluate the use of the XXH-IIA Acupoint Diagnosis and Therapeutic Instrument for routine use on dairy farms to diagnose stages of follicular development to determine optimum insemination times and provide electro-stimulation of specific acupoints to improve pregnancy rates.

MATERIALS AND METHODS

In the diagnostic part of the study, the resistance of 3 acupoints suspected to reflect the phase of follicular development were measured and resistance differences between left and right bilateral acupoints identified. Inclusion criteria for the acupoint resistance part of the study included healthy, black and white dairy cows with visible signs of estrus behavior and whose stage of ovarian follicular development could be determined by rectal palpation. The ovaries of the cows were palpated per rectum at 8 am, 5 pm and 11 pm daily and the phase of ovarian follicular development determined and recorded. Then 60 cows in the 2nd follicular phase, 84 cows in the 3rd follicular phase and 50 cows in the 4th follicular phase were selected by simple randomization to be included in the study, for a total of 194 cows in one of 3 follicular phases. Another 10 dairy cows from the same farm with quiescent ovarian function were also randomly selected to serve as a control group. All cows remained housed at the Lanzhou breeding farm under the same feeding and living conditions and following the Chinese “Guide for the Care and Use of Agricultural Animals in Research and Teaching”. The Gansu Province Institute of Experimental Animals, Gansu, China, oversaw all experiments

Based on the authors’ previous experiences, bilateral acupoints Shen-pang, Yan-pang and Luan-chao were selected for left and right resistance measurements. A reference electrode was placed on the midline at the acupoint Wei-ben (Figure 2). Shen-pang and Yan-pang are 2 new acupoints associated with reproduction in cows discovered by the authors through previous evaluation of low resistance characteristics of other acupoints and surrounding areas. Shen refers to the classical acupoint Shen-jiao and pang means “at the side”. Therefore Shen-pang (not to be confused with classical acupoint Shen-peng) is located lateral and caudoventral to Shen-jiao. Shen-pang can be found by identifying Bai-hui at the lumbosacral space on the midline and moving caudoventrally, along the popliteal groove of the gluteus medius to the depression at the intersection of the grooves of the gluteus medius and the biceps flexor cruris (Figure 2).

For acupoint Yan-pang, the Yan refers to the classical acupoint Yan-zhi and pang means “at the side.” Yan-pang is therefore located lateral and cranioventral to Yan-zhi (on the cranial edge of the wing of the ilium; equine classical acupoint Yan-chi) and can be located in the muscle depression at the intersection of the cranial edge of the transverse processes of the 5th lumbar vertebra (Figure 2). Yan-pang can be found by moving craniodorsally from the dorsal edge of the external angle of the ilium or by identifying Bai-hui at the lumbosacral space on the midline and moving cranioventrally to the muscular depression at a line parallel to the cranial edge of the transverse processes of the 5th lumbar vertebra. Acupoint Luan-chao is located at the craniolateral edge of the transverse process of the 4th lumbar vertebra in line with Yan-pang (Figure 2). The classical acupoint Wei-ben is located on the ventral side of the tail between the 5th and 6th caudal vertebrae (Figure 2).

A 1.5 cm diameter area of hair was shaved at acupoints Shen-pang, Yan-pang, Luan-chao and Wei-ben. The skin was cleaned with gauze and water and then degreased with alcohol. A reference electrode was fastened to acupoint Wei-ben with physiological saline soaked gauze and an alligator clip. The terminal of the 1.5 cm diameter electrode probe (composed of the 9 sub-electrodes) was attached to the XXH-IIA Acupoint Diagnosis and Therapeutic Instrument. Electrode cream was applied to the acupoint to be tested and the electrode probe was pressed onto the skin over the acupoint. In diagnostic mode, the XXH-IIA Acupoint Diagnosis and Therapeutic Instrument automatically calculated a low resistance percentage (P) for each acupoint on left and right sides as follows: P = R / R_{mo} × 100 (R is the resistance (ohms) of the lowest resistance point of the 9 points of the acupoint measured and R_{mo} is the artificial resistance (ohms) associated with XXH-IIA Acupoint Diagnosis and Therapeutic Instrument. The numerical values were recorded and printed by a mini-printer. The P of left and right Shen-pang, Yan-pang and Luan-chao were measured, compared and a balance (B) value automatically calculated by the instrument. If the B value was less than (<) 1500, bilateral acupoints were
considered balanced and if the B value was greater than (> 1500, resistance between left and right acupoints was considered imbalanced. The in cidence of the resistance imbalance of bilateral acupoints was compared using Chi-square tests of crosstabs. The \( p \) value was set at \(< 0.05\) indicating a significant difference between groups.

In the treatment effects on reproductive hormone levels part of the study, the effect of electrical stimulation of Yan-pang, Luan-chao or Shen-pang unilaterally on serum 17\(\beta\)-estradiol were evaluated in cows randomly selected (simple randomization) from each follicular phase group of the first part of the study. Based on the largest percentage of bilateral acupoints that were out of balance in the 1\textsuperscript{st} part of the study, Yan-pang was stimulated in 42 cows in phase 2 of follicular development, Luan-chao in 13 cows during phase 3 and Shen-pang in 25 cows during phase 4.

The point of lowest resistance, within the 1.5 cm area of the acupoint that was identified using the XXH-IIA Acupoint Diagnosis and Therapeutic Instrument was the site deemed most sensitive to electrical stimulation and received electro-stimulation in this study. Only the acupoint, with the lower resistance of the bilaterally unbalanced acupoint pair, received monopolar electro-stimulation as the reference electrode remained at acupoint Wei-ben. In the authors' experience, the side of the lowest resistant acupoint correlates with the side of the active ovary. A pulsed current of 40 Hz and duration of 0.3 seconds was administered for 5 minutes. The intensity was slowly increased to the highest yet tolerable level (average 6-8 volts) for each cow. Before electro-stimulation and 20 minutes afterwards, 10 ml of blood were collected from the jugular vein, centrifuged to collect serum and stored at -20°C. Serum 17\(\beta\)-estradiol levels were measured using a radioimmunoassay reagent kit and LKB-1217 liquid scintillation counter. The mean values of serum 17\(\beta\)-estradiol before and after electro-stimulation were compared using the t-test for independent samples. The \( p \) value was set at \(< 0.05\) as a significant difference and \(< 0.01\) as a very significant difference between pre-stimulation and post-stimulation means plus or minus the standard deviations (M±SD).

Serum progesterone (P4) was also measured before and 20 minutes after acupoint electro-stimulation of 21 cows randomly selected (simple randomization) from the
above Yang-pang-stimulated phase 2 group, 13 cows from the Luan-chao-stimulated phase 3 group and 20 cows from the Shen-pang-stimulated phase 4 group of cows. Serum P4 levels were measured using a radioimmunoassay reagent kits and LKB-1217 liquid scintillation counter.

Five cows were randomly selected from the 25 cows in the Sheng-pang-stimulated group and serum P4 levels were determined before and after electro-stimulation every 20 minutes for 120 minutes. Four different cows in phase 4 of ovarian follicular development from the same herd were randomly selected and Qiang-feng (SI-9) was stimulated and serum P4 levels were also followed before and after every 20 minutes after electro-stimulation. The serum P4 levels of the same 4 cows that received electro-stimulation of Qiang-feng (SI-9) were also evaluated at 3, 5, 7, 9 and 12 hours after electro-stimulation.

In the treatment effects on pregnancy rates part of the study, dairy cows with visible estrus behavior were checked for follicular development by rectal examination at 8 am, 5 pm and 11 pm every day until the follicular phase could be determined. Based on the stage of follicular development, acupoint Yan-pang (phase 2) or Shen-pang (phase 4) was stimulated before and after insemination in 200 cows randomly selected from the group that had unbalanced bilateral acupoints. Again the acupoint with the lowest resistance of the bilateral pair was stimulated with 6-8 volts of pulsed current at 40 Hz for 5 minutes before and 10 minutes immediately after each stimulation. Artificial insemination occurred twice, once in the evening and again the next morning and a total of 4 electro-stimulation treatments of the single acupoint were administered. Another 130 cows were randomly selected to serve controls and were evaluated via rectal palpation and were inseminated 1 evening and the next morning, but did not receive acupoint diagnosis or treatment. Different cows were inseminated each month over a 5-month period (June-October). Pregnancy was determined by rectal palpation 60 days after insemination. The incidence of the pregnancy between the acupoint-stimulated and control groups was compared using Chi-square tests of crosstabs. The \( p \) value was set at <0.05 as a significant difference between groups.

RESULTS

The number and percentage of cows with unbalanced bilateral acupoints for Yan-pang, Luan-chao and Shen-pang in 194 cows in each ovarian follicular development phase and 10 cows with inactive ovaries are outlined in Table 1 and illustrated in Figure 3. All cows in all follicular stages had some incidence of imbalance of all 3 acupoints, but a very significantly higher incidence for each acupoint was found to occur in a different phase of follicular development. In the 2nd phase of follicular development, a resistance imbalance between left and right Yan-pang occurred in 45/60 cows (75%), which was very significantly greater (\( p<0.01 \)) than Yan-pang in any other follicular phase, the other 2 acupoints during the 2nd phase and control cows (0%). In the 3rd phase of follicular development, a resistance imbalance between left and right Luan-chao occurred in 59/84 cows (70.2%), which was very significantly greater (\( p<0.01 \)) than Luan-chao imbalances in any other phase, the other 2 acupoints during the 3rd phase and control cows (10%). In the 4th phase of follicular development, a resistance imbalance between left and right Shen-pang occurred in 39/50 cows (78%), which was very significantly greater (\( p<0.01 \)) than Shen-pang imbalances in any other phase, the other 2 acupoints during the 4th phase and control cows (10%). There was only 1/10 cows (10%) with inactive ovaries (control group) in which the resistance of the bilateral acupoint Luan-chao was not in balance; the resistances of the other 9/10 cows (90%) were in balance.

The M±SD serum 17β-estradiol levels (pg/ml), before and 20 minutes after electro-stimulation of acupoints Shen-pang, Luan-chao and Yan-pang are outlined in Table 2. Electro-stimulation of Shen-pang and Yan-pang produced a significant increase (\( p<0.05 \)) in serum 17β-estradiol levels 20 minutes after electro-stimulation in 14/25 cows (56%) and 13/42 cows (31%) respectively, compared to pre-stimulation levels. Electro-stimulation of Luan-chao produced an almost equal amount of slight increases and decreases in serum 17β-estradiol levels, but these were not significant. Although there were decreases in serum 17β-estradiol levels 20 minutes after electro-stimulation of Shen-pang and Yan-pang, these were not significantly different either.

The M±SD serum P4 levels (pg/ml), before and 20 minutes after electro-stimulation of acupoints Shen-pang, Luan-chao and Yan-pang are outlined in Table 3. Electro-stimulation of Shen-pang and Luan-chao produced a significant increase (\( p<0.05 \)) in serum P4 levels in 7/20 cows (35%) and 9/13 cows (69.2%), 20 minutes after electro-stimulation compared to pre-stimulation levels. Although electro-stimulation of Yan-pang produced a >5% increase in serum P4 levels in 13/21 cows (61.9%), the increase was not significant. Electro-stimulation of Shen-pang, Yan-pang and Luan-chao also produced a >5% decrease in serum P4 levels in some cows, but these also were not significant.

The overall number and percentage of cows to have a >5% change in serum 17β-estradiol and P4 levels 20 minutes after electro-stimulation of Shen-pang, Yan-pang and Luan-chao are outlined in Table 4. Electro-stimulation of Shen-pang either increased (56%) or decreased (32%) serum 17β-estradiol levels in 25 cows 88% of the time. Electro-stimulation of Yan-pang either increased (31%) or decreased (50%) serum 17β-estradiol levels in 42 cows 81% of the time. Electro-stimulation of Luan-chao either increased (53.8%) or decreased (46.2%) serum 17β-estradiol levels in 13 cows 100% of the time. Stimulation of Shen-pang either increased
Figure 3  The resistance unbalance percentages of left and right bilateral acupoints Yan-pang, Luan-chao and Shen-pang at in the 2nd, 3rd and 4th phases of ovarian follicular development

Table 1: The number and percentage of cows with unbalanced resistance between left and right bilateral acupoints Yan-pang, Luan-chao and Shen-pang in the 2nd, 3rd and 4th phases of ovarian follicular development

<table>
<thead>
<tr>
<th>Phase</th>
<th>N</th>
<th>Yan-pang</th>
<th>Luan-chao</th>
<th>Shen-pang</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>%</td>
<td>I</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>45</td>
<td>75.0**</td>
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</tr>
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<tr>
<td>C</td>
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<td>0</td>
<td>1</td>
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</tbody>
</table>

N= total number of cows evaluated in each follicular phase; I= number of cows with imbalanced bilateral acupoints; %= the percentage cows with unbalanced bilateral acupoints; C= control cows with inactive ovaries; **= very significant difference $p<0.01$

Table 2: Mean±standard deviation (M±SD) of serum17β-estradiol levels (pg/ml) before and 20 minutes after unilateral electro-stimulation of acupoints Shen-pang, Yan-pang and Luan-chao

<table>
<thead>
<tr>
<th>Acupoint</th>
<th>Pre-stimulation levels</th>
<th>Level Increased</th>
<th>Level Decreased</th>
<th>Level Unchanged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>M±SD</td>
<td>NI</td>
<td>M±SD</td>
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<td>Shen-pang</td>
<td>25</td>
<td>10.12±0.98</td>
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<td>11.49±1.24*</td>
</tr>
<tr>
<td>Yan-pang</td>
<td>42</td>
<td>10.29±1.24</td>
<td>13</td>
<td>10.98±1.13*</td>
</tr>
<tr>
<td>Luan-chao</td>
<td>13</td>
<td>10.59±1.21</td>
<td>7</td>
<td>11.35±1.03</td>
</tr>
</tbody>
</table>

N= total number of cows; NI= number of cows in which serum17β-estradiol levels (pg/ml) increased; ND= number of cows in which serum17β-estradiol levels (pg/ml) decreased; NU= number of cows in which serum17β-estradiol levels (pg/ml) remain unchanged; *= significant difference $p<0.05$
(35%) or decreased (65%) serum P4 levels in 20 cows 100% of the time. Electro-stimulation of Yan-pang either increased (61.9%) or decreased (38.1%) serum P4 levels in 21 cows 100% of the time. Electro-stimulation of Luan-chao either increased (69.2%) or decreased (23.1%) serum P4 levels in 13 cows 82.3% of the time.

The serum P4 levels (pg/ml) before and 20, 40, 60, 80 and 120 minutes after electro-stimulation of Shen-pang in 5 cows in phase 4 ovarian follicular development and Qiang-feng (SI-9) in 4 cows in phase 4 are outlined in Table 5 and illustrated in Figure 4. Although there were too few cows studied to evaluate statistically, there did seem to be a trend for serum P4 levels to increase over the 120-minute period after Shen-pang electro-stimulation and to decrease after Qiang-feng electro-stimulation.

Table 4: Number and percentage of cows with a >5% increase or decrease in serum 17β-estradiol and progesterone levels, 20 minutes after unilateral electro-stimulation of acupoints Shen-pang, Yan-pang or Luan-chao

The serum P4 levels (pg/ml) before and 3, 5, 7, 9 and 12 hours after electro-stimulation of Shen-pang in 5 cows in phase 4 ovarian follicular development and Qiang-feng (SI-9) in 4 cows in phase 4 are outlined in Table 6. Again, there were too few cows studied to evaluate statistically, but there did seem to be a trend for serum P4 levels to peak between 3-7 hours and then return to or below pre-stimulation levels after Shen-pang electro-stimulation, except in 2 cows that ovulated and experienced a typical sharp rise in P4 levels. Serum P4 levels remained decreased in the 4 cows receiving Qiang-feng electro-stimulation after 12 hours.

The results of electro-stimulation of Shen-pang or Yan-pang on pregnancy rates are outlined in Table 7, and illustrated in Figure 5. In July, the acupoint electro-stimulation group (44 cows) had a significantly (p<0.05) increased pregnancy rate, compared to the control group (43 cows). For the other individual months there was no significance difference, but of all the 200 cows receiving electro-stimulation, 69.5% became pregnant compared to 51% of the 140 un-stimulated control cows. Overall electro-stimulation very significantly increased the pregnancy rate (p=0.001).
Table 5: Serum progesterone (P4) levels (pg/ml) before and 20, 40, 60, 80 and 120 minutes after unilateral electro-stimulation of Shen-pang or Qiang-feng (SI-9)

<table>
<thead>
<tr>
<th>Acupoint</th>
<th>Animal</th>
<th>Pre-Stimulation Levels (pg/ml)</th>
<th>Levels Minutes after Acupoint Stimulation (pg/ml)</th>
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<tbody>
<tr>
<td></td>
<td></td>
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<tr>
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<tr>
<td>D 294</td>
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<tr>
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<td>±22.89</td>
<td>±7.44</td>
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<td>Qiang-feng</td>
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<tr>
<td>GS 266</td>
<td>184</td>
<td>170</td>
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Figure 4: The levels of progesterone (P4) in the plasma before and at time periods up to 12 hours after the electro-stimulation of acupoints Shen-pang and Qiang-feng (SI-9); m= minutes and h= hours
Table 6: Serum progesterone (P4) levels (pg/ml) before and 4, 5, 7, 9 and 12 hours after unilateral electro-stimulation of Shen-pang or Qiang-feng (SI-9)

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<thead>
<tr>
<th>Acupoint</th>
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<td>184</td>
<td>113</td>
<td>144</td>
</tr>
<tr>
<td>GS 128</td>
<td>107</td>
<td>95</td>
<td>101</td>
</tr>
<tr>
<td>H 568</td>
<td>102</td>
<td>80</td>
<td>58</td>
</tr>
<tr>
<td>L 967</td>
<td>140</td>
<td>99</td>
<td>107</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>133.25</td>
</tr>
</tbody>
</table>

^Ovulation occurred and caused the rise in P4 levels

Table 7: The number and percentage of cows with and without unilateral electro-stimulation of Shen-pang or Yan-pang compared to an un-stimulated control group by month inseminated and overall

<table>
<thead>
<tr>
<th>Month</th>
<th>Control Group</th>
<th>Acupoint Stimulation Group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
<td>%</td>
</tr>
<tr>
<td>June</td>
<td>24</td>
<td>11</td>
<td>45.8</td>
</tr>
<tr>
<td>July</td>
<td>43</td>
<td>21</td>
<td>48.8</td>
</tr>
<tr>
<td>August</td>
<td>39</td>
<td>20</td>
<td>51.3</td>
</tr>
<tr>
<td>September</td>
<td>13</td>
<td>9</td>
<td>69.2</td>
</tr>
<tr>
<td>October</td>
<td>11</td>
<td>6</td>
<td>54.5</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td>67</td>
<td>51.5</td>
</tr>
</tbody>
</table>

N= number of cows inseminated, P= number of cows that became pregnant; % = percentage of cows that became pregnant; *significant difference (p<0.05); very significant difference (p<0.01)
DISCUSSION

Using a surface probe electrode, the XXH-IIA Acupoint Diagnosis and Therapeutic Instrument can be used in the diagnostic mode to measure acupoint resistance, detect imbalances between left and right bilateral acupoints and identify the site of lowest resistance within the acupoint location. The instrument can be immediately changed to the treatment mode and provide electro-stimulation to the most sensitive (lowest resistance) site within the acupoint location, as was performed in this study.

In another similar study, the XXH-IIA Acupoint Diagnosis and Therapeutic Instrument was used in 59 horses and donkeys in estrus and showed that follicular development and ovulation were related to an increased rate of resistance imbalance between left and right Shen-jiao acupoints and with a decreased rate of resistance imbalance between left and right Yan-chi acupoints. In another study of cows with traumatic reticulitis, resistance imbalances of bilateral classical acupoints Pi-shu, Wei-shu and Guan-yuan were found.

Based on the results of the acupoint resistance measurements in this study, imbalances of left and right acupoints Shen-pang, Luan-chao and Yan-pang could be used to determine the follicular phase on average 74.4% of the time (Yan-pang imbalances 75.0% during the 2nd phase, Luan-chao imbalances 70.2% during the 3rd phase, and Shen Pang imbalances 78.0% during the 4th phase). These results demonstrate the relationship of these acupoints to ovarian activity, supporting the TCM theory that some acupoints are related to internal organs. Even more importantly to the dairy industry, imbalances of these acupoints may be used to help determine the best time for artificial insemination of dairy cows in estrus to improve pregnancy rates. If the time of insemination is too early, the sperm will die before the ova are released. If insemination occurs too late there is a low rate of fertilization. Estrus behavior of dairy cows and the time of their follicular development and ovulation are not fixed, and the best time to inseminate is not determined just by the estrus behavior, but by the phase of the follicular development. It is best to inseminate immediately pre-ovulation. With the correct instrumentation, acupoint resistance measures are simple, easier to do and less expensive, than using ultrasonography and reproductive hormone levels to diagnose the stage of follicular development and ovulation.

One of the limitations of this part of the study is the difficulty to determine the actual phase of ovarian follicular development via rectal palpation of ovaries. The purpose of this part of the study was to find a simple technique that would be more accurate than rectal palpation alone to determine the follicular phase. Ultrasonography is currently the best way to determine the follicular phase, but this technology was not available. To somewhat overcome this limitation, the ovaries were palpated 3 times a day until a follicular phase could best be determined. Repeating this part of the study, using ultrasonography to determine follicular phase and then looking at acupoint imbalances would ensure the findings in this study based on rectal palpation are accurate.

The results of the study also showed that unilateral
electro-stimulation of acupoints Shen-pang, Luan-chao and Yan-pang affected the release of 17β-estradiol and P4 of dairy cows in estrus. Unilateral electro-stimulation of Shen-pang and Yan-pang produced a significant increase \((p<0.05)\) in serum 17β-estradiol levels in some cows 20 minutes after electro-stimulation. Unilateral electro-stimulation of Shen-pang and Luan-chao produced a significant increase \((p<0.05)\) in serum P4 levels in some cows, 20 minutes after electro-stimulation. Overall electro-stimulation produced at least a 5% change in pre-stimulation levels in the majority of cows. If larger numbers of cows were studied, other significant changes may have been demonstrated. Also in the 5 cows that were studied long term, the P4 serum levels peaked between 3-7 hours (Table 6). Although there were too few animals in this part of the study to statistically compare, there was an obvious rise in serum P4 levels. Further studies on larger numbers of cows are needed to statistically confirm the significance of this trend. Qiăng-feng (SI-9) was selected as a control acupoint thought to have minimal effects on reproductive hormones. Unilateral electro-stimulation of the acupoint Qiăng-feng (SI-9) in 4 cows reduced the serum P4 levels, but again a study with larger numbers of cows are needed in order to statistically confirm the significance of this trend. Acupoint surface electro-stimulation led to increased release of 17β-estradiol and P4 in some cows, but reduced release in others. This was not surprising, as in the author’s experience, acupoint stimulation can produce bimodal balancing and regulatory effects that depend on the initial condition of the cow. As per the results of the serum hormone studies, acupoint electro-stimulation altered serum reproductive hormone levels and this may be 1 of the mechanisms involved in the therapeutic effect.

It was reported that in two groups of 3,148 and 568 dairy cows in China, the pregnancy rate was 38.2-52.3% (depending on the season) and 51.7% respectively. In another report, the annual mean pregnancy rate of 4,634 dairy cows in Shenzhen, China, was 33.8% and as low as 23.1-51.3% depending on the season. The pregnancy rate per artificial insemination (PR/AI) in lactating dairy cows has decreased from 66% in 1951 to about 50% in 1975 and about 40% in 1997, while PR/AI in heifers has remained at 70% during this same period. This disparity in PR/AI cannot therefore be attributed to differences in genetic selection or semen quality between heifers and lactating cows; it is likely due to physiological changes or stresses associated with increased milk production per cow that have occurred during this period. From the results of this study electro-stimulation of Yan-pang or Shen-pang before and after insemination increased the pregnancy rate to 69.5%. In this study that was 18% greater than the pregnancy rate of cows that did not receive acupoint electro-stimulation. In conclusion, the hypothesis of the study was confirmed as the XXH-IIA Acupoint Diagnosis and Therapeutic Instrument was useful to diagnose stages of ovarian follicular development and treat acupoints, resulting in alterations of reproductive hormone levels and improved pregnancy rates in dairy cows. Evaluation of the low resistance characteristics of acupoints followed by electro-stimulation of the most sensitive part of the acupoint using the XXH-IIA Acupoint Diagnosis and Therapeutic Instrument may be a novel, quick, inexpensive and effective diagnostic and treatment technique to improve fertility and subsequently milk production for the dairy industry.

**FOOTNOTES**

a Lanzhou Institute of Husbandry and Pharmaceutics Science, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu, China
b From the Dairy Farm of Baiyin District and Dairy Cow Breeding Farm of Lanzhou, Gansu Province, China
c Model-150 II mini-printer, made by EPSON, Japan
d Statistical Package for Social Science (SPSS) 19 for Windows, IBM
e Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai, China
f LKB Wallac, Sollentuna, Sweden

**REFERENCES**

8. Komatsu S. Research on the acupuncture point Hou
ABSTRACT


Characteristics of acupuncture treatment associated with outcome: an individual patient meta-analysis of 17,922 patients with chronic pain in randomised controlled trials.


BACKGROUND:
Recent evidence shows that acupuncture is effective for chronic pain. However we do not know whether there are characteristics of acupuncture or acupuncturists that are associated with better or worse outcomes.

METHODS:
An existing dataset, developed by the Acupuncture Trialists' Collaboration, included 29 trials of acupuncture for chronic pain with individual data involving 17,922 patients. The available data on characteristics of acupuncture included style of acupuncture, point prescription, location of needles, use of electrical stimulation and moxibustion, number, frequency and duration of sessions, number of needles used and acupuncturist experience. We used random-effects meta-regression to test the effect of each characteristic on the main effect estimate of pain. Where sufficient patient-level data were available, we conducted patient-level analyses.

RESULTS:
When comparing acupuncture to sham controls, there was little evidence that the effects of acupuncture on pain were modified by any of the acupuncture characteristics evaluated, including style of acupuncture, the number or placement of needles, the number, frequency or duration of sessions, patient-practitioner interactions and the experience of the acupuncturist. When comparing acupuncture to non-acupuncture controls, there was little evidence that these characteristics modified the effect of acupuncture, except better pain outcomes were observed when more needles were used (p=0.010) and, from patient level analysis involving a sub-set of five trials, when a higher number of acupuncture treatment sessions were provided (p<0.001).

CONCLUSION:
There was little evidence that different characteristics of acupuncture or acupuncturists modified the effect of treatment on pain outcomes. Increased number of needles and more sessions appear to be associated with better outcomes when comparing acupuncture to non-acupuncture controls, suggesting that dose is important. Potential confounders include differences in control group and sample size between trials. Trials to evaluate potentially small differences in outcome associated with different acupuncture characteristics are likely to require large sample sizes.
Treatment of 220 Cases of Bovine Ovarian Inactivity and 209 Cases of Bovine Persistent Corpus Luteum with Cu Yun Guan Zhu Ye

Dalu Song DVM, Jingbing Song DVM, Yuanliang Hu DVM, PhD, Baokang Zhang DVM, Liuliang Zhang DVM, Guangliang Cao DVM, Hongxing Wu, DVM Bin Yang DVM, Deyun Wang DVM, PhD

ABSTRACT
Ovarian inactivity and persistent corpus luteum are common causes of bovine infertility and result in huge economic losses in the dairy industry. Cu Yun Guan Zhu Ye consists of 40% Yi Mu Cao (Leonurus), 40% Yin Yang Huo (Epimedium) and 20% Hong Hua (Carthamus), and is a patented veterinary Chinese herbal medicine, recorded in the Veterinary Pharmacopoeia of the People's Republic of China. The objective of this case series was to evaluate the effectiveness of 1-3 intra-uterine infusions of sterile Cu Yun Guan Zhu Ye to promote estrus and pregnancy in cows with infertility due to inactive ovaries or persistent corpus luteum. There were 220 cows with ovarian inactivity and 209 cases of persistent corpus luteum from 12 different dairy farms over a 5-year period included in the study. Cu Yun Guan Zhu Ye appeared to be equally effective to produce estrus in cows with ovarian inactivity (93.2%) and persistent corpus luteum (96.7%), usually requiring only 1 intrauterine infusion (76.4% for ovarian inactivity and 82.3% for persistent corpus luteum). Estrus occurred in a mean of 16.6±6.1 and 16.9±6.1 days in cows with ovarian inactivity and persistent corpus luteum respectively. Pregnancy was achieved within 1-3 estrus cycles in 84.9% and 73.8% of cows with inactive ovaries and persistent corpus luteum respectively. Cu Yun Guan Zhu Ye uterine infusion was convenient to use, inexpensive and reliably promoted fertility with no side effects in the current study of 429 cows. Cu Yun Guan Zhu Ye may provide a safe alternative to hormone therapy.

Key words: Chinese herbal medicine, Yi Mu Cao, Leonurus, Yin Yang Huo, Epimedium, Hong Hua, Carthamus, bovine, infertility, ovarian inactivity, persistent corpus luteum

Infertility is a common problem in the dairy industry around the world affecting 15% of adult cows worldwide, 25% in China in 1986 and 12.9% in the United States (US) in 2007. Annual multi-billion dollar losses in the dairy industry due to infertility have prompted researchers all over the world to investigate causes and treatments. In China, ovarian inactivity and persistent corpus luteum are the main causes of infertility. Interest in the control of livestock infertility with Chinese medicinal herbs has increased in China and its use reported in books on livestock obstetrics, including the inconvenience, complications and costs. Hormone therapy can be effective for the treatment of bovine infertility, but estrous cycle disorders often arise from repeated use. The authors have evaluated several Chinese herbal medicines and developed and patented a Chinese herbal medicine consisting 3 Chinese herbs called Cu Yun Guan Zhu Ye that is recorded in the Veterinary Pharmacopoeia of the People's Republic of China, approved by the Ministry of Agriculture (Table 1).

The objective of this case series was to evaluate the effectiveness of 1-3 intra-uterine infusions of Cu Yun Guan Zhu Ye to promote estrus and pregnancy in cows with infertility due to inactive ovaries and persistent corpus luteum.

MATERIALS AND METHODS
Yi Mu Cao (Leonurus), Yin Yang Huo (Epimedium) and Hong Hua (Carthamus) were purchased and verified by Professor Jin Bin Song from Nanjing University of Chinese Medicine. A sterile solution of Cu Yun Guan Zhu Ye was prepared via water decoction and alcohol extraction to form a concentration of 1g/ml.

Cows with anestrous for 3 months or more were identified via production records, vaginal examinations and rectal palpation from 6 main dairy farms and 6 other farms over a 5-year period (Table 2). Cows that were pregnant, had concurrent contagious diseases, parasites or congenital problems or were receiving hormones or...
other treatments were excluded from the study. Cows with ovarian inactivity or persistent corpus luteum on 2 rectal palpations were identified using previously described standard techniques and included in the study. All cows remained housed on their respective dairy farms.

Cu Yun Guan Zhu Ye was infused into the uterus by means of the rectal grasp method used in artificial insemination. The amount varied between 20 ml-30 ml/infusion, depending on the body weight and length of anestrus, with greater amounts given for larger cows with prolonged anestrus. The cows were re-evaluated 10 days after treatment and if no change was found, then the uterine infusion was repeated once or twice more at 10-day intervals. Personnel of the production and techniques section of each farm observed the cows daily for evidence of estrus for 50 days and kept detailed records.

Short-term and long-term effects were evaluated. If estrus appeared within 45 days, after 1-3 infusions without other medical treatments, the short-term effects were assessed as a positive response to treatment. If no signs of estrus appeared within 45 days after 3 infusions, the short-term effects were assessed as a negative response to treatment. Cows that began estrus after Cu Yun Guan Zhu Ye treatment were bred after 3 estrus cycles. Cows were then evaluated for pregnancy by means of rectal palpation 30 days after breeding. If pregnant, the cows were assessed as having a positive long-term response to treatment and if not pregnant after 3 successive estrus cycles and breeding attempts, they were assessed as having a negative long-term response to treatment.

Prior to treatment, 15 lactating cows (6 with ovarian inactivity and 9 with corpus luteum) were selected for determination of milk progesterone levels. At 15:00 hours, at the end of the milking, 5-10 ml of milk were collected into sterile vials and stored at -20°C. The samples were then sent to Zhejiang Agricultural University, where the progesterone content of the milk was determined using radioimmunoassay. Milk progesterone levels were also evaluated using the same techniques at 2 weeks prior to treatment, 3 days after Cu Yun Guan Zhu Ye uterine infusion, at the onset of estrus and 14 and 30 days after breeding.

RESULTS
There were 220 cows with ovarian inactivity and 209 cases of persistent corpus luteum identified and included in the study (Table 2). Of these 429 cows,

Table 1: Ingredients of the Chinese herbal medicine Cu Yun Guan Zhu Ye and their actions

<table>
<thead>
<tr>
<th>Pin Yin Name</th>
<th>English Name</th>
<th>Amount</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hong Hua</td>
<td>Carthamus</td>
<td>0.2 g/ml</td>
<td>Invigorates Blood, removes Stasis, unblocks the Channels, relieves pain</td>
</tr>
<tr>
<td>Yin Yang Huo</td>
<td>Epimedium</td>
<td>0.4 g/ml</td>
<td>Tonifies Kidney Yang, strengthens Yang Qi, eliminates Dampness, expels Wind</td>
</tr>
<tr>
<td>Yi Mu Cao</td>
<td>Leonurus</td>
<td>0.4 g/ml</td>
<td>Invigorates Blood, resolves Stagnation, promotes urination, reduces edema, clears Heat, detoxifies</td>
</tr>
</tbody>
</table>

Table 2: The number of cows with ovarian inactivity and persistent corpus luteum and dairy farms where evaluated

<table>
<thead>
<tr>
<th>Location</th>
<th>Ovarian Inactivity</th>
<th>Persistent Corpus Luteum</th>
<th>Total Cows with Ovarian Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suzhou Dairy Company</td>
<td>57</td>
<td>55</td>
<td>112</td>
</tr>
<tr>
<td>Weigang Dairy Farm of Nanjing</td>
<td>51</td>
<td>35</td>
<td>86</td>
</tr>
<tr>
<td>Xuzhou Dairy Farm</td>
<td>26</td>
<td>6</td>
<td>32</td>
</tr>
<tr>
<td>1st and 2nd Dairy Farms of Xi’an</td>
<td>32</td>
<td>46</td>
<td>78</td>
</tr>
<tr>
<td>Red Flag Livestock Farm of Nanjing</td>
<td>19</td>
<td>27</td>
<td>46</td>
</tr>
<tr>
<td>Experimental Livestock Farm of Nan Nong</td>
<td>5</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>Others*</td>
<td>30</td>
<td>27</td>
<td>57</td>
</tr>
<tr>
<td>Total</td>
<td>220</td>
<td>209</td>
<td>429</td>
</tr>
</tbody>
</table>

*The others include dairy farms in Hangzhou, Liuhe, Huainan, Wanhe, Wuxi and Shanghai, CN.
96.5% had developed infertility between 2-10 years of age and 91.6% had previously had between 1-6 calves. The length of anestrus ranged between 90-986 days, with a mean +/- the standard deviation (M±SD) of 154.3±44.5 days. The M±SD length of anestrus in cows with ovarian inactivity was 130.8±20.6 days and with persistent corpus luteum 177.8±50.7 days.

The short-term effects of Cu Yun Guan Zhu Ye uterine infusion to produce an estrus cycle are outlined in Table 3. Estrus was achieved in 205/220 (93.2%) cows with inactive ovaries after 1-2 uterine infusions of Cu Yun Guan Zhu Ye. In cows with ovarian inactivity, 168/205 (76.4%) required only 1 infusion to induce estrus and 37/205 (16.8%) needed 2 infusions. Estrus began in 2-7 days in 70/205 (31.8%), 8-15 days in 60/205 (27.3%), 16-30 days in 45/205 (20.5%) and 31-45 days in 30/205 (13.6%) cows. The overall M±SD until the first estrus in cows with ovarian inactivity was 16.6±6.1 days

Estrus was achieved in 202/209 (96.7%) cows with persistent corpus luteum after 1-2 uterine infusions of Cu Yun Guan Zhu Ye. In cows with persistent corpus luteum, 172/202 (82.3%) required only 1 infusion to induce estrus and 30/202 (14.4%) needed 2 infusions. Estrus began in 2-7 days in 75/202 (35.9%), 8-15 days in 64/202 (30.6%), 16-30 days in 48/202 (23%) and 31-45 days in 15/202 (7.2%) cows. The overall M±SD until the first estrus in cows with persistent corpus luteum was 16.9±6.1 days. Estrus failed to develop in 15/220 (6.8%) cows with ovarian inactivity and 7/209 (3.3%) cows with persistent corpus luteum.

The long-term effects of Cu Yun Guan Zhu Ye uterine infusion to produce pregnancy are outlined in Table 4. Pregnancy was achieved in 174/220 (84.9%) cows with inactive ovaries and 149/209 (73.8%) cows with persistent corpus luteum within 1-3 estrus cycles. Pregnancy occurred in the 1st estrus cycle breeding (after the 3 estrus cycle waiting period) in 125/174 (61%) cows with ovarian inactivity and 97/149 (48%) cows with persistent corpus luteum. Pregnancy occurred in the 2nd estrus cycle in 34/174 (16.6%) cows with ovarian inactivity and 35/149 (17.3%) cows with persistent corpus luteum. Pregnancy occurred in the 3rd estrus cycle in 15/174 (7.3%) cows with ovarian inactivity and 17/149 (8.4%) cows with persistent corpus luteum. Pregnancy did not occur in 31/220 (15.1%) cows with ovarian inactivity and 32/209 (15.3%) cows with persistent corpus luteum.

### Table 3: The number of cows achieving estrus after 1 or 2 treatments and the time until onset of the first estrus for 220 cows with ovarian inactivity and 209 cows with persistent corpus luteum, treated by intrauterine infusion of Cu Yun Guan Zhu Ye

<table>
<thead>
<tr>
<th>Ovarian disease</th>
<th>Total Number Treated</th>
<th>Estrus Total</th>
<th>One Tx</th>
<th>Two Tx</th>
<th>Estrus 2-7 days</th>
<th>Estrus 8-15 days</th>
<th>Estrus 16-30 days</th>
<th>Estrus 31-45 days</th>
<th>No Estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian Inactivity</td>
<td>220</td>
<td>205</td>
<td>168</td>
<td>37</td>
<td>70</td>
<td>60</td>
<td>45</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>Persistent Corpus luteum</td>
<td>209</td>
<td>202</td>
<td>172</td>
<td>30</td>
<td>75</td>
<td>64</td>
<td>48</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>429</td>
<td>407</td>
<td>340</td>
<td>67</td>
<td>145</td>
<td>124</td>
<td>93</td>
<td>45</td>
<td>22</td>
</tr>
</tbody>
</table>

TX=treatment

### Table 4: The number of cows becoming pregnant in the 1st, 2nd and 3rd estrus cycle (bred after waiting 3 estrus cycles) for 220 cows with ovarian inactivity and 209 cows with persistent corpus luteum, treated by intrauterine infusion of Cu Yun Guan Zhu Ye

<table>
<thead>
<tr>
<th>Ovarian disease</th>
<th>Total Number Treated</th>
<th>Pregnant on 1st Estrus</th>
<th>Pregnant on 2nd Estrus</th>
<th>Pregnant on 3rd Estrus</th>
<th>Total Number Pregnant</th>
<th>Total Number not Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian Inactivity</td>
<td>220</td>
<td>125</td>
<td>34</td>
<td>15</td>
<td>174</td>
<td>31</td>
</tr>
<tr>
<td>Persistent Corpus luteum</td>
<td>209</td>
<td>97</td>
<td>35</td>
<td>17</td>
<td>149</td>
<td>53</td>
</tr>
<tr>
<td>Total</td>
<td>429</td>
<td>222</td>
<td>69</td>
<td>32</td>
<td>323</td>
<td>84</td>
</tr>
</tbody>
</table>

AJTCVM Vol 9, No.1, February 2014
ovarian inactivity and in 53/209 (26.2%) cows with persistent corpus luteum. The conception rate (number of cows in estrus/number of cows that became pregnant) was 84.9% (174/205 cows) in cows with ovarian inactivity and 73.8% (149/202 cows) in cows with persistent corpus luteum. The overall conception rate of cows with both types of ovarian disease was 79.4% (323/407 cows).

The milk progesterone levels 2 weeks before treatment, 3 days after treatment, the 1st day of estrus, and 14 and 30 days after breeding are outlined in Table 5 and illustrated in Figure 1. As expected the 6 cows with ovarian inactivity had much lower milk progesterone levels than cows with persistent corpus luteum. The levels slightly dropped on the day of estrus and then rose at 14 days and 30 days after breeding to 14.2±6.06 ng/ml and 15.6±6.92 ng/ml respectively.

The milk progesterone levels of the 9 cows with persistent corpus luteum were higher than normal (normal 6ng/ml). By the 3rd day after treatment, milk progesterone began to decrease until estrus, when the progesterone level was 4.52 ng/ml. At 14 days and 30 days after breeding, milk progesterone level rose rapidly (Figure 1). All cows in both groups were diagnosed as pregnant by means of rectal examination 30 days after breeding.

**DISCUSSION**

*Cu Yun Guan Zhu Ye* uterine infusion is an orange transparent liquid with a PH value of 4.0-7.0. The main ingredients on chemical analysis are flavonoid glycosides and alkaloids. Previous animal safety tests by

<table>
<thead>
<tr>
<th>Table 5: The milk progesterone levels (ng/ml) 2 weeks before treatment, 3 days after treatment, the 1st day of estrus, and 14 and 30 days after breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian disease</td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>Ovarian Inactivity</td>
</tr>
<tr>
<td>Persistent Corpus luteum</td>
</tr>
</tbody>
</table>

N= number of animals that had milk examined; TX = treatment with an intrauterine infusion of *Cu Yun Guan Zhu Ye*

**Figure 1:** The milk progesterone levels (ng/ml) 2 weeks before treatment, 3 days after treatment, the 1st day of estrus, and 14 and 30 days after breeding for cows with ovarian inactivity (blue line) and persistent corpus luteum (red line).
the authors showed that the LD_{50} of the extract was >40g/kg.\(^6\) No edema, hyperemia or necrosis was found after Cu Yun Guan Zhu Ye was injected into the quadriceps femoris in rabbits, and no inflammation was found after the liquid was placed in the eyes of rabbits. The Cu Yun Guan Zhu Ye extract should be stored in a dark, cool (room temperature) place. Small precipitates may form in the liquid, when stored for longer than 6 months, but the precipitates disappear with warming. Once prepared the authors have stored Cu Yun Guan Zhu Ye uterine infusion for 3 years and found it to still be effective.

Cu Yun Guan Zhu Ye appeared to be equally effective for both ovarian inactivity (93.2%) and persistent corpus luteum (96.7%), primarily requiring 1 infusion (76.4% for ovarian inactivity and 82.3% for persistent corpus luteum). Estrus occurred in a M±SD of 16.6±6.1 days in cows with ovarian inactivity and 16.9±6.1 days in cows with persistent corpus luteum. From these results it can be seen that Cu Yun Guan Zhu Ye has estrus-stimulating and ovulation-accelerating effects for both cows with ovarian inactivity and persistent corpus luteum. The changes in milk progesterone levels provided further objective clinicopathological support of the clinical observations.

Ovarian inactivity and persistent corpus luteum are common in cows as well as other livestock. Ovarian inactivity, in particular, is common in various female animals. The authors have also successfully used Cu Yun Guan Zhu Ye to promote estrus and pregnancy in 32 sows with anestrus and 10 mares with inactive ovaries and persistent corpus luteum in rural areas in Jiang Su and An Hui, China. Cu Yun Guan Zhu Ye is recommended for inactive ovaries and persistent corpus luteum and not other reproductive disorders.

Cu Yun Guan Zhu Ye is an herbal prescription made from 3 Chinese medicinal herbs including Yi Mu Cao (Leonurus), because of their properties to support the Kidney, invigorate circulation to eliminate Blood Stagnation, open the Channels, stimulate estrus and ovulation and promote breeding. In a human study of 95 women with irregular ovulation, Ge Qinsheng et al used Yi Mu Ca (Leonurus) and other Kidney-nourishing Chinese medicine to effectively promote ovulation.\(^7\) Based on these results and those of the current study Yi Mu Cao (Leonurus) can similarly enhance reproductive function of human and animals.\(^7,8\)

In a previous study by the authors, Cu Yun Guan Zhu Ye was shown to increase vaginal keratinization and thickness, uterine thickness and glandular development, promote ovarian follicular development and maturation and endometrial alkaline phosphatase activity in the same way, but significantly less than estradiol, but significantly more than normal saline.\(^8\) Arteriole and venule diameters and the number of connections to capillary beds also increased, improving uterine and ovarian microcirculation, compared to normal saline.\(^8\) In another murine study an alcohol extract of Shu Di (Rehmannia), Dang Gui (Angelica), Yi Mu Cao Zi (Leonurus fruit), Yin Yang Huo (Epimedium), Tu Si Zi (Cuscuta) and He Shou Wu (Polygonum) was injected subcutaneously in ovariectomized mice to nourish the Kidneys, invigorate circulation and eliminate Stagnated Blood and produced estradiol-like effects.\(^9\) The pharmacodynamics of this formula and Cu Yun Guan Zhu Ye are similar.\(^8,9\)

As discussed in a previous study by the authors, Cu Yun Guan Zhu Ye seems to adjust the dynamic equilibrium of the sexual cycle via feedback effects on the hypothalamus and enhance the function of the hypothalamus-pituitary-ovarian axis, as described in traditional Chinese veterinary medicine theory of Kidney-Chong and Ren Tian Gui (ovarian theory).\(^10\) In a previous unpublished study the authors have shown that Cu Yun Guan Zhu Ye improves microcirculation by producing mild uterine contractions in rabbits.\(^8\)

The fertility-promoting effects of Cu Yun Guan Zhu Ye uterine infusion most likely come from an indirect self-adjustment mechanism in the cows, rather than a direct effect as provided by hormone therapy and thus eliminating side effects during clinical use. In conclusion Cu Yun Guan Zhu Ye uterine infusion was convenient to use, inexpensive and reliably promoted fertility with no side effects in the current study of 429 cows. Cu Yun Guan Zhu Ye may provide a safe alternative to hormone therapy.

Acknowledgements
The authors wish to thank Professor Xie Chengxia, Professor Rong Yaofang, Mr Xu Liren and Mr Shen Jiasen for their guidance and assistance with the experiments. The authors also wish to thank the Production and Techniques Section of Suzhou Dairy Company, Wu Qibin, Zuo Lingzhang and Wu Xigui from Weigang Dairy Farm of Nanjing, Sheng Beihai from Xuzhou Dairy Farm, Gao Duanzheng, Gao Congning and Cai Yicai from Red Flag Livestock Farm of Nanjing and Li Fenglin from 1st Dairy Farm of Xi’An for their assistance and cooperation.

REFERENCES


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**Herbal Formula Spotlight**

*Shen Ling Bai Zhu San*

*Shen Ling Bai Zhu San* is a frequently used tonic Chinese herbal medicine. The original source of the formula is from the *Tai Ping Hui Min He Ji Ju Fang* (Imperial Grace Formulary of the Tai Ping Era) by the Imperial Medical Department in 1078-85. *Shen Ling Bai Zhu San* is composed of the following individual herbs: 15g *Ren Shen* (Ginseng), 15g *Bai Zhu* (Atractylodes), 15g *Fu Ling* (Poria) and 9g *Gan Cao* (Glycyrrhiza), 15g *Shan Yao* (Dioscorea), 6g *Sha Ren* (Amomum), 9g *Lian Zi* (Nelumbo), 12g *Bai Bian Dou* (Dolichos), 9g *Yi Yi Ren* (Coix) and 6g *Jie Geng* (Platycodon).1,2

*Shen Ling Bai Zhu San* tonifies Qi and strengthens the Spleen, resolves Dampness, and stops diarrhea. It contains herbs that strongly resolve Dampness in cases of internally generated Dampness from Spleen and Stomach Qi Deficiencies, caused by failure of their transportation and transformation functions. The herbs, *Shan Yao* (Rhizoma Dioscoreae) and *Lian Zi* (Nelumbo) treat diarrhea by assisting *Ren Shen* (Ginseng) in strengthening the Spleen and tonifying Qi. *Bai Bian Dou* (Dolichos) and *Yi Yi Ren* (Coix) strengthen the Spleen and dispel Dampness, *Sha Ren* (Amomum) awakens the Spleen and harmonizes the Stomach to regulate Qi and resolve Stagnation. *Jie Geng* (Platycodon) relieves chest fullness and has an ascending function to help counteract diarrhea.

*Shen Ling Bai Zhu San* is the Chinese herbal medicine of choice in veterinary medicine to treat uncomplicated diarrhea due to Spleen Qi Deficiency and Dampness. A pale or pale pink tongue with scalloped edges and a deep, weak pulse indicate Deficiency. In severe cases the tongue may have a white greasy coating. *Shen Ling Bai Zhu San* is also frequently used for chronic cough and/or chronic gagging with clear Phlegm that arise as a result of Damp accumulation. The dose in horses and cattle is 15-30 grams orally twice daily. For dogs the dose is 0.5–5g (or 0.1g per kg body weight) orally twice daily and for cats the dose is 0.2-0.5 g (or 0.1 g per kg body weight) orally twice daily. The dose for llamas, alpacas, pigs, goats and sheep is 5-15g orally twice daily and for birds 0.1–0.2g per kg body weight, orally twice daily for 3–6 weeks until primary clinical signs resolve.2

According to laboratory experiments in rabbits, administration of *Shen Ling Bai Zhu San* was associated with a regulatory effect on the intestines. Depending on the condition of the subject, it either increased or decreased the intestinal peristalsis.1 In another study *Shen Ling Bai Zhu San* was found to possess significant gastric acid neutralizing effects.3 Using an artificial stomach model it was shown to have antacid effects in vitro similar to the active control drugs used. Clinical studies and research have been performed using *Shen Ling Bai Zhu San* to treat the following disorders: superficial gastritis, diarrhea, chronic colitis, edema, diabetes, immune-deficiency and others.1

Signe E Beebe DVM

**REFERENCES**


Correlation of Acupuncture Point Sensitivity and Lesion Location in 259 Horses

Antonio Alfaro DVM, MSc

ABSTRACT
Palpation of Channels and acupoints is part of the equine traditional Chinese veterinary medicine examination. Sensitivity of acupoints and acupoint combinations have been used to localize disease to certain regions and structures in horses. The objective of this clinical observational study was to scan and record sensitive acupoints of horses presented for lameness, poor performance or pre-purchase examinations and then evaluate the anatomic sites previously suggested for problems, using ultrasound, radiography or nerve blocks. Between January and April 2007, 259 horses, presented for examination, had sensitive acupoints and were evaluated for lesions. Of these, 139/259 (53.7%) horses were presented for lameness and pain, 91/259 (35.1%) horses were presented for general complaints about poor performance and 29/259 (11.2%) horses were presented for pre-purchase examinations. Sensitive acupoints were associated with disorders of tendons (12.9%), ligaments (19.4%), muscles (5.8%), bone (1.4%), joints (23%), hooves (7.2%) and sciatic nerves (10.8%), as well as back pain (13.7%), metacarpal and metatarsal fractures (1.4%) and general poor performance issues (5.81%). Diagnosis was confirmed with ultrasonography (44%), radiography (40%) and/or nerve blocks (16%). In this group of 259 horses, there was 100% correlation with acupoint sensitivity and lesion location reported elsewhere. Integration of palpation of acupoints into the routine conventional examination can be useful to localized lesions in horses with musculoskeletal and performance problems and detect potential problems during pre-purchase examinations.

Key Words: Equine, acupoint sensitivity, acupoint scanning, lameness, poor performance, pre-purchase examination, traditional Chinese veterinary medicine, TCVM

ABBREVIATIONS

<table>
<thead>
<tr>
<th>TCVM</th>
<th>Traditional Chinese veterinary medicine</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMJ</td>
<td>Temporomandibular joint</td>
</tr>
</tbody>
</table>

To competently perform lameness and pre-purchase examinations veterinarians must acquire an accurate history and have excellent skills in observation, inspection, manipulation and gait analysis.1 Gait evaluation is performed during walking, trotting and galloping with and without a saddle and any other appropriate gear. The horse should also be observed while performing the intended sport. The ability to accurately localize lesions and knowledge of conformational defects that can lead to future performance problems are also essential. The nutritional status, especially of young animals, is very important to evaluate, as it may relate to current and future problems. The ability to accurately perform and interpret imaging are also necessary for an accurate diagnosis. The traditional Chinese veterinary medicine (TCVM) evaluation includes the conventional physical examination plus added historical questions, tongue and pulse examination and palpation of Channels and acupoints.1

Acupuncture stimulates neuroendocrine responses via the same somato-somatic and somatic-visceral neural reflexes used to regulate normal physiological processes and heal the body.1 Because of these neural reflexes, sensitivity of specific acupoints have been observed to indicate disease locally, elsewhere along the Channel and of specific internal organs.1,5 Appendicitis in humans has been diagnosed since 1891 by finding pain on palpation of a visceral-somatic reflex point called McBurney’s point.6 McBurney’s point is in the same location as the acupoint ST-30 in traditional Chinese medicine.1,6

Acupoints can be used for both diagnosis and treatment. Methodically palpating and scanning acupuncture Channels with the tip of the finger or a blunt rounded object, such as a plastic hypodermic needle cap, pen or a smooth wooden or metal probe, can be useful to detect sensitivity of specific acupoints and regions of the body.1,5 Many equine TCVM practitioners find acupoint and regional scanning to be an effective tool to detect current and potential problems, during the routine lameness and pre-purchase examinations.1,5

The objective of this clinical observational study was to scan and record sensitive acupoints of horses presented for lameness or pre-purchase examinations and confirm problems at specific anatomic sites with ultrasound, radiography or nerve blocks.
MATERIALS AND METHODS
Horses were presented to the author for examination between January and April 2007 and all horses with sensitive acupoints found on palpation were included in the study. As part of the complete TCVM examination, the author methodically palpated acupoints along the regular TCVM Channels of the head, neck, thoracic limb, dorsolateral vertebral column and pelvic regions (Table 1). Classical acupoints previously suggested to be diagnostic for specific locations were also palpated (Table 1). A 3 ml mepivicane vial with a smooth, rounded metal tip was used to put pressure on acupoints and along the Channels to detect sensitivity. Acupoint sensitivity was graded either +, ++ or +++ based on the criteria outlined in Table 2. Anatomic sites, previously suggested to be associated with sensitive acupoints, were

Table 1: Sensitive acupoints and location of lesions

<table>
<thead>
<tr>
<th>Head and Neck and Thoracic Limb Acupoint Scanning</th>
<th>Location of Disease*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB-1, ST-7, GB-2, SI-19</td>
<td>Temporomandibular joint and teeth</td>
</tr>
<tr>
<td>ST-6, GB-2, SI-19, GB-20, BL-10, SI-16</td>
<td>Atlanto-occipital and atlanto-axial joints</td>
</tr>
<tr>
<td>BL-10, SI-16</td>
<td>Contralateral sacral dysfunction; if ipsilateral Ba-jiao, Lu-gu and Ba-shan also sensitive</td>
</tr>
<tr>
<td>LI-18</td>
<td>Hoof</td>
</tr>
<tr>
<td>TH-16 and SI-16</td>
<td>Fetlock and pastern</td>
</tr>
<tr>
<td>TH-15 and TH-16</td>
<td>Thoracic limb tendons</td>
</tr>
<tr>
<td>TH-15, GB-21, LI-16, SI-9</td>
<td>Shoulder</td>
</tr>
<tr>
<td>LI-17 and C3-C4 or C4-C5 Jing-jia-ji</td>
<td>Carpus</td>
</tr>
<tr>
<td>LI-16 to LI-17</td>
<td>Suspensory ligament</td>
</tr>
<tr>
<td>PC-1</td>
<td>Chronic heel pain and navicular disease (LI-18 usually also reactive and sometimes BL-14, Bl-15)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Paravertebral Acupoint Scanning</th>
<th>Location of Disease*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL-14, BL-15</td>
<td>Medial foot when LI-18 also reactive</td>
</tr>
<tr>
<td>BL-20, BL-21, Qi-hai-shu, ST-7</td>
<td>Behavior disorder associated with equine gastrointestinal disorders (CV-12 usually also reactive)</td>
</tr>
<tr>
<td>BL-51, BL-52, BL-23, GB-24, GB-25, LIV-13</td>
<td>Behavior disorder associated with hormone imbalance</td>
</tr>
<tr>
<td>Hua-tuo-jia-jii</td>
<td>Local Qi/Blood Stagnation at the site of sensitivity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pelvic Acupoint Scanning</th>
<th>Location of Disease*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB-27, SP-13, BL-35, BL-39</td>
<td>Hock</td>
</tr>
<tr>
<td>SP-11, SP-12, ST-31, Dan-tian, Ju-liao, BL-36, BL-37 and BL-38</td>
<td>Stifle</td>
</tr>
<tr>
<td>BL-53, Huan-tiao, Huan-zhong, Huan-hou, Ba-jiao, Lu-gu, Ba-shan</td>
<td>Coxofemoral joint</td>
</tr>
</tbody>
</table>

*Lesions are ipsilateral to the sensitive acupoints unless otherwise indicated; Classical acupoint locations: Jing-jia-ji (above and below the lateral vertebral process), Hua-tuo-jia-jii (½ cun lateral to the dorsal midline between the thoracolumbar dorsal spinous processes), Dan-tian (cranioventral to the tuber coxae), Ju-liao (caudoventral to the tuber coxa), Huan-tiao (2 cun cranial to the greater trochanter), Huan-zhong (2 cun cranial and dorsal to greater trochanter), Huan-hou (dorsal to the greater trochanter), Ba-jiao (1.5 cun lateral to midline between the spinous processes of the sacrum), Lu-gu (⅗ the distance between Bai-hui and the greater trochanter) and Ba-shan (midpoint between the greater trochanter and Bai-hui)
evaluated for lesions with ultrasound, radiography or nerve blocks. The author was the sole examiner of acupoint sensitivity and performed all the diagnostic tests.

RESULTS

A total of 259 horses had 1 or more sensitive (reactive) acupoints to different degrees on palpation. Of these, 139/259 (53.7%) horses were presented for lameness and pain, 91/259 (35.1%) horses were presented for general complaints about poor performance and 29/259 (11.2%) horses were presented for pre-purchase examinations. To obtain a diagnosis at the suspected anatomic lesion location, ultrasonography was performed on 44% of horses, radiography on 40% of horses and nerve blocks on 16% of horses. An overview of the sensitive acupoints, locations and types of lesions and number of affected horses are outlined in Table 3. The percentages of the different types of tissue disorders diagnosed are shown in Figure 1. Sensitive acupoints were associated with disease of tendons (12.9%) ligaments (19.4%), muscles (5.8%), bones (degeneration 1.4%), joints (23%), hooves (7.2%) and sciatic nerves (10.8%), back pain (13.7%), metacarpal and metatarsal fractures (1.4%) and functional performance issues associated with equine ulcerative gastritis complex, temporomandibular (TMJ) disease, occipito-atlanto-axial disease and generalized Bladder Channel imbalances (5.81%). There was 100% correlation with acupoint sensitivity and lesion location suggested in the literature.1,5 No structural lesions were found in 5.81% of the cases with Bladder Channel pain.

DISCUSSION

In the author’s clinical experience and from the results of this study, acupoint palpation can be a valuable diagnostic aid. The author generally begins the palpation of acupoints around the head and TMJ, specifically GB-1, ST-7, GB-2 and SI-19. Pain around this area will normally indicate dental or occipito-atlanto-axial disease and generalized Bladder Channel imbalances (5.81%). Normal palpation of the paravertebral muscles and intervertebral problems, followed by TH-16 and SI-16 for ipsilateral fetlock and pastern disorders. Sensitivity of SI-16 and TH-15 usually indicates ipsilateral tendon disorders. If a very sensitive area between TH-15, GB-21 and LI-16 is found, then ipsilateral shoulder disease is likely and more severe structural damage is suspected if SI-9 is also painful. Sensitivity of LI-17 and Jing-jia-ji at C3-4 or C4-5 indicates ipsilateral carpal disease. If scratching with the probe from LI-16 to LI-17 generates sensitivity, then suspensory ligament disease should be suspected. Acupoint PC-1 is located at the level of the point of the olecranon, in the 5th intercostal space and is more reactive if chronic heel pain, including navicular disease is present. Most of the time in the author’s experience LI-18 will also be reactive.1

Normally, the acupoints along the Bladder Channel are used as “mirror” points to find out whether lesions are more medial or lateral or to support the diagnosis of a hoof problem. For example, if LI-18 and PC-1 are reactive and so are BL-14 and 15, then ipsilateral hoof disease is more likely. If the presenting complaint is a behavioral problem, then sensitivity of Qi-hai-shu, ST-7, BL-20, 21, CV-12 suggests equine gastric ulcerative complex causing the behavioral problems. If sensitivity is found at acupoints BL-51, BL-52, BL-23, GB-24, GB-25 and LIV-13 the behavioral problem may be due to equine hormone-associated syndrome. For segmental intervertebral problems, the author prefers digital palpation over the paravertebral muscles and Hua-tuo-jia-ji acupoints to detect sensitive acupoints and local Qi Stagnation.

In the pelvic area, sensitive acupoints around the tuber coxae have a great deal of clinical significance. Sensitivity of GB-27 and SP-13 (“hock-point”) indicate ipsilateral hock disease, in which case usually BL-35, BL-39a and BL-39b are sensitive also. If acupoints SP-11, SP-12, ST-31, Dan-tian and Ju-liao are sensitive, then stifle disease is likely and more severe if sensitivity is also found at BL-36, BL-37 and BL-38. If BL-53, Huan-tiao, Huan-zhong and Huan-hou are sensitive along with sensitivity at acupoints Ba-jiao, Lu-gu and Ba-shan, then coxofemoral joint disease is likely. The detection of sensitive acupoints during the TCVM examination can be useful to localize lesions to local and distant sites (Table 1). The results of acupoint sensitivity and lesion localization of the 259 horses in

Table 2: Criteria for the grades of acupoint sensitivity

<table>
<thead>
<tr>
<th>Degree of Response</th>
<th>Symbol used</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>+</td>
<td>Palpation generates a short local muscle contraction that stops when palpation is discontinued</td>
</tr>
<tr>
<td>Moderate</td>
<td>++</td>
<td>Palpation generates a local muscle contraction that continues after palpation is discontinued</td>
</tr>
<tr>
<td>Severe</td>
<td>+++</td>
<td>Palpation generates obvious pain as manifested by moving away or acts of aggression (e.g. biting, striking or kicking)</td>
</tr>
</tbody>
</table>
this study outlined in Table 2 were in agreement with suggested sensitivity and locations for tendon and ligament disease, fetlock and pastern, metacarpus and metatarsus, stifles, hocks, hoofs, back, sciatic nerve, equine gastric syndrome, TMJ and atlanto-occipital and atlanto-axial disorders described in Table 1 from other authors.1,5

During acupoint palpation, a superficial or mild response (+) usually indicates superficial functional damage associated with Qi Stagnation (e.g. mild myositis).1,3,5 A moderate response (++) indicates a more serious problem with structural damage (e.g. early degenerative joint disease). Most of the time the lesion can be confirmed by thermography, ultrasonography or radiography. A severe response (+++) is related to damage of tissues and disease of the ligaments, tendons, joints or bones that can be confirmed with ultrasonography or radiography.1,3,5 The author also found this to be the case in this clinical observational study.

Based on the acupoint diagnosis and confirmation with other diagnostic tests, all horses were treated with acupuncture, Chinese herbal medicine and Tui-na alone.

### Table 3: Sensitive acupoints, diagnostic tests and diagnosis in 259 horses

<table>
<thead>
<tr>
<th>Sensitive Acupoints</th>
<th>Diagnostic Tests</th>
<th>Diagnosis</th>
<th>Number of Horses Affected</th>
<th>Percentage of Total Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH-15, SI-16</td>
<td>Ultrasound</td>
<td>Ipsilateral limb tendon disease</td>
<td>33</td>
<td>12.94%</td>
</tr>
<tr>
<td>TH-16 and SI-16</td>
<td>Nerve block, ultrasound and radiography</td>
<td>Degenerative bone disease at each respective site</td>
<td>4</td>
<td>1.43%</td>
</tr>
<tr>
<td>BL-13, BL-25</td>
<td>Radiography</td>
<td>Metacarpal II and metatarsal IV fractures</td>
<td>2 at each site</td>
<td>1.43%</td>
</tr>
<tr>
<td>GB-27, BL-35</td>
<td>Ultrasound, radiography</td>
<td>Joint disease</td>
<td>59</td>
<td>23.02%</td>
</tr>
<tr>
<td>LI-18, PC-1</td>
<td>Ultrasound, radiography</td>
<td>Hoof disorders</td>
<td>18</td>
<td>7.19%</td>
</tr>
<tr>
<td>Local Hua-tuo-jia-ji</td>
<td>Ultrasound</td>
<td>Back problems</td>
<td>35</td>
<td>13.66%</td>
</tr>
<tr>
<td>Shen-shu, Shen-peng, Shen-jiao Ba-jiao, BL-10 contralaterally</td>
<td>Transrectal ultrasound</td>
<td>Sciatic nerve disorders</td>
<td>30</td>
<td>10.79%</td>
</tr>
<tr>
<td>ST-7, BL-20, 21, CV-12</td>
<td>Ultrasound, local anesthesia</td>
<td>Equine gastric ulcer syndrome</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>ST-7, GB-1, GB-2 and SI-19 for temporomandibular disorders</td>
<td>Ultrasound, local anesthesia</td>
<td>Temporomandibular disorder</td>
<td>4</td>
<td>Behavioral issues leading to poor performance 5.81%</td>
</tr>
<tr>
<td>ST-7, GB-1, GB-2 and SI-19 with BL-10 for occipito-atlanto-axial disorders;</td>
<td>Ultrasound, local anesthesia</td>
<td>Occipito-atlanto-axial disorder</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

*LDH = lactate dehydrogenase
or combined with conventional treatment. By the end of 2007, the clinical signs of 94% of the horses in this study had resolved and the horses had returned to national and international competitions. Integration of Channel and acupoint palpation into the routine conventional examination may provide another tool to better evaluate horses with musculoskeletal and performance problems. Acupoint sensitivity may also be useful to better detect potential problems during the pre-purchase examination.

Acknowledgements
Thank-you to Drs. Tania Zeledón Díaz, Ana Catalina Núñez, Paula Cappela Flores and Fernando Moya for their assistance with this study.

REFERENCES

Figure 1: Types of problems diagnosed by acupoint sensitivity in 259 horses
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✓ Concentrated Di Tan Tang*
✓ Concentrated External Wind*
✓ Concentrated Hindquarter Weakness**
✓ Concentrated Liver Happy
✓ Concentrated Shen Calmer
✓ Concentrated Stasis Breaker**
✓ Concentrated Tendon Ligament
✓ Concentrated Wei Qi Booster**

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50-0.25g Capsules  Cats  1 capsule once a day for cat or small dog
3 Oz Biscuits (60 ct)  Dogs  1 biscuit per 20 lbs body weight BID for dog
12 Oz Biscuits (30 ct)  Horses  1 biscuit BID for horse

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Proliferative Effects of a Polysaccharide Extracted from 
Xiang Gu (Shiitake) on Cultured Avian Lymphocytes

Rongrong Liu MS, Zhenzhen Gao PhD, Yuanliang Hu PhD, Deyun Wang PhD, 
Baokang Zhang DVM, Yufeng Xu MS, Dong Cai DVM

ABSTRACT
Xiang Gu (Shiitake) is a culinary mushroom and Chinese herbal medicine. Xiang Gu strengthens the Spleen, benefits Qi, 
eliminates pathogens and regulates Yin and Yang. In numerous studies, lentinan, a polysaccharide isolated from Xiang Gu, 
has been shown to have immunomodulatory effects. The hypothesis of the study was that lentinan, at optimum doses, would 
proliferate chicken lymphocytes in-vitro cell cultures. Eleven concentrations of lentinan were added to 4 wells of 
lymphocyte cell cultures each. Following incubation and processing, the absorbance was measured at 570nm (A$_{570}$ values) 
with an ELISA plate reader to determine non-toxic and optimum concentrations of lentinan, compared to a cell control 
group. From these data, 5 concentrations of lentinan were selected to study the effect on lymphocytes, alone or combined 
with the T-lymphocyte mitogen phytohemagglutinin (PHA). The mean ± standard deviation (M±SD) A$_{570}$ values of 
Groups 3-5 with 37.5, 18.75 and 9.375 μg/mL respectively of lentinan alone were significantly higher (p<0.05) than all 
other groups of lentinan alone and the cell control group. The M±SD A$_{570}$ values of Groups 3-5 with 37.5, 18.75 and 9.375 
μg/mL respectively of lentinan combined with PHA were significantly higher (p<0.05) than all other groups receiving 
lentinan and PHA and a PHA control group. From the results of this study, the hypothesis was accepted since 37.5, 18.75 
and 9.375 μg/mL lentinan significantly produced lymphocyte proliferation alone and synergistically with PHA. This study 
provides a theoretical basis for further in-vivo studies of lentinan as an immune-enhancing treatment for birds and animals.

Key words: Bisideomycetes, Xiang Gu, Shiitake, lentinan, avian, chicken, immune enhancing, lymphocyte proliferation

ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>PHA</td>
<td>Phytohemagglutinin</td>
</tr>
<tr>
<td>M±SD</td>
<td>Mean plus or minus the standard deviation</td>
</tr>
<tr>
<td>MTT</td>
<td>Thiazoyl blue tetrazolium bromide-MTT</td>
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</tbody>
</table>

Xiang Gu (Shiitake) is a bisideomycetes (mushroom, Mo Gu Jun) used as a food source and 
Chinese herbal medicine (Figure 1). From a traditional 
Chinese medicine (TCM) perspective Xiang Gu (Shiitake) strengthens the Spleen, benefits Qi, eliminates 
pathogens and regulates Yin and Yang. Lentinan is a β-1,3;1,6-glucan polysaccharide isolated from Xiang Gu (Shiitake) that has been shown to have anti-inflammatory, immunomodulating, anti-neoplastic, anti-fungal, anti-viral and anti-bacterial effects. Lentinan significantly reduced intestinal inflammation in a murine model of inflammatory bowel disease. In an experimental model of acute myeloid leukemia, rats

Figure 1: Xiang Gu (Shiitake) a bisideomycetes (mushroom) used as food and Chinese herbal medicine. Lentinan, a polysaccharide isolated from Xiang Gu (Shiitake), is suggested to have immune-enhancing effects in humans, animals and birds.
supplemented with lentinan exhibited weight gains, increased white blood cells, monocytes, and circulating cytotoxic T-cells and had reduced anti-inflammatory cytokines Interleukin-4 (IL-4), IL-10 and IL-6 compared to controls. The anti-neoplastic and immunomodulating actions of lentinan and other mushroom polysaccharides is attributed to their ability to stimulate natural killer cells, T-cells, B-cells, neutrophils and macrophage dependent immune system responses at different receptors. A meta-analysis of lentinan studies for gastric cancer showed that lentinan increased survival times of human patients with non-resectable recurrent gastric cancer. In another study of 50 human patients with esophageal cancer, lentinan added to the chemotherapy significantly improved the general condition, symptoms and quality of life of patients. In the same study, serum IL-2, IL-6 and IL-12 levels significantly increased and serum IL-4, IL-5 and IL-10 levels significantly decreased, in patients receiving chemotherapy and lentinan compared to patients receiving chemotherapy alone (p<0.05).

Lentinan have been studied as a fish immune-stimulant for use in aquacultures. Lentinan was added to the feed of rainbow trout for 37 days prior to induction of inflammation by injection with the classic inflammation inducer lipopolysaccharide (LPS). In this study, lentinan decreased the expression of genes involved in acute inflammatory reactions. In a study of chicks, lentinan increased IL-2 production by T-lymphocytes and produced lymphocyte proliferation. In another study of broiler chickens, adding lentinan to the daily diet increased body weights and immune organ indexes. Lentinan is not thought to attack pathogens directly, but instead to stimulate the maturation, differentiation and proliferation of white blood cells and improve overall immune function. The current study was designed to evaluate the ability of lentinan to increase lymphocytes in-vitro. In-vitro studies are commonly used to determine lymphocyte proliferation, as they can be performed rapidly and are easy to control.

The hypothesis of this study was that lentinan alone or combined with a T-cell lymphocyte mitogen would proliferate chicken lymphocytes in-vitro. The objective of this study was to first evaluate various doses of lentinan on chicken lymphocyte cultures in-vitro to determine toxic and optimum doses. This was accomplished by comparing alterations in optical density measurements of cultures containing lentinan with a lymphocyte culture control without lentinan. Next doses of lentinan alone and also mixed with the T-lymphocyte mitogen phytohemagglutinin (PHA) were added to lymphocyte cell cultures and the optical density measurements compared to lymphocyte culture control groups and PHA control groups. An increase in optical density would mean lymphocyte proliferation. The aim of the study was to evaluate the polysaccharide lentinan extracted from Xiang Gu (Shiitake), as a new veterinary immune enhancing agent to provide a theoretical basis for further in-vivo studies of lentinan to enhance immune functions in birds and animals.

MATERIALS AND METHODS

Cardiopuncture was performed on a 60-day-old non-vaccinated healthy white Roman chicken and 5 ml of blood were collected and transferred immediately into a sterile tube containing sodium heparin. The blood was then diluted with an equal volume of Hanks' balanced salt solution and carefully layered on the surface of lymphocyte separation medium, a sterile, iso-osmotic, polysacrose and diatrizoate solution. After 20 min of centrifugation at 2000 rpm, lymphocytes were concentrated in a white band within the plasma. The lymphocytes were collected, washed twice with RPMI-1640, a lymphocyte culture medium, supplemented with 100 IU/mL penicillin and 100 IU/mL streptomycin. The resulting pellet was then re-suspended to 2.5×10^6 mL with RPMI-1640 containing 100 IU/mL each of penicillin and streptomycin and 10% fetal calf serum. The mixture was pipetted into a 96-well cell culture plate (100 µL per well) and incubated at 39.5 ºC with 5% CO₂.

Lentinan was extracted from Xiang Gu (Shiitake) and diluted to 4.8 mg/mL with cell maintenance medium (with RPMI-1640 supplemented with 100 IU/mL penicillin and 100 IU/mL streptomycin), sterilized by boiling and stored at 4 ºC. The toxic, non-toxic and optimum concentrations of lentinan on the lymphocyte cultures was determined using a previously described thiazol blue tetrazolium bromide-MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimic assay to access cellular growth and viability. The lentinan was serially diluted 2-fold with cell maintenance medium into 11 concentrations ranging from 2,400-2.344 µg/mL (Table 1). When the cells had grown into a monolayer, 100 µL of each concentration of lentinan were added to 4 wells each. After incubation at 39.5 ºC for 44 hours, 20 µL of MTT were added into each well and incubation continued for 4 more hours. The supernatant was carefully removed and 100 µL of DMSO were added into each well and the culture plates shaken for 5 minutes to dissolve crystals completely. The absorbance of each well at 570 nm (A_570 value) was measured using a microliter enzyme-linked immunosorbent assay (ELISA) reader and compared to a control group of wells to which lentinan had not been added.

All data were expressed as means plus or minus the standard deviation (M±SD). Duncan's multiple range test was used to determine the difference among groups. Significance was determined as p<0.05. The maximal safe non-toxic concentration of lentinan was identified, when the A_570 values of the lentinan group became the same as the control group on serial dilutions. The maximal safe concentration and 4 other lentinan concentrations that significantly increased the A_570 value,
compared to the control, were selected to further study the effects of lentinan on lymphocyte proliferation.

Lymphocytes were again harvested and prepared as described above, inoculated into a 96-well cell culture plate (80 μL per well) and incubated as previously described until a monolayer of cells grew. Based on the initial cytotoxicity analysis results, lentinan was diluted with cell maintenance medium into 150, 75, 37.5, 18.75 and 9.375 μg/mL solutions. Each solution was then divided into 2 parts. The PHA was dissolved into 0.5 mg/mL of cell maintenance medium and the mixture was then added to 1 part of each of the 5 concentrations of lentinan until the final concentration was 10 μg/mL of PHA. Each lentinan concentration with and without PHA (10 total) was added to 4 wells each (100 μL per well). Cell maintenance medium (100 μL per well) without lentinan was added to 4 wells containing cells to create a cell control group. A PHA control group consisted of PHA alone added to 4 wells of cells (100 μL per well). A blank containing no cells and only cell maintenance media was also prepared. An MTT assay was performed as above and the absorbance of each well at 570 nm ($A_{570}$ value) was measured using the ELISA plate reader.$^6$ All data were expressed as M±SD. Duncan's multiple range test was used to determine the difference among groups. Significance was determined as $p<0.05$. An $A_{570}$ value significantly greater than the cell control group for groups containing lentinan alone or greater than the PHA control group for cell groups containing lentinan plus PHA, indicated the lymphocytes had proliferated in those groups.

RESULTS

The M±SD $A_{570}$ values of 11 concentrations of lentinan and the cell control group without lentinan are listed in Table 1 and illustrated in Figure 2. The M±SD $A_{570}$ values of cells with lentinan at 37.5, 18.75 and 9.375 μg/mL were significantly greater than the M±SD $A_{570}$ values for the cell control group ($p<0.05$), which indicated that lentinan at these concentrations could significantly produce lymphocyte proliferation. The M±SD $A_{570}$ value in the group containing 150 μg/mL lentinan was not significantly different than the M±SD $A_{570}$ value of the cell control group without lentinan ($p>0.05$). The 5 concentrations of lentinan above 150 μg/mL resulted in M±SD $A_{570}$ values significantly less ($p<0.05$) than the cell control group without lentinan. Lentinan at 4.688 and 2.344 μg/mL concentrations also resulted in significantly lower $A_{570}$ values ($p<0.05$) than the cell control group without lentinan.

The M±SD $A_{570}$ values of 5 different concentrations of lentinan with and without PHA compared to a cell control group and PHA control group respectively are outlined in Table 2 and illustrated in Figure 3. The M±SD $A_{570}$ value of Group 1 Lentinan alone at 150 μg/mL was not significantly different than the cell control group. The M±SD $A_{570}$ values of Groups 3-5 with lentinan alone at 37.5, 18.75 and 9.375 μg/mL respectively were significantly higher ($p<0.05$) than the cell control group without lentinan.

<table>
<thead>
<tr>
<th>Lentinan Concentration (μg/mL)</th>
<th>$A_{570}$ value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2400</td>
<td>0.298±0.015d</td>
</tr>
<tr>
<td>1200</td>
<td>0.311±0.014cd</td>
</tr>
<tr>
<td>600</td>
<td>0.313±0.014d</td>
</tr>
<tr>
<td>300</td>
<td>0.313±0.013c</td>
</tr>
<tr>
<td>150</td>
<td>0.332±0.016b</td>
</tr>
<tr>
<td>75</td>
<td>0.341±0.011ab</td>
</tr>
<tr>
<td>37.5</td>
<td>0.348±0.009a</td>
</tr>
<tr>
<td>18.75</td>
<td>0.354±0.007a</td>
</tr>
<tr>
<td>9.375</td>
<td>0.350±0.006a</td>
</tr>
<tr>
<td>4.688</td>
<td>0.320±0.013c</td>
</tr>
<tr>
<td>2.344</td>
<td>0.313±0.014b</td>
</tr>
<tr>
<td>Cell control</td>
<td>0.333±0.014b</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation; different letters indicate significant differences ($p<0.05$); there were no significant differences between $cd$ and $c$, $cd$ and $d$, $ab$ and $a$ and $ab$ and $b$.

Table 1: Effect of 11 different concentrations of lentinan on lymphocyte culture absorbance ($A_{570}$ value) compared to a cell control group without lentinan.
Table 2: Effect of 5 different concentrations of lentinan with and without PHA on lymphocyte culture absorbance ($A_{570}$ value) compared to a cell control group and PHA control group without lentinan respectively

<table>
<thead>
<tr>
<th>Group</th>
<th>Lentinan Concentration (μg/mL)</th>
<th>Lentinan Alone $A_{570}$ value*</th>
<th>Lentinan plus PHA $A_{570}$ value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 Lentinan</td>
<td>150</td>
<td>0.408±0.011&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.432±0.007&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 2 Lentinan</td>
<td>75</td>
<td>0.415±0.002&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.437±0.004&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 3 Lentinan</td>
<td>37.5</td>
<td>0.425±0.010&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.446±0.004&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 4 Lentinan</td>
<td>18.75</td>
<td>0.436±0.007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.456±0.004&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 5 Lentinan</td>
<td>9.375</td>
<td>0.431±0.010&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.449±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 6 Cell control</td>
<td>None</td>
<td>0.408±0.008&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>Group 7 PHA control</td>
<td>None</td>
<td>NA</td>
<td>0.435±0.012&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

PHA = T-lymphocyte mitogen phytohemagglutinin; *Mean ± standard deviation; NA = not applicable; different letters in a column indicate significant differences ($p<0.05$); there were no significant differences between $bc$ and $c$, $ab$ and $bc$ and $ab$ and $a$.

**Figure 2:** The $A_{570}$ value of lymphocyte cultures at different lentinan concentrations compared to a lymphocyte control cell culture containing no lentinan; higher $A_{570}$ values indicate greater lymphocyte numbers; bars marked with different letters differed significantly ($p<0.5$).

**Figure 3:** The $A_{570}$ value of lymphocyte cultures at different lentinan concentrations combined with T-lymphocyte mitogen phytohemagglutinin (PHA) compared to a PHA lymphocyte control culture containing no lentinan; higher $A_{570}$ values indicate greater lymphocyte numbers; bars marked with different letters differed significantly ($p<0.5$).

During evaluation of toxic and optimal doses of lentinan, Group 1 Lentinan alone at 150 μg/mL was not significantly different than the lymphocyte control group, indicating no toxicity, but no lymphocyte proliferation. The highest non-toxic concentration of lentinan was therefore considered to be 150 μg/mL, based on these results. The 5 concentrations of lentinan above 150 μg/mL resulted in $A_{570}$ values significantly less ($p<0.05$) than the cell control group without lentinan, indicating cell loss from toxicity. Lentinan at 4.688 and 2.344 μg/mL concentrations also resulted in significantly lower $A_{570}$ values ($p<0.05$) than the cell control group without lentinan indicating toxicity. Although further studies are needed there appeared to be a dose related toxic effect along with an optimal effective dosage. A dose and time dependent T-lymphocyte transformation was found in another study of chickens by the authors.

**DISCUSSION**

Cellular immunity is mediated by T-lymphocytes. When antigens stimulate T-lymphocytes, the T-lymphocytes proliferate, differentiate and secrete lymphokines. Lymphocyte proliferation therefore reflects the cellular immune status. The $A_{570}$ value is an index to reflect the number of cells in the cell cultures. Increases in $A_{570}$ values indicate lymphocyte proliferation.

Other groups receiving only lentinan and the cell control group. The M±SD $A_{570}$ value of Group 1 Lentinan at 150 μg/mL combined with PHA was not significantly different than the PHA control group. The M±SD $A_{570}$ values of Groups 3-5 with lentinan 37.5, 18.75 and 9.375 μg/mL respectively combined with PHA were significantly higher ($p<0.05$) than all other groups receiving lentinan and PHA and the PHA control group.
following vaccination against New Castle disease and intramuscular administration of polysaccharides, flavones and other fractions of Bai Zhi (Angelica), Huang Qi (Astragalus), Da Qing Ye (Isatis), Yin Yang Huo (Epimedium), and propolis from bees.14

The M±SD A$_{570}$ values of Groups 3-5 with lentinan alone at 37.5, 18.75 and 9.375 μg/mL respectively were significantly higher (p<0.05) than all other groups receiving only lentinan and the lymphocyte control group and were considered the optimum concentrations to promote lymphocyte proliferation. The PHA alone is known to stimulate transformation and mitosis of T-lymphocytes. 15 The M±SD A$_{570}$ values of Groups 3-5 with lentinan 37.5, 18.75 and 9.375 μg/mL respectively combined with PHA were significantly higher (p<0.05) than all other groups receiving lentinan and PHA and the PHA control group. It can be concluded from these results that lentinan at certain concentrations can significantly promote lymphocyte proliferation synergistically with PHA. In other studies by the authors polysaccharides extracted from Bai Zhi (Angelica) and Huang Jin (Polygonatum) were shown to be synergistic with PHA in other studies by the authors.15,16 The results of the current study concurred with the other studies that showed that lentinan could promote the differentiation, proliferation and enhance the immune response of T- and B-lymphocytes.4,17

In conclusion the hypothesis of the study was accepted, based on the results that 37.5, 18.75 and 9.375 μg/mL lentinan could significantly promote lymphocyte proliferation alone and synergistically with PHA and thus enhance cellular immunity. Lentinan derived from Xiang Gu (Shiitake) at the proper concentration provided reliable immune enhancement in-vitro and justifies further in-vivo studies to prevent disease in the poultry and livestock industries.

FOOTNOTES

a Tanquan Poultry Farm, Nanjing, China
c Lentinan prepared by Jiangsu Nanjing Agricultural University Animal Pharmaceutical Co, Ltd, Nanjing, China
d Thiazoly1 blue teteazolium bromide-MTT, AMRESCO Biologicals, Solon, OH; http://www.amresco-inc.com/MTT-0793.cmsx
e DMSO; Suzhou Zhingxing Chemical Research Institute, Suzhou, China
f Model RT-6000, Rayto Life and Analytical Sciences Co, Ltd, Nanshan, Shenzhen, China; http://www.rayto.com/product/show/id/44

REFERENCES

Job Listings

TCVM practice in sunny Albuquerque NM seeks motivated associate.
For details please email:
cwilsondvm@earthlink.net


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6636 E. Virginia Beach Blvd
Norfolk, VA 23502
www.blvdvet.com


Chi Shao (Paeonia)

Common name: Red Peony
Botanical Name: Paeonia lacrifloria
Part Used: Root
Channel/Organ: Liver, Spleen
Taste: Sour, bitter
Energy: Cold
Energetic Functions:
1. Clears Heat and cools Blood
2. Invigorates Blood and reduces swelling
3. Soothes Liver Fire

Comments:
1. Similar to Mu Dan Pi (Moutan), Chi Shao soothes Blood Heat in animals with fever and frank hemorrhage and invigorates the Blood to reduce swelling
2. Chi Shao is related to Bai Shao Yao (Paeoniae or White Peony), but Bai Shao Yao nourishes Blood and Chi Shao invigorates Blood
3. Use caution in patients with Blood Deficiency or Blood Cold patterns and during pregnancy

Haleh Siahpolo DVM
Pearls from TCVM Practice

Traditional Chinese Veterinary Medicine Diagnosis and Treatment of Acquired Infertility in Female Horses and Dogs

Xiuhui Zhong DVM, PhD, Yantao Zhao DVM, PhD

From: The Institute of Traditional Chinese Veterinary Medicine, Agricultural University of Hebei, Baoding, China

ABSTRACT
Acquired female infertility from a traditional Chinese veterinary medical (TCVM) perspective can be associated with Deficiency or Excess patterns. There are 6 patterns of acquired female infertility commonly diagnosed in TCVM practice. The 6 patterns are: 1) Kidney Qi and Blood Deficiency, 2) Kidney Yin Deficiency, 3) Kidney Yang Deficiency or Cold uterus, 4) Excess Phlegm and Damp, 5) Liver Qi and Blood Stagnation and 6) Damp-Heat. Malnutrition or over-working can result in infertility from Kidney Qi and Blood Deficiency or Kidney Yin Deficiency. Obesity will cause Stagnation in the uterus with an Excess of Phlegm and Damp, making it impossible for oocytes to be released or connect with sperm. Cold Deficiency of the uterus or Kidney Yang Deficiency will damage the Chong Channel and the Conception Vessel (Ren Channel) and lead to infertility. Stagnant Qi and Blood in the Liver can impair uterine reproductive function and lead to infertility. Damp-Heat flows downward to the uterus and infertility results. Each pattern has characteristic findings on the TCVM examination, may require treatment of different acupoints and most importantly require different Chinese herbal medicines. Administering the incorrect Chinese herbal medicine could worsen the problems and harm the animal. Thus, when diagnosing infertility, one should attempt to identify the correct pattern, so that proper treatment can be administered. The pathogenesis, clinical signs, treatment principles, acupuncture points and Chinese herbal medicines are described for each pattern. Infertility due to congenital defects and aging are not discussed.

Key words: infertility, Kidney Deficiency, Stagnation, Phlegm, Damp-Heat

ABBREVIATION
TCVM Traditional Chinese veterinary medicine

Acquired female infertility is the inability to conceive after repeated breeding attempts or inseminations, even though the animal is of normal reproductive age.1 Female infertility from a traditional Chinese veterinary medical (TCVM) perspective can be associated with Deficiency or Excess patterns. There are 6 patterns of acquired female infertility commonly diagnosed in TCVM practice. The 6 patterns are: 1) Kidney Qi and Blood Deficiency, 2) Kidney Yin Deficiency, 3) Kidney Yang Deficiency or Cold uterus, 4) Excess Phlegm and Damp, 5) Liver Qi and Blood Stagnation and 6) Uterine Damp-Heat. Malnutrition or over-working can result in infertility from Kidney Qi and Blood Deficiency or Kidney Yin Deficiency. Malnutrition or over-working can result in infertility from Kidney Qi and Blood Deficiency or Kidney Yin Deficiency. Obesity will cause Stagnation in the uterus with an Excess of Phlegm and Damp, making it impossible for oocytes to be released or connect with sperm. Cold Deficiency of the uterus or Kidney Yang Deficiency will damage the Chong Channel and the Conception Vessel Channel and lead to infertility.2 Stagnant Qi and Blood in the Liver can impair uterine reproductive function and lead to infertility. Damp-Heat flows downward to the uterus and infertility results. Each pattern has characteristic findings on the TCVM examination, may require treatment of different acupoints and even more importantly require different Chinese herbal medicines. Administering the incorrect Chinese herbal medicine could worsen the problems. Thus, when diagnosing female infertility, one should attempt to identify the correct pattern so that proper treatment can be administered. Female infertility can be associated with congenital defects or aging TCVM patterns, but will not be discussed here.

PATTERNS OF INFERTILITY AND TREATMENTS

1. Kidney Qi and Blood Deficiency Infertility
   Kidney Qi and Blood Deficiencies result in reduced nourishment of the Chong Channel and Conception Vessel Channel and inability to conceive due to a...
Deficient uterus.

Clinical Signs:
- Poor body condition, emaciation
- Depression
- Weak limb movements (Qi Deficiency)
- Dry hair coat (Blood Deficiency)
- Prolonged estrus or anestrus
- Embryo loss
- Pale tongue with thin coating; wet if Qi Deficiency is prominent or dry if Blood Deficiency prominent
- Weak pulse

Treatment Principle:
- Tonify Qi and Blood to support the embryo

Acupuncture:
- 1-5 treatments, 2-4 weeks apart
- Suggested techniques and basic acupoints are listed in Table 1
- Added acupoints: BL-17 and SP-10 for Blood Deficiency, ST-36, LI-10 or BL-24 for Qi Deficiency

Herbal Medicine:
- Bai Zhu San (Table 2) or Epimedium Formula³ (Sheng Jing San)³
- Dose: 0.5-5g (0.1-0.2g/kg) BID for small animals, 15-60g BID for equine
- Duration: 4-12 weeks until primary clinical signs resolve
- For anestrus can also use: Yin Yang Fang³

Table 1: Acupuncture techniques, potential acupoints to treat and their actions for different infertility patterns

<table>
<thead>
<tr>
<th>Technique</th>
<th>Acupoint</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry needle or Aqua-acupuncture</td>
<td>LIV-3</td>
<td>Soothes Liver Qi, resolves Stagnation</td>
</tr>
<tr>
<td></td>
<td>GB-34</td>
<td>Soothes Liver Qi, resolves Stagnation</td>
</tr>
<tr>
<td></td>
<td>LI-4</td>
<td>Moves Qi and Blood to resolve Stagnation</td>
</tr>
<tr>
<td></td>
<td>ST-40</td>
<td>Influential point for Phlegm</td>
</tr>
<tr>
<td>Electro-acupuncture</td>
<td>Yan-chi + Shen-peng</td>
<td>A special point for infertility (Yan-chi)</td>
</tr>
<tr>
<td></td>
<td>Shen-shu, bilateral</td>
<td>Tonifies Kidney reproductive functions (Shen-peng)</td>
</tr>
<tr>
<td></td>
<td>BL-23 + BL-52</td>
<td>Tonifies Kidney reproductive functions</td>
</tr>
<tr>
<td></td>
<td>BL-26, bilateral</td>
<td>Tonifies Kidney reproductive functions</td>
</tr>
<tr>
<td></td>
<td>BL-21 + BL-18</td>
<td>Strengthens the Spleen and Stomach to enhance post-natal Jing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and tonifies Yuan-Qi for infertility (BL-21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soothes Liver Qi, resolves Stagnation</td>
</tr>
</tbody>
</table>

Table 2: Ingredients of the Chinese herbal medicine Bai Zhu San and their actions

<table>
<thead>
<tr>
<th>Pin Yin Name</th>
<th>English Name</th>
<th>Amount (g)</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bai Shao</td>
<td>Paeonia</td>
<td>20</td>
<td>Nourishes Liver Blood and Yin</td>
</tr>
<tr>
<td>Bai Zhu</td>
<td>Atractylodes</td>
<td>30</td>
<td>Tonifies Spleen Qi, removes Stagnation</td>
</tr>
<tr>
<td>Chen Pi</td>
<td>Citrus</td>
<td>25</td>
<td>Eliminates Damp, reinforces the Spleen and Stomach</td>
</tr>
<tr>
<td>Chuan Xiong</td>
<td>Ligusticum</td>
<td>15</td>
<td>Removes Stagnation</td>
</tr>
<tr>
<td>Dang Gui</td>
<td>Angelica</td>
<td>25</td>
<td>Facilitates Blood flow, removes Stagnation</td>
</tr>
<tr>
<td>E Jiao</td>
<td>Asinum</td>
<td>30</td>
<td>Nourishes Blood, calms the fetus</td>
</tr>
<tr>
<td>Gan Cao</td>
<td>Glycyrrhiza</td>
<td>15</td>
<td>Invigorates Spleen, replenishes Qi, harmonizes formula</td>
</tr>
<tr>
<td>Dang Shen</td>
<td>Codonopsis</td>
<td>30</td>
<td>Nourishes Spleen Qi and calms the fetus</td>
</tr>
<tr>
<td>Huang Qin</td>
<td>Scutellaria</td>
<td>25</td>
<td>Cools Hot Blood and calms the fetus</td>
</tr>
<tr>
<td>Sha Ren</td>
<td>Amomum</td>
<td>20</td>
<td>Promotes Qi flow and calms the fetus</td>
</tr>
<tr>
<td>Shu Di Huang</td>
<td>Rehmannia</td>
<td>30</td>
<td>Tonifies Blood, nourishes the fetus</td>
</tr>
</tbody>
</table>
2. Kidney Yin Deficiency Infertility

Kidney Yin Deficiency may be caused by Jing exhaustion, blood loss or Body Fluid loss. This usually occurs in chronic febrile diseases or other chronic disorders that exhaust Kidney Yin. The Deficient Kidney fails to nourish the marrow to generate bone and the animal will also have a weak and painful lumbar region. Kidney Yin Deficiency results in uterine Blood Deficiency and infertility.

Clinical Signs:
- Emaciation
- Weakness and/or lumbar pain
- Low fever in the afternoon
- Dry feces
- Weak estrus and difficulty conceiving
- Dry mouth
- Red tongue
- Weak pulse

Treatment Principle:
- Nourish Kidney Yin

Acupuncture:
- 1-5 treatments, 2-4 weeks apart
- Suggested techniques and basic acupoints are listed in Table 1
- Added acupoints: For Yin Deficiency, add KID-3, KID-6, SP-6 to tonify Yin and GV-14 to clear Heat

Herbal Medicine:
- Liu Wei Di Huang (Rehmannia 6\textsuperscript{a}) (Table 3) or Zuo Gui Wan\textsuperscript{a5}
- Dose: 0.5-5g (0.1g/kg) BID for small animals, 15-60g BID for equine
- Duration: 4-16 weeks until primary clinical signs resolve

3. Kidney Yang Deficiency (Cold Uterus) Infertility

Deficient Kidney Yang fails to warm the uterus, resulting in infertility from a Cold uterus that is unable to nourish the sperm.

Clinical Signs:
- Cold ears, nose, back and limbs
- Loss of appetite
- Increased bowel movements and watery diarrhea
- Delayed estrus or anestrus or mucous vaginal discharge
- Whitish tongue with a thin coating
- Deep and slow pulse

Treatment Principle:
- Warm the uterus and disperse Cold

Acupuncture:
- 1-5 treatments, 2-4 weeks apart
- Suggested techniques and basic acupoints are listed in Table 1
- Added acupoints: For Yang Deficiency, add moxibustion at GV-3, GV-4

Herbal Medicine:
- Ai Fu Nuan Gong Wan (Table 4) or You Gui Wan\textsuperscript{5}
- Dose: 0.5-5g (0.1g/kg) BID for small animals, 15-60g BID for equine
- Duration: 4-12 weeks until primary clinical signs resolve

4. Excess Phlegm and Damp (Fatty Type) Infertility

Obesity can cause Stagnation in the ovaries and uterus resulting in failure of oocyte release and lack of connection to sperm.

Clinical Signs:
- Over-conditioned
- Obesity
- Disturbed estrus cycle
- Large amounts of mucous vaginal discharge (Damp type)
- Reddish tongue with white and lipid coating
- Slippery pulse

Treatment principle:
- Dry the Damp and disperse Phlegm

---

Table 3: Ingredients of the Chinese herbal medicine Liu Wei Di Huang and their actions

<table>
<thead>
<tr>
<th>Pin Yin Name</th>
<th>English Name</th>
<th>Amount (g)</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di Huang</td>
<td>Rehmannia</td>
<td>80</td>
<td>Nourishes Kidney Yin</td>
</tr>
<tr>
<td>Shan Yao</td>
<td>Dioscorea</td>
<td>40</td>
<td>Nourishes postnatal Jing by nourishing Spleen Qi</td>
</tr>
<tr>
<td>Shan Zhu Yu</td>
<td>Cornus</td>
<td>40</td>
<td>Nourishes Liver and Kidney to store Kidney Jing</td>
</tr>
<tr>
<td>Dan Pi</td>
<td>Moutan</td>
<td>30</td>
<td>Removes Deficiency Heat</td>
</tr>
<tr>
<td>Fu Ling</td>
<td>Poria</td>
<td>30</td>
<td>Nourishes the Spleen by removing Damp</td>
</tr>
<tr>
<td>Ze Xie</td>
<td>Alisma</td>
<td>30</td>
<td>Facilitates urination to support Kidney Yin</td>
</tr>
</tbody>
</table>

---
Acupuncture:
- 1-5 treatments, 2-4 weeks apart
- Suggested techniques and basic acupoints are listed in Table 1
- Added acupoints: For Damp and Phlegm add ST-40 and SP-9

Herbal Medicine:
- Cang Zhu San (Table 5) or Phlegm Fat Formulaa (modified Er Chen Tang)3
- Dose: 0.5-5g (0.1g/kg) BID for small animals, 15-60g BID for equine
- Duration: 4-12 weeks until primary clinical signs resolve

5. Liver Qi and Blood Stagnation Infertility
Stagnant Qi and Blood can impair uterine reproductive function leading to infertility.

Clinical Signs:
- Anestrus, short luteal phase or an irregular estrous cycle
- During estrus, thick discharge from vagina
- Intolerance to pressure on the abdomen
- Petechia under tongue or on gingiva (especially during estrus)
- Purple tongue with a thin coating
- Wiry pulse

Treatment principle:
- Regulate Liver Channel and eliminate Stagnation

Acupuncture:
- 1-5 treatments, 2-4 weeks apart
- Dry or aqua-acupuncture: LIV-3, GB-34, LI-4, ST-40 and SP-9
- Electro-acupuncture: 20 Hz for 10 min and 80 to 120 Hz for another 10 min at the following pairs: 1) Yan-chi + Shen-peng, 2) Shen-shu, bilaterally, 3) BL-23 + BL-52, 4) BL-26, bilaterally and 5) BL-21 + BL-18
- LIV-3, GB-34 and BL-18 soothe Liver Qi
- LI-4 and ST-40 move Qi and Blood to resolve Stagnation
- BL-21 Strengthens the Spleen and Stomach to enhance post-natal Jing for infertility

Table 4: Ingredients of the Chinese herbal medicine Ai Fu Nuan Gong Wan and their actions

<table>
<thead>
<tr>
<th>Pin Yin Name</th>
<th>English Name</th>
<th>Amount (g)</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ai Ye</td>
<td>Artemesia</td>
<td>24</td>
<td>Warms Channels, anti-abortive</td>
</tr>
<tr>
<td>Dang Gui</td>
<td>Angelica</td>
<td>30</td>
<td>Facilitates Blood flow, removes Stagnation</td>
</tr>
<tr>
<td>Chuan Xu Duan</td>
<td>Dipsacus</td>
<td>24</td>
<td>Tonifies Kidney and calms the fetus</td>
</tr>
<tr>
<td>Chuan Xiong</td>
<td>Ligusticum</td>
<td>15</td>
<td>Removes Stagnation</td>
</tr>
<tr>
<td>Bai Shao</td>
<td>Paeonia</td>
<td>24</td>
<td>Nourishes Liver Blood and Yin</td>
</tr>
<tr>
<td>Xiang Fu</td>
<td>Cyperus</td>
<td>24</td>
<td>Regulates Qi flow and calms the fetus</td>
</tr>
<tr>
<td>Wu Zhu Yu</td>
<td>Evodia</td>
<td>24</td>
<td>Warms the Spleen and Middle Burner (Zhong-Jiao)</td>
</tr>
<tr>
<td>Huang Qi</td>
<td>Astragalus</td>
<td>30</td>
<td>Tonifies Qi and Spleen</td>
</tr>
<tr>
<td>Guan Gui</td>
<td>Cinnamomum</td>
<td>30</td>
<td>Warms the Kidney</td>
</tr>
<tr>
<td>Gan Cao</td>
<td>Glycyrrhiza</td>
<td>15</td>
<td>Invigorates Spleen, replenishes Qi, harmonizes formula</td>
</tr>
</tbody>
</table>

Table 5: Ingredients of the Chinese herbal medicine Cang Zhu San and their actions

<table>
<thead>
<tr>
<th>Pin Yin Name</th>
<th>English Name</th>
<th>Amount (g)</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cang Zhu</td>
<td>Atractylodes</td>
<td>45</td>
<td>Eliminates Damp and reinforces the Spleen</td>
</tr>
<tr>
<td>Zhi Ban Xia</td>
<td>Pinellia</td>
<td>30</td>
<td>Eliminates Damp</td>
</tr>
<tr>
<td>Fu Ling</td>
<td>Poria</td>
<td>30</td>
<td>Eliminates Damp and nourishes the Spleen</td>
</tr>
<tr>
<td>Shen Qu</td>
<td>Massa</td>
<td>45</td>
<td>Promotes digestion</td>
</tr>
<tr>
<td>Chen Pi</td>
<td>Citrus</td>
<td>34</td>
<td>Eliminates Damp, reinforces the Spleen and Stomach</td>
</tr>
<tr>
<td>Zhi Xiang Fu</td>
<td>Cyperus</td>
<td>30</td>
<td>Promotes Qi flow and regulates the Channels</td>
</tr>
<tr>
<td>Gan Cao</td>
<td>Glycyrrhiza</td>
<td>24</td>
<td>Invigorates Spleen, replenishes Qi, harmonizes formula</td>
</tr>
<tr>
<td>Chuan Xiong</td>
<td>Ligusticum</td>
<td>24</td>
<td>Removes Stagnation</td>
</tr>
</tbody>
</table>
• Yan-chi is a special point for infertility
• Shen-shu, Shen-peng, BL-23, BL-52 and BL-26 tonify Kidney reproductive functions

**Herbal Medicine:**
• *Tiao Jing San* (Table 6) or Lotus Formula\(^a\) (*Huo Xue Yu Zi Tang*)\(^b\)
• Dose: 0.5-5g (0.1g/kg) BID for small animals, 15-60g BID for equine
• Duration: 4-16 weeks until primary clinical signs resolve

### 6. Uterine Damp-Heat (Metritis) Infertility
Strong Damp Stagnates in the Liver Channel and transforms into Damp-Heat that flows down to the uterus causing a yellowish vaginal discharge and infertility.

**Clinical Signs:**
• Yellowish and sticky vaginal discharge, turbid

**Treatment Principle:**
• Dispel Damp-Heat, soothe the Liver

**Acupuncture:**
• 1-5 treatments, 2-4 weeks apart
• Suggested techniques and basic acupoints listed in Table 1

**Herbal Medicine:**
• *Long Dan Xie Gan* decoction (Table 7) or Phellodendron and Plantago Combination\(^a\) (*Bai*

---

### Table 6: Ingredients of the Chinese herbal medicine *Tiao Jing San* and their actions

<table>
<thead>
<tr>
<th>Pin Yin Name</th>
<th>English Name</th>
<th>Amount (g)</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bai Shao</td>
<td>Paeonia</td>
<td>30</td>
<td>Nourishes Blood, restrains dissipation of Yin fluids</td>
</tr>
<tr>
<td>Dang Gui</td>
<td>Angelica</td>
<td>30</td>
<td>Removes Stagnation</td>
</tr>
<tr>
<td>Di Huang</td>
<td>Rehmannia</td>
<td>15</td>
<td>Tonifies Blood, nourishes the fetus</td>
</tr>
<tr>
<td>Gou Qi Zi</td>
<td>Lycium</td>
<td>10</td>
<td>Tonifies the Kidney and nourishes the embryo</td>
</tr>
<tr>
<td>Fu Pen Zi</td>
<td>Rubus</td>
<td>25</td>
<td>Nourishes sperm, tonifies the Kidney</td>
</tr>
<tr>
<td>Nu Zhen Zi</td>
<td>Ligustrum</td>
<td>30</td>
<td>Reinforces Kidney and nourishes Yin</td>
</tr>
<tr>
<td>Gan Cao</td>
<td>Glycyrhiza</td>
<td>15</td>
<td>Invigorates Spleen, replenishes Qi, harmonizes formula</td>
</tr>
<tr>
<td>Chuan Xiong</td>
<td>Ligusticum</td>
<td>10</td>
<td>Removes Stagnation</td>
</tr>
<tr>
<td>Hong Hua</td>
<td>Carthamus</td>
<td>25</td>
<td>Removes Stagnation</td>
</tr>
<tr>
<td>Tu Si Zi</td>
<td>Cuscuta</td>
<td>25</td>
<td>Tonifies the Kidney, anti-abortive</td>
</tr>
<tr>
<td>Zhi Mu</td>
<td>Anemarrhena</td>
<td>15</td>
<td>Removes Heat Toxin, nourishes Yin</td>
</tr>
<tr>
<td>Ze Xie</td>
<td>Alisma</td>
<td>20</td>
<td>Eliminates Damp and Heat</td>
</tr>
<tr>
<td>Zhi Xiang Fu</td>
<td>Cyperus</td>
<td>10</td>
<td>Harmonizes the Liver, modulates the Channels</td>
</tr>
</tbody>
</table>

### Table 7: Ingredients of the Chinese herbal medicine *Long Dan Xie Gan Tang* and their actions

<table>
<thead>
<tr>
<th>Pin Yin Name</th>
<th>English Name</th>
<th>Amount (g)</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long Dan Cao</td>
<td>Gentiana</td>
<td>45</td>
<td>Dispels Liver Damp-Heat</td>
</tr>
<tr>
<td>Che Qian Zi</td>
<td>Plantago</td>
<td>30</td>
<td>Dispels Damp, diuretic</td>
</tr>
<tr>
<td>Chai Hu</td>
<td>Bupleurum</td>
<td>30</td>
<td>Soothes Liver</td>
</tr>
<tr>
<td>Dang Gui</td>
<td>Angelica</td>
<td>30</td>
<td>Harmonizes Blood by dispelling Stagnation</td>
</tr>
<tr>
<td>Zhi Zi</td>
<td>Gardenia</td>
<td>30</td>
<td>Eliminates Heat</td>
</tr>
<tr>
<td>Di Huang</td>
<td>Rehmannia</td>
<td>45</td>
<td>Nourishes Blood and removes Heat</td>
</tr>
<tr>
<td>Gan Cao</td>
<td>Glycyrhiza</td>
<td>15</td>
<td>Harmonizes formula</td>
</tr>
<tr>
<td>Huang Qin</td>
<td>Scutellaria</td>
<td>30</td>
<td>Removes Damp-Heat</td>
</tr>
<tr>
<td>Ze Xie</td>
<td>Alisma</td>
<td>45</td>
<td>Dispels Damp, diuretic</td>
</tr>
<tr>
<td>Mu Tong</td>
<td>Akebia</td>
<td>20</td>
<td>Dispels Damp, diuretic</td>
</tr>
</tbody>
</table>
Che San\(^3\)

- Dose: 0.5-5g (0.1g/kg) BID for small animals, 15-60g BID for equine
- Duration: 2-6 weeks until primary clinical signs resolve

As described above, there are 6 patterns of acquired infertility. Regardless of the pattern, it is suggested to wait for at least 1 normal heat cycle before again attempting to breed. That way recovery of hormonal imbalances is more likely and the animal will have the best chance for conception.

FOOTNOTE
a Jing Tang Herbal inc; http://www.tcvmherbal.com/

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<th>B&amp;W or Color Fee</th>
</tr>
</thead>
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</tr>
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</tr>
</thead>
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The Kidney is the root of prenatal life. It stores Jing, governs water metabolism, dominates the bones and marrow and controls urination and defecation. The Kidney is almost always Deficient if there is a problem. Thus, the Kidney Jing should always be stored or conserved. The Bladder is the storage place for urine. The Bladder is almost always in Excess if there is a problem.

The Kidney and Bladder Patterns include diseases such as urinary incontinence, renal failure, intervertebral disc disease, arthritis, geriatric diseases and infertility. Any chronic diseases may eventually lead to Kidney Deficiency.

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<table>
<thead>
<tr>
<th>Eight Principles</th>
<th>Zang-Fu Syndromes (Pattern)</th>
<th>Herbal Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney Yang Deficiency</td>
<td>General with Edema</td>
<td>Jin Gui Shen Qi</td>
</tr>
<tr>
<td>Kidney Qi Deficiency</td>
<td>with Male Infertility</td>
<td>Epimedium Formula*</td>
</tr>
<tr>
<td>Kidney Yin Deficiency</td>
<td>with Female Infertility</td>
<td>Yin Yang Fang</td>
</tr>
<tr>
<td>Kidney Jing Deficiency</td>
<td>with Muscle Pain (Cold/ Damp)</td>
<td>Fennel Formula*</td>
</tr>
<tr>
<td>Excess</td>
<td>with Bi Syndrome (Equine)</td>
<td>Equine Du Huo*</td>
</tr>
<tr>
<td></td>
<td>with Bi Syndrome (Canine)</td>
<td>Dok's Formula*</td>
</tr>
<tr>
<td></td>
<td>with Urinary Dribbling</td>
<td>Suo Quan Wan</td>
</tr>
<tr>
<td></td>
<td>with Urinary Incontinence</td>
<td>Jin Suo Gu Jing</td>
</tr>
<tr>
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<td>with Rear or Back Weakness</td>
<td>Bu Yang Huan Wu</td>
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<tr>
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<td>with Fractures, Weak Bones</td>
<td>Jie Gu San</td>
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<tr>
<td></td>
<td>with Heaves/Dyspnea</td>
<td>Breathe Easier B*</td>
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<tr>
<td></td>
<td>General</td>
<td>Liu Wei Di Huang Wan</td>
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<tr>
<td></td>
<td>with False Heat</td>
<td>Zhi Bai Di Huang</td>
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<tr>
<td></td>
<td>with Bony Bi Syndrome</td>
<td>Di Gu Pi</td>
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<td></td>
<td>with Acute UTI</td>
<td>Ba Zheng San</td>
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<tr>
<td></td>
<td>with Urinary Crystals/Stones</td>
<td>Crystal Stone Formula*</td>
</tr>
<tr>
<td></td>
<td>with Hematuria</td>
<td>Red Front Door*</td>
</tr>
</tbody>
</table>

Adapted from Xie H. Chinese Veterinary Herbal Handbook 3rd Ed. Reddick, FL: Jing Tang Publishing 2011; *Jing Tang herbals at www.tcvmherbal.com