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(December, 2019)

## Avian Pathology

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### Note

Ecuadorian mainland industrial poultry production is free of H5/H7 Avian influenza virus: National surveillance program in 2016

José Luis MEDINA, Katherine LUGO, Javier VARGAS, Nataly MORALES, Ana BURGOS, Evelyn Pamela MARTÍNEZ, David ORTEGA-PAREDES, María REVELO  
J Vet Med Sci. 2019 Nov; 81(11): 1597-1600.

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## Bacteriology

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J Vet Med Sci. 2019 Nov; 81(11): 1601-1605.

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## Internal Medicine

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Next-generation sequencing analysis of bacterial flora in bovine protothecal mastitic milk and feces

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Evaluation of visceral fat mass in dogs by computed tomography

Itsuma NAGAO, Koichi OHNO, Takuro NAGAHARA, Nozomu YOKOYAMA, Taisuke NAKAGAWA, Reina FUJIWARA, Kie YAMAMOTO, Yuko GOTO-KOSHINO, Hirotaka TOMIYASU, Hajime TSUJIMOTO  
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Left atrial anteroposterior diameter in dogs: reference interval, allometric scaling, and agreement with the left atrial-to-aortic root ratio

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Enhanced angiogenic activity of dimethyloxalylglycine-treated canine adipose tissue-derived mesenchymal stem cells

Sang-Min KIM, Qiang LI, Ju-Hyun AN, Hyung-Kyu CHAE, Ji-In YANG, Min-Ok RYU, Aryung NAM, Woo-Jin SONG, Hwa-Young YOUN  
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Clinical and postmortem findings of pentalogy of Fallot in an 18-month-old Holstein heifer

Dai ISHIYAMA, Eiji MAKINO, Yoshihiro NAKAMURA, Makoto UCHIDA, Yukio ONODERA, James K. CHAMBERS, Kazuyuki UCHIDA, Fuko MATSUDA  
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Effects of a selective casein kinase 1 $\delta$  and  $\epsilon$  inhibitor on Fc $\epsilon$ RI expression and IgE-mediated immediate-type cutaneous reactions in dogs

Hikaru OHNO, Kaho TAKAHASHI, Nanako YANUMA, Misato OGAWA, Ayana HASEGAWA, Koji SUGITA, Koji KAWANO, Kazuaki SASAKI, Junsuke SHIRAI, Kentaro NAGAOKA, Keitaro OHMORI  
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## Laboratory Animal Science

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### Full Paper

Protective effect of *Corchorus capsularis* L. leaves on ethanol-induced acute gastric mucosal lesion in rats

Chiu-Fang LEE, Ching-Wen FAN, Ni-Na CHIANG, Hsiou-Chuan CHANG, Chun CHEN, Yu-Syuan HUANG, Hui-Yun WANG, Wen-Chuan LIN, Fu-An CHEN  
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Takao IRIE, Kyoji YAMADA, Yasuyuki MORISHIMA, Kinpei YAGI  
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Pigmented viral plaque and basal cell tumor associated with canine papillomavirus infection in Pug dogs

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### Pharmacology

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#### Full Papers

Release of urinary aquaporin-2-bearing extracellular vesicles is decreased in pregnant Japanese Black cattle

Thitaporn SINLAPADEELERDKUL, Hiroko SONODA, Kazuyuki UCHIDA, Go KITAHARA, Masahiro IKEDA  
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Possible anti-oxidative effects of long-term administration of Juzen-taiho-to in dogs

Yuta SHINOHARA, Ayaka OYAMA, Tatsuya USUI, Kazuaki SASAKI  
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Interaction of cyclosporine with phenobarbital in cats: a preliminary study

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J Vet Med Sci. 2019 Nov; 81(11): 1621-1623.

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### Physiology

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#### Full Paper

Reduced fibrillar collagen accumulation in skeletal muscle of secreted protein acidic and rich in cysteine (SPARC)-null mice

Sanae OMI, Keitaro YAMANOUCI, Katsuyuki NAKAMURA, Takashi MATSUWAKI, Masugi NISHIHARA  
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### Surgery

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## Theriogenology

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### Full Paper

Factors affecting estrus and ovulation time in weaned sows with induced ovulation by GnRH administration in different seasons

Pachara PEARODWONG, Chanyuth TRETIPSKUL, Noline M. SOEDE, Padet TUMMARUK  
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Double ovulation rate of the first follicular wave follicles is higher in the first follicular wave dominant follicle in the ovary contralateral to the corpus luteum treated with human chorionic gonadotropin five days after estrus in lactating dairy cows

Ryotaro MIURA, Nobu MATSUMOTO, Shingo HANEDA, Motozumi MATSUI  
J Vet Med Sci. 2019 Nov; 81(11): 1685-1687.

Breeding performance of Indonesian beef cattle as recipients for embryo transfer

Tita Damayanti LESTARI, Ismudiono ISMUDIONO, Trilas SARDJITO, Osamu YAMATO, Mitsuhiro TAKAGI, Akira YABUKI, Pudji SRIANTO  
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Dichlorodiphenyltrichloroethane (DDT) levels in rat livers collected from a malaria vector control region

Kodai MOTOHIRA, Yoshinori IKENAKA, Yared Beyene YOHANNES, Shouta M. M. NAKAYAMA, Victor WEPENER, Nico J. SMIT, Johan H. J. VAN VUREN, Ana Catarina SOUSA, Alex Ajeh ENUNEKU, Emmanuel Temiotan OGBOMIDA, Mayumi ISHIZUKA  
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## Wildlife Science

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### Full Papers

Effects of feeding on plasma concentrations of vitamin A in captive African penguins (*Spheniscus demersus*)

Kentaro UEDA, Fumiko AKASHI, Motoki KAWASAKI, Tatsuya SUGAWARA, Yuki MANABE, Tohru MATSUI  
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Cellular distribution of the prion protein in palatine tonsils of mule deer (*Odocoileus hemionus*) and Rocky Mountain elk (*Cervus elaphus nelsoni*)

Matthew M. HILLE, Jean E. JEWELL, E. Lee BELDEN  
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First isolation of voriconazole-resistant *Candida albicans*, *C. tropicalis*, and *Aspergillus niger* from the blowholes of bottlenose dolphins (*Tursiops truncatus*)

Yoshito OHNO, Yuichiro AKUNE, Yasuo INOSHIMA, Rui KANO  
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### Note

Intralesional methylprednisolone injection as an adjunct treatment for peri-cloacal pyogranuloma in an African penguin (*Spheniscus demersus*)

Shangzhe XIE, Gabrina Shuang-Li GOH, Chia-Da HSU  
J Vet Med Sci. 2019 Nov; 81(11): 1632-1635.

# Breeding performance of Indonesian beef cattle as recipients for embryo transfer

Tita Damayanti LESTARI, Ismudiono ISMUDIONO, [...], and Pudji SRIANTO

Additional article information

## Abstract

The objective of this study was to evaluate the breeding performance of Indonesian beef cattle (Ongole cross) as recipients for embryo transfer using Limousin embryos. As a result, the pregnancy rate was 35% (7 out of 20 cows). There was a significant difference ( $P < 0.01$ ) in the serum progesterone concentration between the nonpregnant and pregnant cows at the time of the embryo transfer (day 7 after the estrus). The pregnancy rate in the Indonesian beef cows was low, which may be due to their insufficient genetic quality and/or low physical conditions caused by the poor management, like a low-nutrition diet. The low progesterone concentration in the nonpregnant cows on day 7 may be associated with the failure of embryo implantation.

**Keywords:** embryo transfer, Indonesian beef cattle, pregnancy rate, progesterone

The increased production of high-quality beef is required to meet the demand of the big population in Indonesia, but there has always been a gap between supply and demand of beef, with national beef production only satisfying less than half of demand [5]. The beef cattle breed mainly kept in Indonesia is Ongole cross, followed by Simmental, Limousin, and the native cattle like Madura breed. The weight and quality of Ongole cross's carcass are not so good; therefore, the numbers of Limousin and Simmental cattle, as beef cattle, are expected to increase in Indonesia. Most beef cattle are mated naturally, whereby a bull is released into a cowherd approximately six weeks after calving period. In some cases, beef cattle can also be bred through artificial insemination. Natural mating and artificial insemination are not effective to increase the numbers of superior beef cattle, such as Limousin and Simmental cattle; whereas embryo transfer (ET) can directly produce such beef cattle if a sufficient number of Ongole cross cows are utilized effectively as recipients for ET. The problems associated with beef production in Indonesia strongly require collaborative research with some policy makers and stakeholders, including central and local governments, investors, and cooperative and farmers' communities. It is necessary to break through the plateau in the beef production system in Indonesia using ET technology.

It is generally accepted that ET programs constitute an important approach to rapidly multiply genetically superior animals, and consequently, improve livestock production [3]. The success of ET depends on factors associated with the embryo, the recipient, or an interaction among factors of the embryo and recipient, such as morphologically poor quality of embryos, inconsistency of the estrous cycle of the donors with that of the recipients, nutrition of recipients, high temperature environments, and luteal insufficiency in recipients [1, 2, 6, 9,10,11].

Progesterone (P<sub>4</sub>) is the principal hormone responsible for the maintenance of pregnancy. The synchrony between the uterine status and embryo development is important for maximizing embryonic survival and chances of pregnancy maintenance, and it is partially influenced by the ability of P<sub>4</sub> to modulate both the uterine environment and embryo growth [3, 4, 8]. However, plasma or serum P<sub>4</sub> levels during ET were related to the pregnancy rate to some extent in some studies [7, 10], while others found no relation [8, 11]; thus, the contradictions in the arguments may be due to the interaction of these factors. Therefore, it may be necessary to evaluate the effect of P<sub>4</sub> concentration under the same management and environmental conditions, especially on the cattle herd in a tropical environment like Indonesia.

The objectives in the present study were to evaluate the breeding performance of Indonesian beef cattle (Ongole cross) as recipients for ET and to compare their breeding performance with that of dairy cows (Holstein-Friesian) evaluated in the previous study [4].

The use of animals in the present study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia. The experiment was conducted in a local government farm in Tuban, East Java, Indonesia. Twenty Ongole cross cows reared in the same station and in the second parity (60 to 90, mostly 90 days after the last parturition) were selected based on the general healthy condition, body condition score (BCS; 2.5 to 3 out of 5 scores, 2.75  $\hat{\pm}$  0.26 as mean  $\hat{\pm}$  standard deviation) [12]. Normal reproductive organs and ovaries with normal corpus luteum (20 mm or more in diameter) were examined by an ultrasound device. All the beef cows were maintained under the same management. They were housed in well-ventilated roofed sheds with an anti-slippery cemented floor. They were fed with fresh grasses (mainly 30 kg/day king grass) and concentrated diet (18% crude protein) twice a day at about 9:30 A.M. and 3:30 P.M. with clean drinking water and mineral blocks provided *ad libitum*.

Estrus of all the cows was synchronized by 25 mg of an intramuscular injection of dinoprost tromethamine (prostaglandin F<sub>2 $\beta$</sub> ; Lutalyse<sup>®</sup>, Zoetis, Parsippany, NJ, U.S.A.) twice at 11-day intervals according to the manufacturer's protocol. Estrus signs were observed in the morning and afternoon twice a day. Estrus was confirmed



by the mucous and cervix dilatation using a vaginoscope. The day when estrus signs were observed was interpreted as day 0. The cows were implanted on day 7 with thawed frozen-stored embryos (Limousin breed), which were purchased from Balai Embryo Ternak Cipelang (West Java, Indonesia), using a general ET technique. During ET, the cows were administered intramuscularly with 1,500 IU of human chorionic gonadotropin (Chorulon<sup>®</sup>, Intervet, Boxmeer, Netherlands). A rectal examination was done on day 90 to judge pregnancy. The difference of the pregnancy rate between the Ongole cross beef cows and Holstein-Friesian dairy cows was statistically analyzed using the Chi-square test and odds ratio. Differences with  $P < 0.05$  were considered statistically significant.

Whole blood samples were collected from jugular vein of the cows at the onset of estrus (day 0), at the time of the ET (day 7), and on day 21. Serum was separated from the whole blood by centrifugation. Serum progesterone concentration was measured by an enzyme-linked immunosorbent assay using an analyzer (DRG:HYBRiD-XL<sup>®</sup>, DRG, Marburg, Germany) and a kit (17-OH Progesterone, DRG). Data were shown as mean  $\hat{\pm}$  standard deviation. Statistical analyses were performed using the Mann-Whitney  $U$  test. Differences with  $P < 0.05$  were considered statistically significant.

In the present study, 7 out of 20 beef cow recipients got pregnant. Based on this result, the pregnancy rate was 35%. All the calves (three male and four female) born by ET were normally healthy, weighing 32 kg on an average. One of the Limousin calves and one of Ongole cross dam are shown in [Fig. 1](#).

#### Fig. 1.

An Ongole cross dam (A) used as a recipient for embryo transfer and a Limousin calf (B) born by embryo transfer.

The serum progesterone concentration ( $ng/ml$ ) was  $0.43 \hat{\pm} 0.39$ ,  $2.84 \hat{\pm} 1.70$ , and  $2.59 \hat{\pm} 1.05$  on days 0, 7, and 21, respectively, in the nonpregnant beef cows, whereas  $0.39 \hat{\pm} 0.55$ ,  $7.06 \hat{\pm} 3.98$ , and  $13.7 \hat{\pm} 6.40$  on days 0, 7, and 21, respectively, in the pregnant beef cows ([Fig. 2A](#)). There was a significant difference in the progesterone concentration between the nonpregnant ( $n=13$ ) and pregnant beef cows ( $n=7$ ) on days 7 ( $P < 0.01$ ) and 21 ( $P < 0.001$ ).

## Fig. 2.

Comparison of serum progesterone concentration between nonpregnant (N) and pregnant groups (P) in beef (A) and dairy (B) cows as recipients for embryo transfer. All data are represented as box and whisker plots. \* $P < 0.05$ , \*\* $P < 0.01$ , and ...

Before the present study, the same research group performed an experiment using Holstein-Friesian dairy cows (60 to 90 after the last parturition) as recipients for ET using Simmental embryos purchased from the same company with the same materials and protocol as those in the present study [4]. Based on the data of the previous experiment, the pregnancy rate was 55% (11 out of 20 dairy cows), which is high compared with that (35%, 7 out of 20 beef cows) in the present study. However, there was no significant difference ( $P = 0.204$  on the Chi-square test and  $P = 0.124$  on the odds ratio) of the pregnancy rate between these two types of cows probably due to a small number of animals. The change in the progesterone concentration in the dairy cows (Fig. 2B) was similar to that in the beef cows in the present study (Fig. 2A). The serum progesterone concentration (ng/ml) was  $0.44 \pm 0.40$ ,  $3.53 \pm 2.06$ , and  $2.44 \pm 1.05$  on days 0, 7, and 21, respectively, in the nonpregnant dairy cows, whereas  $0.19 \pm 0.45$ ,  $6.64 \pm 3.87$ , and  $18.7 \pm 11.7$  on days 0, 7, and 21, respectively, in the pregnant dairy cows. There was a significant difference in the progesterone concentrations between the nonpregnant ( $n = 9$ ) and pregnant dairy cows ( $n = 11$ ) on days 7 ( $P < 0.05$ ) and 21 ( $P < 0.001$ ). The difference of the progesterone concentration between the nonpregnant and pregnant groups in the dairy cows was similar to that in the beef cows in the present study, although the pregnancy rate was high (55%) in the dairy cows compared with that (35%) in the beef cows, as mentioned above.

Successful implantation of ET depends on several factors, such as quality of embryos and recipient cows, and skills of operators [1, 2, 5, 6, 9,10,11]. The quality of embryos depends on the age of donors, types of embryos (fresh or frozen), freezing method, and contamination of infectious agents. The recipients should be generally healthy without any defect and infectious agent in the reproductive system, respond appropriately to the estrous synchronization, have enough forage, and have a moderate BCS of  $2.8 \leq 3.5$ . The operators should transfer embryos precisely in the apex cornu of the uterus in the correct time and with less stress to recipient animals.

There could be a difference in the quality of embryos between the previous [5] and present experiments because Simmental and Limousin embryos were used in the previous and present experiments, respectively. However, the embryos were processed similarly by the same company. The skills of operators were the same between the two experiments because both were performed by the same research group. The biggest difference between the two experiments are the quality of recipient

cows. Therefore, the difference in pregnancy rate between the two experiments can be attributed to the difference in breeding performance as recipients for ET between dairy and beef cows in Indonesia.

Holstein-Friesian (*Bos taurus*) dairy cows were used as the recipients for ET in the previous study [5], whereas Ongole (*Bos indicus*) cross beef cows were used in the present study. Embryos of Simmental and Limousin breeds (*Bos taurus*) were used in the previous and present experiments, respectively. The genetic difference between Ongole cross recipient cows and Limousin embryos may affect the pregnancy rate of Ongole cross recipient cows as a species barrier, although these two species can be routinely interbred in many areas of the world.

Another difference between dairy and beef recipient cows is physical conditions: BCS in dairy cows was moderate ( $3.50 \pm 0.24$ ; an author's personal information, TDL) in the previous study [5], whereas BCS in beef cows was lower ( $2.75 \pm 0.26$ ) in the present study. This lower physical condition in beef cows is probably caused by the poor management, such as a low-nutrition diet, which may affect the response to estrous synchronization and the endocrine status, including the secretion of  $P_4$ . The insufficient secretion of  $P_4$  in the beef cows on day 7 in the present study may be associated with the failure of embryo implantation. This result suggests that serum  $P_4$  concentration plays an important role in embryo implantation to increase the pregnancy rate; some studies agree with this view [7, 10], while others disagree with it [8, 11].

Based on this assumption, methods to increase the pregnancy rate in Indonesian beef cows by ET should include selecting recipients with moderate BCS (e.g., 2.8–3.5) and/or implanting embryos in recipients with a sufficiently high concentration of  $P_4$  (e.g.,  $7.06 \pm 3.98$  ng/ml) on the day of ET. Further studies are required to achieve an ideal pregnancy rate in Indonesian beef cows using ET.