

学 位 論 文 要 約

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題 目 Studies on the Development of Piggyback Programmable Syringe Pump and Multiple pFSH Injection Regimen for Superovulation in Cows
(インテリジェントマイクロシリンジを用いた豚卵胞刺激ホルモン製剤の多回投与による牛の過剰排卵に関する研究)

牛の胚生産を目的として過剰排卵処置が行われてきたが、処置 1 回当たりの胚生産数は 6.5 個未満に留まっている。牛の卵胞を過剰に発育させる為には、豚卵胞刺激ホルモン (pFSH) 製剤を反復して投与することが必要であるが、効率的な卵胞発育を促進する過剰排卵誘起法は未だに確立されていない。pFSH 製剤の 1 回投与や 1 日 2 回 4 日間の反復投与によって牛の過剰排卵を誘起する事が可能であるが、これらの方法は牛に対するストレスが大きく、投与手法も煩雑で手間のかかるものになっている。そこで本研究においては、ホルスタイン種乳牛において 1) 卵胞発育刺激処置に小型シリンジポンプを用いる事の有用性の評価、および 2) 過剰排卵処置の成績の著しい個体差が発生する要因の解明、を目的として研究を行った。

pFSH 製剤を用いて牛に過剰排卵を誘起する為には、血中半減期の短い pFSH 製剤を持続的に感作させる事が必要である。そこで投与量と投与間隔を予め設定可能なシリンジポンプを牛に背負わせて装着して、背部皮下に留置した皮下注射針から pFSH 製剤を予め設定した投与量と投与間隔で反復投与する術式を開発し、この手法を用いて牛の過剰排卵処置が可能か否かを検討した。

本研究においては、シリンジポンプによる pFSH 製剤投与で誘起した過剰排卵の卵胞発育動態を超音波診断装置で追跡した。超音波診断装置の利用によって直径 2mm 以上の卵胞を可視化する事が可能であった。過剰排卵処置に対する卵巣の反応は個体毎にばらつきが大きかったが、同一個体に反復して処置をした場合には個体毎にほぼ一定の反応がみられた。この原因は明らかではないが、個体毎の卵胞リザーブの差に起因するものと思われた。また、牛の年齢、卵巣状態、投与するホルモンの用量、投与プロトコルや栄養状態等、多様な要因が過剰排卵処置の成績に影響を与えている事が示唆された。

次いで、シリンジポンプによる 1 日 4 回投与と従来法である 1 日 2 回の漸減投与法が卵巣の反応性に及ぼす影響を検討したところ、シリンジポンプによる 1 日 4 回投与法の方が効率よく卵胞発育を誘導出来る事が示された。また、1 日 4 回投与法の場合には、発情から 1 週間後に卵巣を検査した際に残存卵胞が少なく、効率よく排卵が行われている事が示唆された。FSH の半減期 (4-6 時間) を考慮すると、1 日 4 回投与法は pFSH の血中濃度を一定に維持する投与法として適切なものと思われた。

更に、1 日 4 回投与法と 1 日 6 回投与法による過剰排卵処置の成績を比較したところ、1 日 4 回投与法の方が好成績であった。

本研究の成績から、牛に背負わせて投与間隔と投与量をプログラム出来るシリンジポンプを用いる事によって、過剰排卵が誘起出来る事が示された。シリンジポンプを用いた 1 日 4 回投与法による牛の過剰排卵処置は、従来法である 1 日 2 回投与法に比べて、発育卵

胞数が多く、かつ残存卵胞数が有意に少なかった。よって本研究で開発したシリンジポンプを用いた1日4回投与方法による過剰排卵処置は、牛に対するストレスが少なく、処置の手技が簡便で、かつ胚生産効率に優れている事が示された。

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Superovulation has been used to stimulate follicular growth dynamics as the results embryos can be collected by in vivo procedure in cows. However, as long as mean embryo production with in vivo system in cows remain an average less than 6.5. Multiple injections of pFSH are needed to develop a group of follicles from the original cohort. Despite, several researchers has been made their efforts to establish suitable treatment patterns of pFSH for follicular development to ovulate. Based on our knowledge, a successful protocol for follicular growth dynamics has not been established. Superovulation program based on a single injection of pFSH or twice-daily injections induced superovulation, which are stressful to the cows, consuming time, and required labor. Therefore, the objectives of the study were 1) to assess the usefulness of the micro syringe pump on the follicular growth dynamics in Holstein cows, 2) to determine the reason why such variable ovarian response is happened during superovulation treatment in cows.

This is the first time that such experiments has been examined by multiple injections via micro syringe pump and our results demonstrate the fundamental nature of the micro syringe pump that interestingly, in both experiments at certain intervals pump driven pFSH successfully, and induced ovarian response in cows. Certainly, hormone is necessary be available for long time into the blood circulation of cow to stimulate the follicular growth dynamics, or by other hands if given pFSH frequently in order to give a better ovarian response in cows, this can be done by using micro syringe pump. One of the advantages of micro syringe pump is that it can be programmed at certain intervals with various volumes per each injection to pump.

We investigated the numbers of the different follicles types in the ovaries of cows which was monitored by ultrasonography. Ovaries were scanned and counted a follicle approximately 2mm in diameter and characterized well during the follicular waves. The results of present studies demonstrate that the numbers of follicles that grow during follicle waves are varied and repeatable among individual cows. But the degrees of variation in the terms of the follicular growth dynamics during follicular waves between the cows are unknown. This variation is perhaps caused by ovarian reserved during fetal life in cows. The ratio of FSH/LH is also important; when FSH/LH ratio is lower superovulatory response is higher. Factors involving, age of cow, ovarian status, dose of hormone, superovulation protocol and nutrition may all of them affect the superovulatory response

as well as repeated superovulation, the response of cow will decrease between the first and second ovarian superstimulation.

In experiment one, the results showed that micro syringe pump driven pFSH successfully but the follicular growth dynamic was not developed as we expected in those cows treated with multiple six times daily-injection than that twice-daily injections regimen of pFSH. In study group fewer large unovulated follicles were detected on D21 compared with the control group, which was the major finding of our study. This research was needed for further investigation to find suitable treatment patterns of treatments for cows.

In experiment two, the results suggest that superovulatory response was increased by four times daily-injection of pFSH which was successfully driven through micro syringe pump compared with the twice-daily injections regimen in the control group. As can be seen from the data interestingly, an increased the numbers of follicles, ovulation rate and fewer unovulated follicles were detected on D21 in study group. The data clarify that an increase of armour units of pFSH from 28 to 30 AU, meanwhile increased the interval of injection from four to six h effectively induced superovulatory response in cows.

In conclusion, outcome of the studies are to clarify that superstimulation was successfully induced by administrating exogenous pFSH at certain intervals through micro syringe pump. However, we should also mention that fluctuated multiple injections were cased to increased the follicular growth dynamics, ovulation rate, formation of CLs, and fewer unovulated large follicles were detected on D21. Significant difference was revealed in the terms of large unovulated follicles between the two treatment regimens, which was the key findings of our researches. The major task of these researches was to provide an alternative for superovulation in cows, so we began this ovarian superstimulation from six times daily-injections and this was followed by four times daily-injections of pFSH via micro syringe pump. It is important to note that the four times daily-injection treatment pattern was more suitable than that of six time daily-injection treatment pattern of pFSH in non-lactating Holstein cows. Porcine FSH injection should be timed in order to mimic the physiological normal pattern of hormone into the circulation of cows as we have programmed. It is, therefore, important to note that this is one of the major development and powerful tool to induce superovulation in cows, so without doubt we took a valuable step toward success and represented an alternative to induce ovarian superstimulation in cows. In order to establish a suitable treatment patterns for follicular growth dynamics in cows, having this micro syringe pump enabled us to examine several new treatments patterns of pFSH for follicular development in cows.