Animal Reproduction

Abstracts - 37th Annual Meeting of the Association of Embryo Technology in Europe (AETE)

Practitioner's and clinical reports

Influence of body condition score and lactation status on oestrus response and pregnancy rate in dairy and beef cows inseminated with sex-sorted or non-sex-sorted semen

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Oestrous synchronization and artificial insemination (AI) are two advanced reproductive technologies that dairy and beef cattle productions are increasingly using to enhance their herds' reproductive performance. However, oestrus synchronization can be influenced by many intrinsic and extrinsic factors. Therefore, the objectives of this study were to compare the effects of body condition score (BCS) and lactation status on oestrus response rate and pregnancy rates of dairy and beef cows submitted to timed artificial insemination (TAI) with sex-sorted or non-sex-sorted semen. For this study 231 cows (dairy; n = 134 and beef; n = 97) with BCS of ≤2.5, 3 and ≥3.5 (scale 1: emaciated to 5: obese), lactating or dry and at 90 days postpartum were used. On any given day throughout the oestrous cycle (Day 0) the cows received a controlled intravaginal drug release (CIDR®, Pfizer Laboratories) device, with 2 mL intramuscular (i.m.) of estradiol benzoate® (EB; VTech). On Day 5, 2.5 mL i.m. of pregnant mare serum gonadotrophin (Chronogest®, Intervet International B.V.). On Day 8, 2 mL i.m. of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) (Estrumate®, Intervet, South Africa), with adhesive tail-head heat mount detectors (HMD) (Kamar®, USA) and CIDR® was removal. On Day 9, 1 mL i.m. of EB. TAI was performed by the same inseminator 55 hours following CIDR® removal using frozen-thawed X-sorted or non-sex sorted semen from eight sires (4 Holstein Friesian and 4 Angus). At AI, oestrus behaviour was assessed by activation of the HMD colour either as are red (oestrus/activated patch) or white (no oestrus/ not activated patch). Pregnancy diagnosis was performed 95 days following TAI using transrectal ultrasound scanner (5.0- MHz linear transducer; Ibex pro™, USA). Chi-square test was used to compare the proportion of oestrus response and pregnancy. The model included sires as a fixed effect. The proportion of oestrus response by BCS of ≤2.5 (79.0%), 3 (89.0%) and ≥3.5 (92.6%) were higher in dairy cows as compared to ≤2.5 (68.4%), 3 (61.1%) and ≥3.5 (70.8%) beef cows (P< 0.05). Lactating (86.2%) and dry (81.5%) dairy cows had higher oestrus responses, compared to beef lactating (67.7%) and dry (59.4%) cows (P< 0.05). The proportion of pregnancy was higher in BCS ≥3.5 in dairy (64.3%) cows compared to beef (40.0%) cows inseminated with non-sex-sorted semen (P< 0.05). However, BCS of 3 on beef cows had higher (41.9%) pregnancy rate compared to dairy (31.6%) cows inseminated with sex-sorted semen. Lactating dairy cows inseminated with sex-sorted (42.5%) or non-sex-sorted (50.0%) semen, had higher pregnancy rate compared to beef (sex-sorted; 31.2% and non-sex-sorted; 34.4%) (P< 0.05). However, pregnancy rate in dry cows was higher in beef (sex-sorted; 47.4% and non-sex-sorted 46.1%) cows compared to dairy (sex-sorted; 36.4% and non-sex-sorted; 36.4%) (P< 0.05). In conclusion, BCS and lactation status of dairy and beef cows do affect negatively on oestrus response and pregnancy rate.

Keywords: synchronization, pregnancy, cattle

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Practitioner's and clinical reports

Differences in reproductive parameters between two close related bovines, buffalo and cattle raised in the same environment conditions

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Buffalo and cattle are bovines that belong to the subfamily Bovinae, used worldwide to produce milk and meat. Reproductive parameters are different *in vivo* and *in vitro,* mainly when applying reproductive technologies. It has been reported that buffalo has a smaller number of primordial follicles than cattle do (10 000–19 000 vs. 150 000, respectively), smaller antral follicles, and a higher incidence of atresia (82–92%) (Kumar A. Anim Reprod Sci. 1997;47:189) as a consequence buffalo ovaries are smaller than cattle., few reports compare the consequences over reproduction of these differences, especially those produced natural mating system and their reproductive parameters. This report performed in 2021, aims to compare reproductive parameters in two bovine species located in the same geographical area, with the same reproductive management, cattle (brahman breed N=912).and buffaloes (crossbred N=262). The farms are located in Middle Magdalena Region in Colombia (6°18'48"N 73°57'00"O). Data from reproductive parameters (calving, inter calving period (IEP), days open (DO) were collected and pregnancies were diagnosid by rectal palpation, and compared. Cattle and buffaloes were culled at 10 and 15 years. Pregancies were diagnosed by rectal palpation.Data were analyzed using descriptive statistics and comparison using the student T-test. $P<0.05$ was considered statistically significant. Calving numbers were 3.04 and 4.36 for cattle and buffaloes, respectively ($P< 0.05$). Calving, IEP, and DO were 67.8% vs. 96.5%, 528 days vs. 420, and 275 days vs. 133 days, respectively, for cattle and buffaloes and statistically significant.For other parameters such as first calving age and abortion rate based on pregnancy detection was 42.76 months vs. 35.09 months, and 10.80% vs. 6.04% were are also statistically significant. If the results in reproductive parameters are associated with differences in calving number, they remain to be clarified, but there are undoubtedly paradoxical because the specie with lower quantity of primordial follicles has better reproductive performance. To date, there are no explanations for this phenomenon. Many reports that show the differences in many other reproductive parameters *in vivo* and *in vitro*. Indeed, the reproductive parameters of buffaloes and cows are different; and from this resutls are better than cattle. This performance makes the production of buffaloes more attractive to breeders specially in lands where cattle dont produce well (wet lands and humid foresr) and for reproductive biology researchers to study the causes of this observation,

Keywords: Reproductive parameters, buffaloes, cattle, differences

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Practitioner's and clinical reports

Difference in performance between OPU and slaughterhouse derived oocytes

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CRV is continuously working on further improvement the IVP embryo production process. In that process new commercial media or modification of in the standard CRV (in house made) media are tested. These media are always first tested in several experiments using slaughterhouse derived oocytes. If the embryo production rate of those experiments look good, i.e. are at least similar or better than the control (=CRV standard medium), a trial using OPU derived oocytes is started.

The aim of this study is to check whether the results of these media trials obtained with slaughterhouse oocytes are representative for those obtained with OPU derived oocytes.

Oocytes for the slaughterhouse trials are derived from ovaries of animals (mainly HF) that are slaughtered because of low production, fertility problems, etc.. These ovaries are transported (transport time ~6Hrs) at 30 °C to the IVP lab. Upon arrival oocytes are collected and quality 1 and 2 oocytes (50:50 ratio) are used for the experiments.

Oocytes for the OPU trials, are collected from animals (mostly heifers) at our own CRV station. In this case also mainly quality 1 (28%) and 2 (66%) is used in the experiments (remaining part are some quality II and IV).

Subsequently, both groups of oocytes (slaughterhouse and OPU derived) are used for in vitro production of embryos using the standard CRV media (=control) or using a new serum free medium (=test medium). The standard CRV protocol starts with a 24 hr maturation in M199 supplemented with 10% FCS, LH&FSH plus cysteamine, followed by a fertilisation for 24 hrs and a culture of 7 days in SOF-BSA medium with 0.2% serum. In the test group we used serum free media (both IVM and IVC), but supplemented the IVM and IVC media with EGF. For the slaughterhouse we did 5 different sessions for the test and control medium. In the OPU experiments we did 125 different sessions for the test medium and 178 for the control.

The results of the slaughterhouse clearly indicated that the test medium is better than the control (% total embryos 29% and 24%, respectively, $P<0.05$ Chi square test). However, the OPU results were completely different. In that case the test group was significantly worse than the control group (36% compared to 43% total embryos, $P<0.05$ Chi square test). If you look at only the quality 1+2 embryos (i.e., the transferable ones) the same pattern was observed.

From these results it is clear that the test medium, when used in combination with slaughterhouse derived oocytes, is significantly better than the control medium. However, when OPU derived oocytes are used, the test medium is significantly worse than the control medium. This is a phenomena we also see in other trials with different media.

It is difficult to explain these results. It might be because of the higher percentage of class 2 oocytes in the OPU derived group. Potentially class 2 oocytes are more sensitive to different (potentially less optimal) media.

We therefore conclude that you have to be very careful in making a decision to switch to new IVP medium based on slaughterhouse experiments only.

Keywords: IVP, OPU, slaughterhouse

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Practitioner's and clinical reports

Efficiency of repeated in-vivo embryo collections in Holstein heifers

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Worldwide, the in-vitro production (IVP) of embryos within Holstein breeding programs has increased significantly in recent years. Overall, the collection of embryos after superovulation and flushing is declining (IETS,2020). Compared to IVP embryos, embryos obtained in vivo are characterized by higher pregnancy rates, lower losses until calving and higher cryotolerance (Hansen,2020). The aim of this study is to show that larger numbers of embryos transferable for transfer can be obtained within a short time by repeated flushing. For this purpose, the data of four ET teams in Schleswig-Holstein, Lower Saxony and Hessen from the period January 2019 to March 2022 were evaluated. The calculation was carried out with Excel ®. The means were presented as arithmetic mean (AM) and median (MED). The first ($Q_{0.25}$) and third quartiles ($Q_{0.75}$) were also calculated. 1.353 embryo collections (day 7) of 712 different heifers of the Holstein breed were taken into account. The embryos were classified according the IETS standard. The frequencies of the flushings were distributed as follows: 347 heifers 1x; 223 heifers 2x; 76 heifers 3x; 35 heifers 4x; 15 heifers 5x; 8 heifers 6x; 4 heifers 7x and 1 heifer each 8x, 9x, 10x or 14x.The superovulation was done with Folltropin® (Vetoquinol) in 676 flushes and with Pluset® (Calier) in 677 flushes. 446 recoveries took place on a donor station and 907 on the farm of the breeder. On the donor station the animals were usually given an intravaginal progesterone releasing device (CIDR®; Zoetis) after each collection.

In the 1.353 flushings 12.783 embryos/oocytes (AM=9,4;MED=9; $Q_{0.25}$=5; $Q_{0.75}$=13) were recovered. 9.129 embryos (AM=6,4; MED=6; $Q_{0.25}$=3; $Q_{0.75}$=10) were suitable for transfer. At the first collection the heifers had an average age of 13.2 (MED = 13 $Q_{0.25}$=12; $Q_{0.75}$=14). Animals flushed more than four times were on average 11 months old on the day of first embryo collection (MED = 11; $Q_{0.25}$=10.0; $Q_{0.75}$=12.0). The interval between the collections averaged 51 (MED =46; $Q_{0.25}$=35; $Q_{0.75}$=59) days. 52.7% of the heifers were superovulated again at intervals of 31 to 50 days. In this group, 5,5 embryos suitable for transfer could be obtained per flush (MED=5;$Q_{0.25}$=2; $Q_{0.75}$=8). In heifers on a donor station, the average intervall was only 43 days (MED=36 $Q_{0.25}$=35; $Q_{0.75}$=48).

When the heifers were flushed four times, 25 transferable embryos could be obtained within 152 days.

It could be shown that a sufficient number of embryos can be obtained as part of a breeding program using conventional embryo transfer. The better pregnancy results of the embryos obtained in vivo (fresh and frozen) help the farms to use their limited number of recipients economically and effectively.

An increase of the effectiveness of embryo recovery seems possible. In many cases it seems to make sense to start using the donor animals even earlier. In many cases, the interval between flushings can also be shortened further (see flushings on station). Here, the farms need to be advised on rearing intensity and measures to shorten the intervals between embryo collections. IETS (2020), Data Retrieval Report 2020; https://www.iets.org Hansen (2020), Journal of Animal Science, 2020, Vol. 98, No. 11, 1–20

Keywords: in-vivo collection, bovine, breeding program

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Fertility of heifers after superovulation and embryo collection – breaking the myth

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There exists a myth among farmers in Finland, that superovulation and embryo flushing can jeopardize further reproductive performance of embryo donors. Data of 866 heifers of Holstein and Ayrshire breeds, which had undergone superovulation and embryo collection on Finnish dairy farms (donor group) were analyzed. The control group (n=824) consisted of untreated herd mates, which had their first service at the same time period as the donors. The average age at first service was 16.1 and 15.5 months for donors and controls, respectively. The following reproductive parameters were analyzed: first service conception rate, services per conception, length of the breeding period, and culling due to infertility. Independent samples t-test was used to analyze the differences between the groups.

Conception rate at the first service was similar in both groups, 52.2 and 52.1% for donors and controls, respectively. When donors were separated into two groups according to their embryo production, conception rate was 43.5% for those which didn't produce any viable embryos (n=92) and 53.5% for those which produced embryos (n=720). Double inseminations (>1 AI per estrus) were performed for 8% of the donors and 4% of the controls. Because of the higher frequency of double inseminations in the donor group, consecutively a more services per conception were required for donors compared to the controls (1.95±1.35 vs. 1.73±1.05, $P<0.001$). However, there was no difference in the duration of the breeding period for donors and controls (26.2±43.8 and 25.3±48.1, $P=0.693$). Within the donor group, 6.2% of heifers never calved and were culled. In the control group, 10.3% of heifers were culled without a preceding calving.

These findings indicate that farmers need not to be concerned of embryo production being a risk for reproductive performance of donor dairy heifers. More services per conception were needed after superovulation, but this was because of more double AIs. The reason(s) for increased frequency double AIs in the donor group were not investigated in this study. It is possible that the farmers' fertility management strategies were different for donors, as they were slightly older at the time of first service compared to their herd mates and represent the top animals of the herd, resulting to multiple AIs in order to ensure the pregnancy possibility. Also, the estrous behavior after a superovulatory treatment could be more vague and thus timing of insemination more difficult to predict. Further research is needed to investigate if there is a relationship between viable embryo production and conception rate, as the poor-responding donors showed a lower conception rate at first service. However, the most important parameter from the practical and economical point of view, the time from first service to conception, was similar for the donors and untreated controls. Also, superovulation didn't increase the risk of the heifer being culled before first calving.

Keywords: superovulation, reproductive performance, embryo donor