Surgical Embryo Transfer Method in the Native Iraqi Cows

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Abstract

Three virgin cows of 5-6 years old were selected as Donors of embryos, extensively, their genitalia was examined rectally, with a history of 1-2 normal pregnancy. After using $PGF_2\alpha$ analogue (loprosteol), field observation for the normal cyclicity was conducted twice daily. The donors was super ovulated with a single injection of 3500 IU of PMSG at day 10 of the cycle (E=0), their ovaries was examined by next day rectally for the achievement of action of the hormone, donors were re-injected with $PGF_2\alpha$analogue (loprosteol) on day 12 and inseminated as the signs of the cycle appeared. Embryos were collected non-surgically on day 6 after the insemination, examined and graded by stereomicroscope and directly transferred to synchronized recipient and follow them to term. 12 recipients of native breed, mainly SHARABI and GENOBI**.** Iraqi cows were selected; their genitalia were examined rectally and observed twice daily for normal cycle then synchronized with the donors by two injections of $PGF_2\alpha$ analogue 11days apart. Both donors and recipients were already synchronized, embryos surgically transferred to the horn epsilateral to the ovary with corpus luteum. The results showed that 8 recipients stayed without signs of estrus until day 45 after transferring, one of them continued the term to parturition, 4 recipients showed estrus signs on days 24-36. The study showed that there is a good ability to apply this technique with a simple inquiries and facilities and easily attempt in field condition and on the native breed safely and can be used it as a tool of breeding performance.

نقل الأجنة بالطريقة الجراحية في الأبقار العراقية المحلية

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الخلاصة

ثلاثة أبقار نوع فريزيان الهولندي مرباة في كلية الطب البيطري- جامعة بغداد تراوحت أعمارها بين 5-6 سنة اعتبرت كواهبات، فحصت بالجس عبر المستقيم لتقيم كفاءتها التناسلية، لها على الأقل 1-2 ولادة طبيعية، تم إجراء المراقبة العينية لمعرفة سلامة دوراتها التناسلية، حقنت عن طريق العضلة بأحد النظائر المصنعة للبروستوكلاندين وتمت مراقبتها حقليا بواقع مرتين في اليوم للتأكد من ظهور علامات الشبق عليها، اجري لها إفراط الاباضة بواسطة جرعة واحدة وبتركيز 3500 وحدة دولية من هرمون مصل الفرس الحامل في اليوم العاشر من دورة الشبق، فحصت بطريقة الجس عبر المستقيم للتأكد من تأثير الهرمون على مبايضها أعطيت في اليوم الثاني عشر من الشبق هرمون للبروستوكلاندين ولقحت صناعيا عند ظهور علامات الصراف وفي الوقت المحدد. اجري لها جمع الأجنة بالطريقة غير الجراحية في اليوم السادس من التلقيح، فحصت الأجنة بواسطة المجهر المجسم وتم تقييم الأجنة، ثم ونقلت الأجنة جراحيا إلى قرن الرحم لمستقبلة متزامنة شبقيا مع الواهبة. تم اختيار 12 بقرة كمستقبلات من الأصول العراقية (شرابي وجنوبي) فحصت أجهزتها التناسلية بالجس عبر المستقيم وروقبت عينيا

للتأكد من دوراتها التناسلية اجري لها التزامن الشبقي مع الواهبات بهرمون البروستوكلاندين بواقع جرعتين وبفترة زمنية أمدها 11 يوما بين الجرعتين. نقلت الأجنة التي تم جمعها مباشرة إلى المستقبلات المتزامنة شبقيا مع الواهبة بواقع جنين واحد أو اثنين حسب توفر الأجنة يوم الجمع جراحيا بطريقة فتح جدار البطن في منطقة الخاصرة بالجهة المقابلة للمبيض الحاوي على الجسم الأصفر. بينت النتائج إن (8 من 12) من المستقبلات لم يظهرن علامات الشبق لغاية اليوم 45 وواحدة منها استمر الحمل فيها للنهاية، بينما اظهرن (4 من 12) مستقبلات علامات الشبق في الأيام بين (24–36). أظهرت النتائج إمكانية تطبيق تقنية نقل الأجنة بنجاح على الأبقار المحلية وبالظروف والإمكانيات المتوفرة حقليا مع زيادة النجاح فيها إن استمر العمل بها مما يزيد من كفاءة العاملين في هذا المجال وجعلها واحدا من البرامج ذات الإمكانيات المستقبلية في تطوير الثروة الحيوانية.

Introduction

The extensive investigations of mammalian embryos conducted during the last two decades have increased our depth of under-standing of normal bio physiological events taking place during fertilization and early embryonic development (1). Recent studies explained that mammalian ovaries contained hundreds of thousands of oocytes as compared with the smaller number of progeny produced (2). Embryo transfer is important to save some of those follicles by stimulating the ovary to produce more and more follicles to be used in the programs of embryo transfer (3). Since the first successful embryo transfer was reported in rabbit done by Heapeon1890 (4).Many researchers were conducted in this technique until the first successful transfer on (1949) in sheep, and followed by many trail till (1956) when first successful embryo transfer in pig. The successful result was obtained by Willet (7) to get a live calf by surgical transfer of embryo in bovine. Many studies and experiment were conducted in this technique in different animals and on human (8) when first baby born in 1978. A group of commercial companies for embryo transfer in farm animals have been established in Australia, Argentina, Canada, New Zealand, USA and several European coteries (9). Embryo can be collected from the oviducts or uteri after slaughtering of the animal or excision of the reproductive tract, or can be removed either surgically or non-surgically from the intact animal (1). Surgical technique have been used to collect embryos from farm animals (10), with some advantage in getting more embryos and some disadvantages as adhesions, inflammations and we can't use the animal second time. The non-surgical technique of collection with little harmless, easy to applied, and can be repeated more than one time on the same donor after a period of rest (11). Shelton (12) showed that there was a chance to repeat the non-surgical collection without any risk to the donor and its reproductive efficiency. The catheter, the site of collection was studied well by (13). The surgical method of transferring to the horn epsilateral to the ovary with the corpus luteum in synchronized recipient studied well by (2) and said that, whatever this method have some disadvantage but still gave an elevated pregnancy rate. Evans (14) Described the site of the opening in the abdominal wall of synchronized recipient and declared that this can be applied in the field with a limited tools and labor and to be more gentile in handling the genitalia. Newcomb (15) describe the Para lumber area with the same site to the ovary with C.L in standing position with (79%) pregnancy, we pull the genitalia to the edge of the incised wound, by P. pipette puncher the wall of the horn toward the last third, the embryo was logged there, then genitalia replayed to the normal position with care.

Materials and Methods

Three donors were selected according to their records, have 5-6 years old with a history of 1-2 normal birth. Rectal examination was done to evaluate. Their genitalia. Twice daily observations for watching the cyclicity, then injected with 2ml. PGF2α (loprosteol)* I/M, to induced estrus, all observations are recorded, signs of estrus as mentioned by Williamson (16). Super ovulation was carried by single injection of 3500 IU PMSG (Folligon) ** I/M, on day 10 of the cycle (E=0) (17), their ovaries examined on day 11(E=11) to confirm the action of the Gonadotropin, on day 12 (E=12) the donors injected with 2ml. loprosteol (Prosolvin) and inseminated at the suitable time of estrus. Embryos were collected non-surgically using two-ways Foley catheter on day 6 (E=6), examined by stereomicroscope and graded depending on the number of blastomeres, size and arrangement, dead or alive, color vasculisations, as bad, good or very good (18). (*) (**)= Intervet international B. V. Boxmere-Holland. Twelve recipients of native breed (Sharabi and Genobi) were selected depending on the healthy status of their genitalia which intensively examined rectally, their aged supposed to be between 4-5 years, field observations twice daily for normal cyclicity then synchronized with 2ml loprosteol (Prosolvin), twice injection with 11 days apart (19). The donors and the synchronized recipients on day 6 (which is the collecting day) and also on the day of transferring. Synchronized recipients on the same day of collection, rectally examined to determined the side of the ovary with C.L., on standing position the Para lumber fossa shaved and anesthetized by infiltration, lidocaine 2% used, then the skin was incised together with the fascia, the abdominal muscles were diverged by hand and the genitalia were pulling toward the edge of wound. By using Pasture pipette loaded with one or two embryos attached to plastic syringe the uterine wall puncturing toward the last third where the embryos lodged, the genitalia repack to abdominal cavity with care and the wound sutured. The recipients were observed for returning to the cycle on the expected days, rectally examined on days 45, 60 and 90 to confirm pregnancy.

Results

The three donors showed a good response to the effects of the treatment with Gonadotrophine hormone through the rectal examination to their ovaries, and also showed a good response when synchronized with the recipients. Seventeen embryos were collected in this study, all appeared to be asthe same degree of growth (morulla, early blastocyst), only (14) embryos of very good grade transferred to the recipients surgically (Table 1). The results showed that the pregnancy was confirmed in (10) recipients due to non returning to the cycle in the expected days after the transfer, and one of them continued her pregnancy to the final term and give birth to a fresien calf, while (4) recipients showed signs of estrus on days (24-36) and found not pregnant.

Table (1) No. of donors, attempts of flushing and no. of collected embryos

Donor	No. of flushing	No. of collected embryos	Type of embryo	Grading
1	3	8 embryos	5 morulla	6: very good
			3 early blast.	2: bad
2	2	5 embryos	3 morulla	4: very good
			2 early blast.	1: bad
3	2	4 embryos	2 morulla	4: very good
			2 early blasts.	

Table (2) Site of transferring, type and no. of embryos and the fellowship details after transferring

Recipient	Site of transfer	Type and No. of Embryos	Details followed the transferred
1	Rt. Horn	One Morulla	Estrus on day 24
2	Rt. Horn	One Morulla	No sigs, not pregnant on day 45
3	L. Horn	One early Blastocyst	Not pregnant on day 45
4	L. Horn	One Morulla	Estrus on day 36
5	L. Horn	Two Morulla	No signs, not pregnant on day 45
6	L. Horn	One early blas.	Estrus on day 24
7	Rt. Horn	Two Morulla	Pregnant on day 45,60 & 90
8	L. Horn	One early Blas.	Not pregnant on day 45
9	Rt. Horn	One Morulla	Estrus on day 30
10	L. Horn	One Morulla	Not pregnant on day 45
11	L. Horn	One early Blas.	Not pregnant on day 45
12	L. Horn	One Morulla	Not pregnant on day 45

1. **Embryos were transferred to the horn of the same site to the ovary of the C.L.**
2. **The conformation of the pregnancies achieved by rectal examination.**
3. **The type and no. of transferred embryos depending on the no. of collected embryos on day of transferring.**
4. **Estrus detection manifested by field observation on the expected days.**

Discussion

The study showed that the gonadotropin have a good effect of ovarian tissue, and could be used in superovulation regime (20). The use of PGF2α analogue (Loprosteol) showed a good result in induction of estrus and in synchronization regime. The result agreed with (21) and this might be to the effect of PGF2α to induce estrus. The non-surgical method of embryo collection could be applied with high success under field conditions without any harmful manipulations to the super ovulatory donors (15) and we can use the same dam as a donor after considerable period of rest, without any droop in its reproductive efficiency (22). The result obtained with in agreements and dis agreement the surgical method, those within agreements appear to be more accurately in restricted the horn with same hygienic precaution. It was concluded from this study that the possibility of doing the technique of embryo transfer in local Iraqi cattle with high success when the requirement was present. The disagreement might be harmful to the animals and adhesion may result and cannot use the same animal again (2). The low pregnancy rate might be due to bad managements with imbalanced ration (23), the degree of the compatibility between the age of the embryos and the physiological status of the recipient's uterus, the maturity and physiological activity of the C.L. (24). Un known physiological events of the affect ting pregnancies (25). Early embryonic mortality and their causes related to this reason, the period from conception to days 45 of pregnancy known as the embryonic stage and all the losses takes place within (26), and also mentioned that if the losses happened before day 15 estrus return with the same time but if it is after so estrus may be delayed.

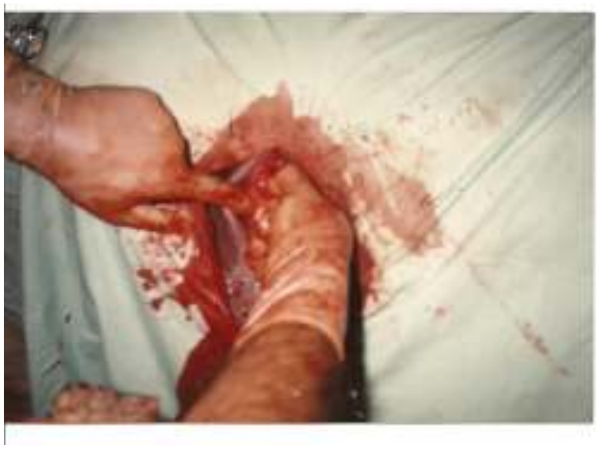

Fig. (1) surgical embryo transfer, the exploration of the horn parallel to the ovary with Corpus Luteum

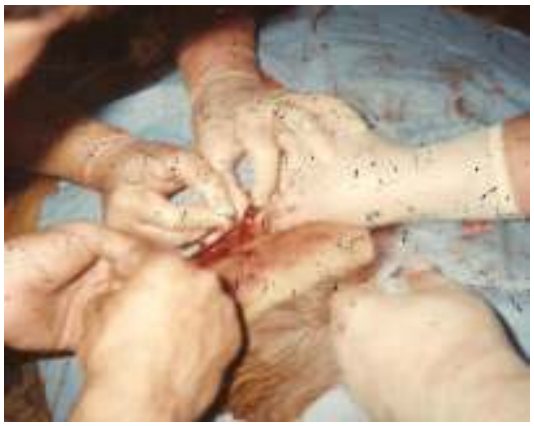

Fig. (2) Surgical embryo transfer, the technique of lodging of embryo inside the horn, see the plastic syringe connected to P. pippete loaded with embryo

Fig. (3) Surgical embryo transfer, two of 6 days embryos in the Morulla stage

Fig. (4) Surgical embryo transfer, the newborn freshen calf

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